Solutions to Analytical Chemistry Problems with Clean Water Act Methods

March 2007

Solutions to Analytical Chemistry Problems with Clean Water Act Methods

Prepared by

Analytical Methods Staff
Engineering and Analysis Division
Office of Science Technology
Office of Water
U. S. Environmental Protection Agency
Washington, DC

EPA 821-R-07-002

March 2007

Disclaimer

This technical document recommends ways to document and resolve analytical chemistry problems encountered in the analysis of wastewater samples. This advice is not a substitute for the Clean Water Act (CWA) or EPA regulations, nor is this document a regulation. The advice in this document does not alter any otherwise applicable statutory or regulatory requirements and does not, and may not, impose legally binding requirements on EPA, States, Tribes, or the regulated community.

Our advice may not apply to your case-specific circumstances. EPA, State and other decision makers retain the discretion to adopt other approaches on a case-by-case basis where appropriate, or when additional information is available to them. It is recommended that commercial laboratories convey analytical problems to their customers and permittees communicate problems to their regulatory authority and regional EPA water program offices.

Staff of the Engineering and Analytical Support Branch within the Engineering and Analysis Division of the EPA Office of Water have reviewed this document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This document was reformatted in 2014 to address errors created when the original PDF version was prepared and to replace the appendices with more readable copies. During that effort, the content of the document was not changed, but the reformatting affected some page breaks in the earlier document.

Foreword

"*Solutions to Analytical Chemistry Problems with Clean Water Act Methods*" is an update of the document titled "G*uidance on Evaluation, Resolution, and Documentation of Analytical Problems Associated with Compliance Monitoring"*, which was published in 1993. The 1993 document has been referred to as the "Pumpkin Book" because of its pumpkin-colored cover. The material and technical advice in the Pumpkin Book, and this document are based on questions and situations directed to EPA's Clean Water Act chemists by the EPA regions, state agencies and other users of our methods. The questions and situations discussed in this document concern analytical challenges encountered in the conduct of compliance monitoring under the Clean Water Act (CWA) for chemical pollutants.

The purpose of this document is to recommend ways to document the existence of a matrix or analytical problem with a CWA sample analysis, and mitigate these problems.

This document is organized as follows:

EPA's Engineering and Analysis Division (EAD) is solely responsible for the content of this document. Comments and suggestions should be directed to:

CWA Analytical Methods Staff
Engineering and Analysis Division (4303T)
Office of Science and Technology
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
E-mail: OSTCWAMethods@epa.gov

Table of Contents

Appendices

Chapter 1
Introduction

Pollutants Regulated Under the Clean Water Act

The Federal Water Pollution Control Act (FWPCA) Amendments of 1972, later amended as the Clean Water Act (CWA), classifies each pollutant as a "conventional pollutant," "toxic pollutant," or "non-conventional pollutant." The five "conventional pollutants" are codified at Title 40, Part 401.16 of the *Code of Federal Regulations* (40 CFR Part 401.16). (For information on how to access the CFR, see Chapter 9 of this document). The five conventional pollutants are:

- biological oxygen demand (BOD)
- total suspended solids (TSS)
- fecal coliform
- pH
- oil and grease

There are 65 "toxic pollutants" listed at 40 CFR Part 401.15 and this group of pollutants has been further refined to a list of 126 "priority pollutants" at 40 CFR Part 423, Appendix A. The priority pollutants can be subdivided into:

- cyanide
- asbestos
- 13 metals pollutants
- 25 pesticide/PCB pollutants
- 86 non-pesticide/non-PCB organic pollutants

By definition, all pollutants other than "conventional pollutants" or "toxic pollutants" are "non-conventional pollutants." Examples of non-conventional pollutants are:

- toxicity (acute or chronic)
- chemical oxygen demand (COD)
- metals and organic compounds not on the priority pollutant list
- radioactivity
- color

Analytical Methods Approved Under the Clean Water Act

CWA Section 304(h) requires EPA to publish test procedures (analytical methods) appropriate for the measurement of pollutants. These methods are commonly known as the "304(h) methods".

CWA Section 402 establishes a National Pollutant Discharge Elimination System (NPDES) to control the discharge of pollutants to surface waters of the U.S. NPDES is implemented through regulations at 40 CFR Parts 100 - 135 and the effluent guidelines and pretreatment regulations at 40 CFR Parts 400 - 500. The CWA prohibits any discharge of a pollutant except in compliance with the Act, including Section 402. EPA regulations implementing Section 402 generally require facilities that discharge wastewater directly to surface waters of the U.S. to obtain an NPDES permit. The regulations refer to the facility or person that discharges pollutants as "discharger," "permittee," or "applicant." Facilities that discharge

wastewater to a publicly owned treatment works (POTW) are known as indirect dischargers and subject to pretreatment requirements. EPA's pretreatment program regulations are found at 40 CFR Part 403. The term "discharger" will be used in this document to mean a discharger, permittee, applicant, or other entity regulated under EPA's wastewater regulations. Under the regulations, each discharger is required to monitor its effluent for compliance with any and all relevant Federal and State discharge limitations, and use the 304(h) methods to demonstrate compliance with NPDES and pretreatment program limitations. Regulatory authorities have accepted primacy for implementing the Clean Water Act and with that responsibility have authority to be more restrictive than the federal regulations.

The 304(h) methods are published or incorporated by reference at 40 CFR Part 136 or 40 CFR Parts 405 – 500 and are commonly known as the "Part 136 methods." For many analytes, these methods include methods published by EPA, by the U.S. Geological Survey (USGS), by voluntary consensus standards bodies such as ASTM International (formerly known as the American Society for Testing and Materials International), and by manufacturers of instruments and testing devices.

The Part 136 methods include methods approved for use in all of EPA's wastewater and ambient water programs (e.g., methods for general use). Methods approved for general use are listed in tables at 40 CFR Part 136.3(a), including:

- Table IA - List of approved biological methods
- Table IB - List of approved inorganic test procedures
- Table IC - List of approved test procedures for non-pesticide organic compounds
- Table ID - List of approved test procedures for pesticides
- Table IE - List of approved radiological test procedures
- Table IF - List of approved methods for pharmaceutical pollutants
- Table IG - Test methods for pesticide active ingredients
- Table IH - List of approved microbiological methods for ambient water

Methods approved for use in a single industrial category are published in tables at 40 CFR Part 136 or at 40 CFR Parts 405 - 500. If the methods are not published in tables at 40 CFR Part 136, they are not approved for general use, and may only be used for discharges from the industrial category for which they are approved. These special category methods are for uses when the nature of the discharge from a particular industry poses unique analytical challenges, or when the pollutants to be regulated are specific to that industry. For example, methods approved for use in the Pharmaceuticals industrial category are listed in Table IF at 40 CFR Part 136, while methods approved for use in the Pesticides Manufacturing industrial category are listed in Table 7 at 40 CFR Part 455.

At present, there are 75 pollutants listed in Table IB, including common inorganic anions, metals, and many of the conventional pollutants named above. To simplify discussions in the remainder of this document, the term "classical pollutant" will refer to all the pollutants listed in Table IB, *except* the metals (i.e., the conventional pollutants listed in Table IB and all other non-metals in the table).

Scope of This Document

We presume that you have knowledge of, and access to the relevant Part 136 analytical methods. These methods cover a wide range of pollutants and analytical technologies. The method descriptions range from a few pages for simple tests to lengthy and detailed documents covering hundreds of analytes. Many of these methods and accompanying documents are available on various CD-ROM products or at the Office of Science and Technology (OST) web site at www.epa.gov/waterscience/methods. Methods from other organizations are often available from those organizations for a fee (see Chapter 9.)

Our goal is to address a broad range of analytical problems and sample types. As a result, some level of detail was sacrificed and some situations have not been addressed in this document. However, the approaches to resolve matrix interferences that are described in this document may be applied to issues not specifically addressed in this publication. States and EPA have laboratories with experts to answer some questions regarding analytical problems.

This document does *not* cover analyses of oil and grease, metals requiring the use of "clean" sampling and analysis techniques, whole-effluent toxicity (WET), biological (microbiological), or radiological pollutants. EPA has provided guidance for some of these categories, including:

- *Analytical Method Guidance for EPA Method 1664A Implementation and Use (40 CFR Part 136)*, EPA 821-R-00-003, February 2000, (Oil & Grease)

- *Guidance for Implementation and Use of EPA Method 1631B (40 CFR Part 136)*, EPA 821-R-01-023, March 2001, (Mercury)

- *Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels*, EPA 821-R-96-011, July 1996,

- *Guidance on Establishing Trace Metal Clean Rooms in Existing Facilities*, EPA 821-B-95-001, January, 1996,

- *Trace Metal Cleanroom*, prepared by the Research Triangle Institute, RTI/6302/04/02 F,

- *Evaluating Field Techniques for Collecting Effluent Samples for Trace Metals Analysis*, EPA-821-R-98-008, June 1998,

- *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring*, EPA 821-B-96-004, July, 1996,

- *Water Quality-Based Permitting for Trace Metals Fact Sheet*, April 1996 (no EPA number),

- *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136)* (EPA 821-B-00-004, July 2000; the "WET Methods guidance"), and

- *Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program* (June, 2000).

Guidance for microbiological methods can be found in Section 9000 of *Standard Methods for Examination of Water and Wastewater*.

Guidance for radiochemistry measurements can be found in the *Multi-Agency Radiological Laboratory Analytical Protocols Manual* (EPA 402-B-04-001A to C (in three volumes); the "MARLAP Manual") published in Volume 69, page 77228 of the Federal Register (69 FR 77228) on December 27, 2004.

The documents listed above are available from the sources in Chapter 9.

Chapter 2
Sampling Requirements

Sample Collection

The collection of the sample can have significant effects on the overall analytical process. In addition, to ensure some degree of consistency and representativeness, EPA requires that a sample for compliance monitoring be collected in a prescribed fashion. Sampling requirements for the NPDES and pretreatment programs are spelled out in Parts 122 and 403 of Title 40 of the CFR.

Even when the analyst or other laboratory personnel are not responsible for collecting the sample, it is important for them to understand EPA's sampling requirements in order to provide acceptable and cost-effective analytical results (e.g., there may be little point in analyzing an improperly collected sample if the results may not be used for compliance monitoring). Ideally, laboratory personnel will have ready access to the relevant sections of the CFR. However, recognizing that this is not always practical, a reasonable level of detail is provided below.

NPDES Sampling Requirements

The sampling requirements under NPDES are given at 40 CFR Part 122, as part of the requirements for applying for an NPDES discharge permit. The requirements are broken out by type of industry and discharge. For example:

- 40 CFR Part 122.21(g)(7) provides the requirements for sampling existing manufacturing, commercial, mining, and silvicultural dischargers,

- 40 CFR Part 122.21(h)(4)(i) provides the requirements for sampling manufacturing, commercial, mining and silvicultural facilities that discharge only non-process wastewater.

- 40 CFR Part 122.21(j)(4)(viii) provides the requirements for sampling new and existing publicly owned treatment works (POTWs).

Although many of the other permit application requirements differ among these types of dischargers, they have in common the requirements for collecting grab samples for certain pollutants and how composite samples for other pollutants must be collected, namely:

> *"Grab samples must be used for pH, temperature, cyanide, total phenols, residual chlorine, oil and grease, fecal coliform, fecal streptococcus, E. coli, Enterococci, and volatile organics, unless specified otherwise at 40 CFR Part 136. For all other pollutants, a 24-hour composite sample, using a minimum of four (4) grab samples, must be used unless specified otherwise at 40 CFR Part 136."*

Providing acceptable data for NPDES compliance samples requires that the sample be collected in the required fashion. Therefore, laboratory personnel should recognize that grab samples are required for the 12 pollutants listed above and 24-hour composite samples are to be used for all other pollutants monitored under an NPDES permit.

Pretreatment Program Sampling Requirements

Sampling requirements for the pretreatment program are found at 40 CFR Part 403, specifically at 40 CFR Part 403.7(b)(2)(iii), 40 CFR Part 403(b)(2)(iv), 40 CFR Part 403.12(b)(5)(iii), and 40 CFR Part 403, Appendix E. Similar to NPDES program requirements, these sections list the pollutants for which grab sampling is required. Appendix E to Part 403 gives details on the collection of grab and composite samples for the pretreatment program.

Trace Metals Sampling Guidance

Sampling for trace metals presents a unique challenge to avoid sample contamination. EPA has guidelines for sampling ambient water for trace metals (See Chapter 9).

Compositing Samples for Volatiles

As specified in 40 CR 122.21 and noted above, samples to be analyzed for volatile organics must be collected as grab samples and not with an automated compositing device. This stands to reason, since the compositing equipment is at least partially open to the atmosphere and volatile contaminants could be lost from the equipment during the lengthy sample collection period. While using grab samples for volatiles preserves the integrity of the individual sample, it raises the overall analytical cost when multiple samples of the same discharge have to be collected and analyzed.

EPA has studied the differences between the analysis of individual grab samples, and analysis of a composite sample prepared at the laboratory from grab samples collected in the field. The study was not conclusive so the EPA has not recommended VOA compositing procedures.

Sample Preservation and Holding Times

Sample preservation and holding time requirements are listed by analyte or analyte group in Table II at 40 CFR Part 136, and are detailed in the analytical methods. The information listed in Table II is often generic, as it applies to a large group of analytes, e.g., metals. The information in the methods is often more specific because preservation and holding times generally are studied as a part of method development. However, in some cases, there are footnotes in the table for specific analytes that provide additional information or requirements that are critical to compliance monitoring. The footnotes to Table II at 40 CFR Part 136 often are quite detailed and address, but are not limited to:

- Sample containers,
- Sample holding times,
- Sample preservation, including instances in which the sample must be held for a shorter time than the stated holding time if the shorter time is necessary to maintain sample stability,
- Department of Transportation (DOT) shipping requirements,
- Interferences specific to certain parameters; e.g., interferences specific to cyanide.

Because the footnotes may change with each update to Part 136, the current version of the CFR should be consulted for the latest information. The order of precedence for the sample preservation and holding time requirements is:

- Table II with footnotes,
- The individual method

Conflicts between these requirements can arise, particularly if new methods are brought into use and the generic requirements in Table II are inadvertently not revised. If you discover a potential conflict between the holding time requirements in Table II and in a method, please notify the Engineering and Analysis Division at the e-mail address given in Chapter 9, and your permitting authority or your client.

Chapter 3
Flexibility to Modify an Analytical Method

Balancing Flexibility and Performance

EPA provides analysts with the flexibility to deal with interferences, or otherwise improve method performance. This flexibility dates back to the inception of EPA's wastewater method approval program. In December 1979, when EPA proposed the majority of the test methods for organic pollutants, the Agency requested comments on the relationship between flexibility in methods and the approach to quality control. After reviewing those comments, EPA decided to allow limited flexibility in both the sample preparation and analysis portions of its methods. The major flexibility options are discussed in the preamble of the October 26, 1984 final rule promulgating the organic methods at 40 CFR Part 136. That discussion, which is reproduced in the appendix of this document, specifically cites the ability to change chromatographic conditions such as column packings and detectors and changes to sample concentration procedures. The preamble also states that:

> *"However, the primary objective underlying this flexibility is to enhance precision and accuracy for each analysis. Flexibility should not be permitted if the altered technique would be less precise or less accurate than the standard approved analytical method. Thus, a corollary of increased flexibility was an increased need for a rigorous and unambiguous quality control procedure."*

All of the EPA methods approved at 40 CFR 136 since 1984 have incorporated a rigorous and standardized approach to quality control. If unsure, the permittee should contact the regulatory authority with questions and guidance on what constitutes allowable flexibility.

EPA's Alternate Test Procedure (ATP) Program

In addition to balancing limited flexibility in the methods against a more rigorous quality control procedure, EPA included a process for obtaining approval of an alternate test procedure (ATP) on a nationwide basis or on a site- or discharge-specific basis (40 CFR Parts 136.4 and 136.5).

The ATP program is intended to encourage development of new or improved analytical methods and to give analysts options for resolving analytical problems that may be unique to specific wastewaters. If you want to use a method other than those specified at 40 CFR Part136, you should apply to the Engineering and Analysis Division for approval of a nationwide ATP, or to the State or Regional EPA authority for approval of a limited-use ATP e.g. an approval for method changes from those listed in 40 CFR Part 136 which are granted to a specific site/facility as opposed to all permittees.

As part of the ATP program, EPA developed protocols to assist applicants seeking EPA approval of alternate test procedures or new methods for use in monitoring wastewater, ambient water, and drinking water. There are protocols for organic and inorganic contaminants, and microbiological contaminants. The changes instituted in 1999 made the process simpler. These protocols are available at www.epa.gov/waterscience/methods. (EPA 821-B-98-002 Protocol for EPA Approval of Alternate Test Procedures for Organic and Inorganic Analytes in Wastewater and Drinking Water March 1999)

Flexibility in the EPA Methods

As noted above, flexibility is permitted in many EPA analytical methods. For example, the methods for organic pollutants published at 40 CFR Part 136, Appendix A note that the analyst is permitted to "improve separations or lower the costs of analyses" provided that the results obtained are as or more accurate than the results obtained using the unmodified method. Recent EPA methods for other analytes may also include specific allowances for flexibility.

The flexibility to make changes in approved methods without prior approval from EPA is described at 40 CFR Part 136.6. The full text of Part 136.6 is reproduced in the appendix of this document. It is strongly recommended that analysts consult the full text of 40 CFR Part 136.6 before undertaking method modifications. Briefly, Part 136.6 (b)(1) describes allowable method modifications, including:

- Changes between automated and manual discrete instrumentation,
- Changes between automated and manual sample preparations such as digestions, distillations, and extractions (provided that the temperatures and/or exposure times are maintain same as manual method to achieve same performance),
- Changes in the calibration range (provided that the modified range covers any relevant regulatory limit),
- Changes in equipment such as using similar equipment from a vendor other than that mentioned in the method,
- Changes in equipment operating parameters such as minor changes in the monitoring wavelength of a colorimeter or modifying the temperature program for a specific GC column,
- Changes to chromatographic columns, including the use of a capillary (open tubular) GC column with EPA Methods 601 - 613, 624, 625, and 1624B, and
- Increases in purge-and-trap sample volumes,
- Adjusting sample sizes or changing extraction solvents to optimize method performance in meeting regulatory requirements.

Such changes are only allowed if the modified method produces equivalent performance for the analyte(s) of interest, and the equivalent performance is documented. Part 136.6 provides detailed requirements for both the demonstration and documentation of the performance of a modified method.

Note: The allowance for modifications does **not** apply to a method for a method-defined analyte or a change that would result in measurement of a different form or species of an analyte (e.g., a change to a metals digestion or total cyanide distillation). It also does not apply to changes in sample preservation and/or holding time.

In addition to the flexibility provided by the ATP program and in the analytical methods, EPA suggests that regulatory authorities allow flexibility in the spirit of method improvement. Because it is not possible to address all matrix interferences in all wastewaters, it may be necessary to tailor a method modification to a specific matrix interference problem. For example, the solid-phase and continuous liquid/liquid extraction have been shown to be effective in reducing emulsions formed with separatory funnel extraction, and microwave and bomb digestions have been shown to be more effective in solubilizing some metals than mineral acid digestions. The spirit of allowing a method modification is that the change results in improved method performance such as accuracy (e.g. recovery) a lower detection limits, or better precision.

Demonstrating Equivalency of a Method Modification

Your objective in modifying a method should be to make it more specific for a given pollutant, more sensitive, more accurate, or to improve the method in some other way *without compromising the performance of the method for the intended use.* Such improvements could include reducing the overall cost of the analysis, or reducing the volumes of wastes produced by the analysis. However, some laboratories have interpreted the provision to modify a method solely as a means of increasing the speed of analysis, thus reducing the analysis time, or taking other "shortcuts" to reduce cost. This is not EPA's approach.

EPA has addressed this issue by:

1. providing limited flexibility within the methods, so that improvements can be made, and
2. requiring the analyst to demonstrate that the results produced by a modification will be equal or superior to results produced by the unmodified method.

The yardsticks by which this performance is to be measured are precision and recovery, but can be extended to include detection limit, chromatographic resolution, mass spectral resolution, and other measures of method performance. For compliance analyses, clearly note that the method is modified and communicate the modifications to the regulatory authority. If in doubt contact your local regulatory authority.

Initial Demonstration of Method Performance

To prove the modification is appropriate, the laboratory should first perform an initial precision and recovery test (IPR) with the unmodified method, and record the results. The initial demonstration provides validation of the performance of a method by a specific laboratory. The procedure is described in detail in Section 8 or 9 of the 600-series and 1600-Series wastewater methods and also is in ASTM International methods and other methods systems. For some methods systems, the IPR may be termed an "initial demonstration of method performance" or "initial demonstration of capability" (IDC). A typical test consists of an analysis of four or more replicate volumes of reagent water, or other appropriate reference matrix, spiked with the pollutants of interest at the concentration specified in the method or at 5–10 times the detection limit of the method. The final demonstration should be done in the actual wastewater matrix of concern.

For each analyte, the precision of analysis of the replicates, as determined by the standard deviation or relative standard deviation (RSD) of the measurements, should be less than the standard deviation or RSD specified in quality control (QC) acceptance criteria in the method. Similarly, for each analyte, the average percent recovery of the measurements should fall within the range of percent recovery specified in the method. If either the precision or recovery test is failed, the test is repeated until the laboratory is able to meet precision and recovery requirements.

Include a minimum of one blank in the initial demonstration, and the concentration of the analyte(s) in the blank should be less than the level(s) specified in the method.

If you modify a method, repeat the initial demonstration with the modification as an integral part of the method, until the QC acceptance criteria in the method for precision and recovery and for the blank are met. Otherwise, the modification is not permitted. Maintain records that document that the initial demonstration was performed on the modified method and those requirements for precision and recovery and the blank were met.

Application of a Method Modification to a Sample Matrix

In addition to the initial demonstration in a reference matrix such as reagent water, the method modification is applied to the specific discharge or sample matrix to which the modified method will be applied in monitoring. The modified method is tested by spiking the analytes of interest into duplicate aliquots of the sample matrix at a concentration of 5 - 10 times the background concentration of the pollutant in the sample, 1 - 5 times the quantitation limit, or 1 -5 times the regulatory limit, whichever is greatest. The recoveries of the analytes from these matrix spike/matrix spike duplicate (MS/MSD) tests are compared to the QC acceptance limits in the original method. Likewise, the relative percent difference (RPD) of the MS/MSD results is calculated and compared to the QC limits for RPD in the approved method. The modification is acceptable if the recovery and RPD meet the respective limits. Methods from some sources may use terms other than MS/MSD for these QC samples, but the concept and use remain the same.

Suggested QC Acceptance Criteria for Criteria Not Stated in Approved Methods

Many of the older methods listed in the tables at 40 CFR Part 136 do not contain standardized QC or QC acceptance criteria. To fill this gap, EPA proposed standardized QC tests and analyte-specific QC acceptance criteria for all 75 contaminants in Table IB at 40 CFR Part 136 in a "Streamlining Initiative" in 1997. The initiative was proposed on March 28, 1997 (62 FR 14975) and a correction was published on June 26, 1997 (62 FR 34573). The QC acceptance criteria published in the Streamlining Initiative were developed from interlaboratory data or from single-laboratory data with an allowance for interlaboratory variability (see Section III.B.2 of the proposal at 62 FR 14983). Although that initiative was not completed, EPA remains committed to the intent of this initiative. EPA suggests use of the QC tests and QC acceptance criteria in the Streamlining Initiative as a starting point for evaluating method modifications when the approved method is absent of such tests and criteria.

Intractable Samples

Method flexibility permits pollutant identities and concentrations to be determined in nearly all wastewaters, but EPA recognizes that there may be a few intractable sample matrices that do not yield readily to extensive analytical efforts. Please let EPA or your regulatory authority know about modifications that you have made that have worked or not worked with difficult matrices. Reporting to the permitting authority that "the sample couldn't be analyzed" is not sufficient and will not be accepted as justification for a claim of matrix interference. See Chapter 4 for the information that will document a matrix interference and Chapter 8 for possible relief when a matrix interference is shown.

Chapter 4
How to Document Matrix Interference

This chapter outlines the analytical data and other information that the EPA recommends be provided to evaluate a discharger's claim that a complex matrix precludes measurement of a pollutant. Generally, the data are the same as data gathered by EPA in developing the Agency's regulations.

Because different analytical techniques provide different data (e.g., gas chromatography/mass spectrometry (GC/MS) procedures produce plots of mass intensities while colorimetric procedures do not), the specific form of the data will differ according to the method. The following items describe the minimum data that should be developed to support a claim of compliance.

1. The identity of the method used for the measurement.

In order to support a claim of a matrix interference, the analyst should, of course, use a method that is approved for the pollutant of interest for NPDES compliance monitoring. Therefore, the most basic information an analyst should submit is the identity of the method used for the measurement e.g., separatory funnel or continuous liquid/liquid extraction. This information should include the source of the method (e.g., EPA, *Standard Methods*, or ASTM), the method number, complete with any letter suffixes or "point" designations (e.g., 1613B or 350.1), and the date of issue of the method (for EPA methods) or the edition of the method compilation (e.g., *Standard Methods*, 18th edition). The tables at 40 CFR Part 136 illustrate the level of detail required to unambiguously identify a particular method. The date of an EPA method revision or the edition from which a *Standard Method* is drawn are often critical because not all EPA method revisions are approved at 40 CFR Part 136 and different editions of *Standard Methods* may use different letter suffixes for the same technique as methods are added or removed from the manual (e.g., SM 4500-S^{-2} E in the 18th edition is the iodometric method, but in the 19th and 20th editions, the iodometric method is SM 4500-S^{-2} F).

2. A detailed narrative discussing the problems with the analysis, corrective actions taken, and the changes made to the approved method identified.

The discharger should describe the reasons for the change to the approved method, the supporting logic behind the technical approach to the change, and the result of the change.

Many compliance monitoring analyses are performed by contract laboratories on behalf of the discharger. However, the responsibility for providing the information to EPA rests with the discharger. The discharger should, therefore, impress upon its contract laboratory the need for detailed technical communication of problems experienced and solutions attempted. The narrative should be authored by an analyst and written in terms that another analyst can understand.

3. A summary level report or data reporting forms giving the pollutants for which analyses were conducted and the concentrations detected. For the pollutants that were not detected, the detection limits or estimated detection limits should be provided.

Such results should be provided for each field sample analyzed, including any dilutions and reanalyses. If not specified in the approved method, the means for estimating the detection limit of each pollutant should be provided in the narrative. If the laboratory uses "flags" in its data reporting, the definition of each flag should be provided with the data.

4. A summary of all quality control results required by the approved method.

These results include, but are not limited to the following:

- Instrument tuning, if applicable
- Calibration
- Calibration verification
- Initial precision and recovery test, as described in Chapter 3
- Ongoing demonstration of laboratory capability (i.e., ongoing precision and recovery, laboratory control sample, laboratory fortified blank)
- Matrix spike/matrix spike duplicate (MS/MSD) or equivalent spiked sample matrices
- Surrogate recovery, if applicable
- Labeled compound recovery (isotope dilution methods)
- Blank results

5. Raw data that will allow an independent reviewer to validate (reconstruct) each determination and calculation performed by the laboratory.

This validation would consist of tracing the instrument output (peak height, area, or other signal intensity) to the final result reported. The raw data are method specific and may include any of the following:

- Sample numbers or other identifiers used by the both the discharger and the laboratory
- Extraction or digestion date
- Analysis date and time
- Sequence of analyses or run log
- Sample volume
- Extract volume prior to each cleanup step
- Extract volume after each cleanup step
- Final extract volume prior to injection
- Digestion volume
- Titration volume
- Percent solids or percent moisture
- Dilution data, differentiating between dilution of a sample and dilution of an extract or digestate
- Instrument and operating conditions
- GC and/or GC/MS operating conditions, including detailed information on
  - columns used for determination and confirmation (column length and diameter, stationary phase, solid support, film thickness, etc.)
  - analysis conditions (temperature program, flow rate, etc.)
  - detector (type, operating conditions, etc.)
- Chromatograms, extracted ion current profiles, bar graph spectra, library search results
- Quantitation reports, data system outputs, and other data to link the raw data to the results reported. (Where these data are edited manually, explanations of why manual intervention was necessary should be included.)
- Direct instrument readouts; i.e., strip charts, printer tapes, etc., and other data to support the final results
- Laboratory bench sheets and copies of all pertinent logbook pages for all sample preparation and cleanup steps, and for all other parts of the determination

The raw data required should be provided not only for the analysis of samples, but also for all calibrations, calibration verifications, blanks, matrix spikes and duplicates, and other QC analyses required by the approved method. Data should be organized so that an analyst can clearly understand how the analyses were performed.

6. **Example calculations that will allow the data reviewer to determine how the laboratory used the raw data to arrive at the final results.**

   Useful examples include both detected compounds and undetected compounds. If the laboratory or the method employs a standardized reporting level for undetected compounds, this should be made clear in the example, as should adjustments for sample volume, dry weight reporting (solids only), dilutions, etc.

7. **Possible submission of raw data in electronic format.**

   For GC/MS and other instruments involving data systems, the discharger should be prepared to submit raw data in electronic format or current permanent format upon request by EPA.

8. **The names, titles, addresses, and telephone numbers of the analysts that performed the analyses and of the quality control officer that assured and will attest to the results.**

   If a contract laboratory collected the data, it is the discharger's responsibility to see that the contract laboratory met all of the requirements in the methods and that the pertinent data listed above are provided.

9. **Describe attempts to minimize interference.**

   It is important that the laboratory describe all attempts to eliminate or minimize the interference, e.g., use of simple dilution or use of a totally different 40 CFR Part 136 method that still allows reliable measurements at the permit level.

10. **Document modifications.**

    The lab should also lists how any modifications made were demonstrated by the supporting data to give equivalent performance over the reference method as written.

Chapter 5
Reports of Matrix Interferences

Chapter 4 described the kind of information that in the EPA's view should be provided to demonstrate that a matrix problem precluded measurement of a pollutant regulated under a NPDES permit limitation. This chapter provides case histories of selected reports of matrix interference problems submitted by dischargers regulated under the Organic Chemicals, Plastics, and Synthetic Fibers (OCPSF) rule. Additional details of matrix interferences reported by dischargers and others, and how to overcome these interferences, are given in Chapter 6 of this document.

In the early 1990s, the Engineering and Analysis Division (EAD) of EPA reviewed data provided by at least 15 dischargers regulated under the categorical pretreatment standards for the OCPSF industry. In each instance, the discharger reported that the facility's wastewater could not be monitored for compliance with the pretreatment standards because of interferences. EAD was asked by either the Region or State permitting authority to review these reports of matrix interferences. Over the years, dischargers have reported similar matrix interferences in other industrial categories and many of the documents cited in this document were developed by EPA to address these reports.

EAD's review focused on each facility's reported inability to determine the organic analytes in its wastewater because of interferences. This chapter presents six case histories of EAD's review of data submitted by dischargers reporting interference problems and provides further detail as to how these dischargers might resolve matrix interference problems. None of the dischargers nor any of the laboratories involved are identified in this document.

Prior to EAD reviewing the data, each of the permitting authorities was provided with:

- A draft checklist of laboratory data required to support a claim that the discharger was unable to measure pollutants due to matrix problems. That draft checklist resembled the *Data Required to Document a Matrix Interference* in Chapter 4 of this document.

- Draft guidance for analysts attempting to identify and quantify pollutants in wastewaters discharged from plants manufacturing organic chemicals, plastics, and synthetic fibers. That draft guidance was ultimately incorporated into the 1993 "Pumpkin" book.

- Draft guidance for permit writers and others reviewing data from the analysis of organic compounds determined using the 600- and 1600-Series methods, similar to that found in Chapter 7 of this document.

It was EAD's intention that these draft documents be provided to the dischargers and in turn to their laboratories, as needed. However, the review revealed that the States and Regions had either not provided the draft documents or had not followed them. In general, EAD's review of the reports submitted by the dischargers revealed the following:

- In nearly all instances where data were submitted, the dischargers and/or their contract laboratories used incorrect analytical methods or did not follow the procedures required in 40 CFR Part 136.

- In other instances, the dischargers and/or their contract laboratories did not submit data necessary to document that the methods were followed.

- Finally, the dischargers and/or their contract laboratories did not submit documentation regarding the nature of interferences and the attempts (if any) to resolve these interferences.

Case Histories

Case #1: This discharger used a contract laboratory for its analytical work. Information submitted by the laboratory revealed inconsistencies with the stated analytical methods.

The discharger allowed the laboratory to either:

(1) Use methods other than the 40 CFR Part 136 methods, or
(2) Modify methods 624 and 625.

At the time the data were evaluated by EAD, alternative methods were allowed under the ATP program described at 40 CFR Part 136.4 and 136.5, but required prior approval from EPA. Otherwise, alternative methods were not allowed. EAD found no reference to the approval of the laboratory's modified methods.

If Methods 624 and 625 were modified under the spirit of the 40 CFR Part 136.6, the laboratory did not document these modifications and did not demonstrate their equivalence. Modifications that the laboratory made to Methods 624 and 625 included:

- Combining acid and base/neutral fractions for samples analyzed by Method 625,
- Using a fused-silica capillary column for the analysis of acid and base/neutral fractions (since approved by EPA, Appendix B and C)
- Using alternative internal standards,
- Using alternative surrogates,
- Using higher detection limits,
- Using fewer matrix spike compounds, and
- Using matrix spike amounts inconsistent with regulatory compliance, background, or method-specified levels.

The October 26, 1984 preamble to the 40 CFR Part 136 methods states that a method is considered to be equivalent, if its performance has been demonstrated to meet or exceed the specifications in the original method. None of the submitted data provided any evidence supporting method equivalence.

The use of multiple internal standards and a fused-silica capillary column for the base/neutral and acid fractions represent improvements. EPA has provided letters recommending approving of these modifications. 40 CFR Part 136.6 explicitly allows for changes to the chromatographic column and use of alternative internal standards and surrogates without prior approval from EPA.

However, at that time EPA did not accept combining fractions, higher detection limits, alternative matrix spike compounds, and matrix spike amounts inconsistent with background or regulatory compliance levels represent improvements. It was determined also at that time these changes degrade method performance and are therefore in violation of both the spirit and letter of the flexibility permitted in the 600- and 1600-Series 40 CFR Part 136 organic methods.

The matrix spike compounds and spiking levels used by the laboratory appeared to have been from Office of Solid Waste (OSW) SW-846 methods or from Superfund Contract Laboratory Program (CLP)

methods. The 600- and 1600-Series wastewater methods require the matrix spike compounds to be the compounds regulated in the discharge (e.g., 40 CFR Part 136, Appendix A: Method 624, Section 8.3) and require that the spike levels be at:

(1) The regulatory compliance level,
(2) 1–5 times the background level of the analyte in the sample, or
(3) The level specified in the method (e.g., Method 624, Section 8.3.1).

The compounds spiked were not those regulated, and the spikes were not at the levels required. Therefore, the results were not useful in demonstrating performance of the method for the problem analytes.

The matrix spike was performed on a diluted sample. Had the matrix spike been performed as specified in Method 624 or 625, the spike would likely have failed the specifications in the method and the associated sample result could not have been reported for regulatory compliance purposes. This should have triggered cleanup procedures, the use of alternative methods, or modification of Method 624 or 625 to improve method performance.

The QC specifications for matrix spike recovery used by the laboratory were not the specifications given in Methods 624 and 625. The specifications in the wastewater methods (40 CFR Part 136, Appendix A: Method 624, Table 5; and Method 625, Table 6) must be used for compliance monitoring. While tighter specifications from a documented source may be acceptable if met, use of wider limits without documentation is not acceptable.

The detection limits reported for semivolatiles were, for the most part, twice the minimum levels given in Method 625 and were approximately 10–20 times the method detection limits (MDLs) given in Method 625. No explanation for the increased detection limits was given, nor could the limits be derived from the data provided.

The laboratory made no attempt to clean up the samples using pH change, gel permeation chromatography, or the other techniques described in the 600- and 1600-Series methods or in the draft guidance provided by EPA.

Even with the increased and explicit allowance for flexibility provided at 40 CFR Part 136.6, the majority of the modifications made by this laboratory did not improve performance; did not see the analytes at the regulatory limits, and thus the modifications were not acceptable.

EAD has since recommended (Appendix C) allowing several acceptable modifications to EPA Method 625 for environmental permitting and compliance monitoring under the EPA's CWA program.

Case #2: Information provided with data submitted by this discharger was insufficient for a detailed review.

Despite the general lack of data, it appeared the discharger submitted samples to a contract laboratory for analyses by a GC/MS method which failed to produce useful results. The discharger and/or the laboratory attributed the problems to large concentrations of acetone in the discharge, though this problem could not be confirmed from the information provided. The analytical contractor proposed to the discharger that Methods 601 and 602 be used for the volatiles analysis in an attempt to overcome the interference problems. Both of these methods are approved at Part 136 methods and they are more sensitive and more selective than a GC/MS method. Therefore, the regulated analytes should be measurable in the presence of a large concentration of acetone. The discharger ignored the laboratory's proposal and submitted

a report of matrix interferences. EPA reported that the approach proposed by the laboratory was workable and appropriate, and should have been attempted.

Case #3: This discharger used several contract laboratories for analyses. The reports from these laboratories consisted of summary reporting forms showing detection limits that were 10–50 times greater than the MDLs in Methods 624 and 625.

There were no QC results, no details of how the analyses were performed, and no documentation of interference problems or steps taken to overcome interference problems, and therefore, no documentation that an interference existed. The laboratory may have chosen to dilute samples for convenience. The discharger and its laboratory should have provided the data listed in Chapter 4 of this document, and attempted to solve interference problems using the techniques discussed in Chapter 6 of this document.

Case #4: This discharger submitted a report from one contract laboratory that contained insufficient information for evaluation; and two letters from a second contract laboratory describing a problem with 4,6-dinitro-*o*-cresol.

The report provided by the first laboratory indicated no results for spikes of the OCPSF-regulated analytes into samples, no details of how the analyses were performed, what interference problems were encountered, or what steps were taken to overcome interference problems. In addition, it appeared that the contract laboratory combined acid and base/neutral extracts, thus exacerbating interference effects.

The letters from the second laboratory describing the problem with 4,6-dinitro-*o*-cresol asked for suggestions on how to determine this compound in the presence of interferences. Chapter 6 of this document provides general suggestions for overcoming matrix interference problems and specific suggestions for determination of phenol. The specific suggestions for determination of phenol can be applied to 4,6-dinitro-*o*-cresol.

Other reports by the contract laboratory showed high detection limits for the substituted phenols because of a huge quantity of phenol in the sample. The analytical laboratory should have used the procedures for determination of phenol detailed in the Chapter 6 of this document.

Case #5: This discharger submitted letters and reports from several contract laboratories.

Data items that were present and are required for a thorough review were instrument tunes, run chronologies, chromatograms, calibration data, calibration verification data, results for blanks, quantitation reports for samples, and matrix spike data run against the QC limits for Methods 624 and 625. The initial precision and recovery (IPR) data that demonstrate method equivalence were missing.

The semivolatile matrix spike data were inconsistent. Results of analysis of unspiked samples indicated that some of the acids and base/neutrals were not detected, yet results for the spiked samples showed large concentrations of some analytes that were not spiked into the samples.

The volatiles matrix spike had been diluted by a factor of 200 and spiked after dilution. Diluting and spiking will not show matrix interferences, and thus these data are of no value in evaluating the undiluted sample results.

Cases #6: Several dischargers simply submitted summary reports from their laboratories.

None of the materials contained the information required in Chapter 4 of this document, and none contained explanations of the nature of the interferences found or descriptions of attempts to overcome these interferences. These facilities should have followed the guidance in Chapters 4 and 6 of this document, and reviewed the data produced using the data review guidance provided in Chapter 7 of this document.

Chapter 6
Solutions to Matrix Interferences

Solving Matrix Problems

The inability to measure the concentration of a pollutant in a specific wastewater is often attributed to a "matrix problem." Matrix problems are caused by substances in the water that interfere in some way in the analysis. These substances can be suspended materials, dissolved salts, polymeric materials, and highly acidic or caustic waters. Examples of solutions to matrix problems are described below and given in references in this chapter. The examples are not intended to be exhaustive, but rather representative enough to help the analyst understand how to overcome typical matrix interference problems.

In addition to the information below and in the guidance documents referenced in this document, the means to overcome matrix interferences can often be found in the technical literature and in sets of methods and individual methods published by other EPA Offices and by other organizations, most notably *Standard Methods for the Examination of Water and Wastewater* (Standard Methods) and methods published by ASTM International. Both of these method sets contain extensive means for overcoming matrix interference problems, and Standard Methods and ASTM methods should be consulted before contacting EPA for a solution to a matrix interference problem.

Solutions Applicable to Nearly All Analytes

Selective Reaction and/or Removal of the Interferent

The best solution to a matrix interference problem caused by a particular substance is to first identify the substance, then selectively remove it from the sample or from the sample extract or digestate. Selective removal can be accomplished by reaction with another substance that will not interfere or by physical separation from the analyte of interest by adsorption on an ion exchange or chromatographic column. The selective reaction/removal technique is described in some of the suggested solutions to matrix interference problems below, and is described in further detail in Standard Methods and ASTM methods.

Method of Standard Additions (MSA)

A common means of resolving matrix interferences that can be applied to nearly all analytes in all matrices is the "method of standard additions" (MSA). MSA for metals is described in *Methods for Chemical Analysis of Water and Wastes* (MCAWW Revised March 1983, NTIS PB 84-128677). MSA for organics is described in ASTM Standard D 5788. Also, instrument manufacturers may provide MSA procedures in instruction manuals and/or application notes. In MSA, increasing concentrations, typically at factors of 2, 4, and 8 times the concentration of the analyte in the sample, are added to separate aliquots of the sample. The aliquots are analyzed and a regression or plot of response versus concentration is used to determine the concentration of the analyte in the sample.

Solutions Applicable to Classical Pollutants

Oil and Grease

Oil and grease is the pollutant for which the most matrix interferences have been reported to EPA, and nearly all of the reports have been about the formation of emulsions in the extraction of oil and grease. EPA has published advice on these problems in *Analytical Method Guidance for EPA Method 1664A Implementation and Use,* EPA 821-R-00-003, February 2000 (see Chapter 9).

Cyanide

Next to oil and grease, cyanide is the pollutant for which the most matrix interferences have been reported to EPA. Cyanide chemistry is very complex, and resolving matrix interferences with cyanides may involve considerable investigation. Fortunately, companies that work with cyanides are usually very familiar with the cyanide chemistry used in their products/processes and wastewaters, and have addressed cyanide interference issues. Suggested means for mitigating or overcoming cyanide interferences are presented in Section 4500-CN^- of *Standard Methods for the Examination of Water and Wastewater*, in ASTM D2036, *Standard Test Methods for Cyanides in Water*, and in OIA Method 1677, approved for use at 40 CFR Part 136. Standard Method 4500-CN^- and ASTM D2036 devote large sections to overcoming cyanide interferences.

The most common interfering species in the determination of cyanides is sulfur, primarily in the form of sulfide. Footnotes to Table II at 40 CFR Part 136.3 address cyanide interferences other than sulfide, including:

- sulfur,
- sulfite,
- oxidants (including chlorine and hypochlorite),
- thiocyanate,
- aldehydes, and
- carbonate.

The footnotes also address the preservatives that may be used and how to deal with particulate matter in the sample. The following text is based on footnotes to Table II at 40 CFR Part 136.3 and input from ASTM:

"Add a reducing agent only if an oxidant (e.g., chlorine) is present. Reducing agents shown to be effective are sodium thiosulfate ($Na_2S_2O_3$), ascorbic acid, sodium arsenite ($NaAsO_2$), or sodium borohydride ($NaBH_4$). However, some of these agents have been shown to produce a positive or negative cyanide bias, depending on other substances in the sample and the analytical method used. Therefore, do not add an excess of reducing agent. Methods recommending ascorbic acid (e.g., EPA Method 335.4) specify adding ascorbic acid crystals, 0.1 - 0.6 g, until a drop of sample produces no color on potassium iodide (KI) starch paper, then adding 0.06 g (60 mg) for each liter of sample volume. If $NaBH_4$ or $NaAsO_2$ is used, 25 mg/L $NaBH_4$ or 100 mg/L $NaAsO_2$ will reduce more than 50 mg/L of chlorine (see method "Kelada-01" and/or Standard Method 4500-CN^- for more information). After adding reducing agent, test the sample using KI paper, a test strip (e.g. for chlorine, SenSafe™ Total Chlorine Water Check 480010) moistened with acetate buffer solution (see Standard Method 4500-Cl.C.3e), or a chlorine/oxidant test method (e.g., EPA Method 330.4 or 330.5), to make sure all oxidant is removed. If oxidant remains, add more reducing agent. Whatever agent is used, it should be tested to assure that cyanide results are not affected adversely.

[6] Sample collection and preservation: Collect a volume of sample appropriate to the analytical method in a bottle of the material specified. If the sample can be analyzed within 48 hours and sulfide is not present, adjust the pH to >12 with sodium hydroxide solution (e.g., 5 % w/v), refrigerate as specified, and analyze within 48 hours. Otherwise, to extend the holding time to 14 days and mitigate interferences, treat the sample immediately using any or all of the following techniques, as necessary, followed by adjustment of the sample pH to >12 and refrigeration as specified. There may be interferences that are not mitigated by approved procedures. Any procedure for removal or suppression of an interference may be employed, provided the laboratory demonstrates that it more accurately measures cyanide. Particulate cyanide (e.g., ferric ferrocyanide) or a strong cyanide complex (e.g., cobalt cyanide) are more accurately measured if the laboratory holds the sample at room temperature and pH >12 for a minimum of 4 hours prior to analysis.

(1) Sulfur: To remove elemental sulfur (S_8), filter the sample immediately. If the filtration time will exceed 15 minutes, use a larger filter or a method that requires a smaller sample volume (e.g., EPA Method 335.4 or Lachat Method 01). Adjust the pH of the filtrate to 12 - 13 with NaOH, refrigerate the filter and filtrate, and ship or transport to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate from the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in µg or mg), and divide by the original sample volume to obtain the cyanide concentration.

(2) Sulfide: If the sample contains sulfide as determined by lead acetate paper, or if sulfide is known or suspected to be present, immediately conduct one of the volatilization treatments or the precipitation treatment as follows: Volatilization - Headspace expelling. In a fume hood or well-ventilated area, transfer 0.75 liter of sample to a 4.4-L collapsible container (e.g., Cubitainer™). Acidify with concentrated hydrochloric acid to pH < 2. Cap the container and shake vigorously for 30 seconds. Remove the cap and expel the headspace into the fume hood or open area by collapsing the container without expelling the sample. Refill the headspace by expanding the container. Repeat expelling a total of five headspace volumes. Adjust the pH to >12, refrigerate, and ship or transport to the laboratory. Scaling to a smaller or larger sample volume must maintain the air to sample volume ratio. A larger volume of air will result in too great a loss of cyanide (> 10%). Dynamic stripping: In a fume hood or well-ventilated area, transfer 0.75 liter of sample to a container of the material specified and acidify with concentrated hydrochloric acid to pH < 2. Using a calibrated air sampling pump or flowmeter, purge the acidified sample into the fume hood or open area through a fritted glass aerator at a flow rate of 2.25 L/min for 4 minutes. Adjust the pH to 12 - 13, refrigerate, and ship or transport to the laboratory. Scaling to a smaller or larger sample volume must maintain the air to sample volume ratio. A larger volume of air will result in too great a loss of cyanide (> 10%). Precipitation: If the sample contains particulate matter that would be removed by filtration, filter the sample prior to treatment to assure that cyanide associated with the particulate matter is included in the measurement. Ship or transport the filter to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate from the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a

higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in µg or mg), and divide by the original sample volume to obtain the cyanide concentration. For removal of sulfide by precipitation, raise the pH of the sample to >12 with NaOH solution, then add approximately 1 mg of powdered cadmium chloride for each mL of sample. For example, add approximately 500 mg to a 500-mL sample. Cap and shake the container to mix. Allow the precipitate to settle and test the sample with lead acetate paper. If necessary, add cadmium chloride but avoid adding an excess. Finally, filter through 0.45 micron filter. Cool the sample as specified and ship or transport the filtrate and filter to the laboratory. In the laboratory, extract the filter with 100 mL of 5% NaOH solution for a minimum of 2 hours. Filter the extract and discard the solids. Combine the 5% NaOH-extracted filtrate with the initial filtrate, lower the pH to approximately 12 with concentrated hydrochloric or sulfuric acid, and analyze the combined filtrate. Because the detection limit for cyanide will be increased by dilution by the filtrate form the solids, test the sample with and without the solids procedure if a low detection limit for cyanide is necessary. Do not use the solids procedure if a higher cyanide concentration is obtained without it. Alternatively, analyze the filtrates from the sample and the solids separately, add the amounts determined (in µg or mg), and divide by the original sample volume to obtain the cyanide concentration. If a ligand-exchange method is used (e.g., ASTM D6888), it may be necessary to increase the ligand-exchange reagent to offset any excess of cadmium chloride

(3) Sulfite, thiosulfate, or thiocyanate: If thiocyanate is known or suspected to be present, use UV digestion with a glass coil (Method Kelada-01) or ligand exchange (Method OIA-1677) to preclude cyanide loss or positive interference. If sulfite and thiosulfate are present there is no way to accurately determine cyanide if heat is applied. In these situations a non-distillation method such as D6888-04, or method OI-1677 may be used.

(4) Aldehyde: If formaldehyde, acetaldehyde, or another water-soluble aldehyde is known or suspected to be present, treat the sample with 20 mL of 3.5% ethylenediamine solution per liter of sample.

(5) Carbonate: Carbonate interference is evidenced by noticeable effervescence upon acidification in the distillation flask, a reduction in the pH of the absorber solution, and incomplete cyanide spike recovery. When significant carbonate is present, adjust the pH to ≥12 using calcium hydroxide instead of sodium hydroxide. Allow the precipitate to settle and decant or filter the sample prior to analysis (also see Standard Method 4500-CN.B.3.d)".

Solutions Applicable to Metals Pollutants

In environmental testing, samples analyzed for metals are digested with strong mineral acid(s), or the metal is chelated and extracted, followed by determination of the metals by:

- flame atomic absorption spectrophotometry (FLAA),
- graphite furnace atomic absorption spectrophotometry (GFAA),
- cold-vapor atomic absorption spectrophotometry (CVAA),
- hydride generation atomic absorption spectrophotometry (HGAA),
- direct-current plasma atomic absorption spectrophotometry (DCPAA),
- inductively coupled plasma/optical emission spectrometry or mass spectrometry (ICP/OES and ICP-MS),
- atomic fluorescence spectrophotometry (AF)
- or colorimetric, titrimetric, voltammetric, or gravimetric techniques.

The full list of techniques approved for metals analysis is listed in Table IB at 40 CFR Part 136.

The most commonly used of these techniques for determination of metals in wastewater are GFAA, HGAA, and ICP-MS. HGAA is only approved at 40 CFR Part 136 for arsenic and selenium. CVAA is used exclusively for determination of mercury. The introduction of EPA Method 1631, an atomic fluorescence method, has caused a shift in technology because of the ability to measure to lower levels than CVAA, and with fewer interferences.

Clean Room

Although not strictly a matrix interference problem, contamination of metals samples, particularly at or near ambient water quality criteria (WQC) levels, can be a significant problem in sampling and in some laboratories. The problem is particularly common for mercury, which is a volatile metal and, therefore, can be transported throughout a building through heating, ventilating, and air conditioning systems. However, clean room techniques have been successful at eliminating contamination sources for many other metals, including lead and zinc.

To mitigate laboratory contamination problems with mercury and other metals, EPA published *Guidance on Establishing Trace Metal Clean Rooms In Existing Facilities*, EPA-821-B-96-001, April, 1995 and *Trace Metal Cleanroom*, RTI/6302/04-02F, Research Triangle Institute, October, 1995. These guidance documents detail how the laboratory can modify existing facilities to reduce contamination to the lowest levels and, thereby, prevent contamination from interfering in the analysis.

General Matrix Interferences

Matrix interferences in metals determinations by AA, ICP, and other techniques result in a decrease or increase in the signal (response) from what the signal would be if the interference were not present. For analysis of environmental samples, the most common forms of matrix interference are caused by dissolved materials in the sample digestate. These interferents change the characteristics of the solution that is injected into the instrument.

One means of resolving matrix problems with metals is use of MSA, described in the section on MSA above. Another means of overcoming matrix interferences from dissolved materials is to match the blank and the matrix containing the standard used for instrument calibration with the characteristics of the sample or digestate. Matrix matching can involve matching the pH, acid concentration, and dissolved solids content of the blank and standards. If chelation is used, or if the sample contains significant concentrations of organic compounds, the standards and blank should be chelated and/or otherwise matched to contain the organic compounds also.

Chromium VI

Although chromium VI (also known as “hexavalent chromium,” and colloquially known as “chrome 6”) can be considered a metal pollutant, it has historically been treated as a classical pollutant because it is usually determined using classical wet-chemistry techniques. Interferences in the determination of chromium VI have been overcome by use of ion chromatography with methods such as EPA Method 218.6.

Mercury

The dual amalgam purge-and-trap and fluorescence system in EPA Method 1631 is less susceptible to matrix interferences, particularly at low levels, than cold vapor atomic absorption and other mercury analysis techniques. Therefore, if a matrix interference is encountered in the determination of mercury, EPA Method 1631 should be used. Generally, use of this method will resolve most matrix interferences. Recommended approaches for addressing any remaining interferences can be found in Chapter 3 of EPA's *Guidance for Implementation and Use of EPA Method 1631B,* EPA 821-R-01-023, March 2001, which specifically addresses matrix interferences in the determination of mercury. This guidance is applicable to subsequent versions of the method, e.g. 1631E.

Solutions Applicable to Organic Pollutants

Many of these solutions focus on the pollutants regulated under the OCPSF rule, but are applicable to pollutants in other effluent guidelines as well.

Volatiles - The 304(h) methods for volatiles include Methods 601, 602, 603, 624, and 1624.

1. Use of Selective GC Detectors

   The effluent limits in the OCPSF regulation and any other industry regulations involving volatiles are all greater than 10 µg/L (10 ppb). The selective GC detectors in Methods 601 and 602 cover all OCPSF volatile pollutants regulated, and allow detection at levels well below the effluent limits in the OCPSF regulation. The specificity provided by the electrolytic conductivity detector and by the photoionization detector allows detection of the halogenated and aromatic analytes, respectively, in complex matrices.

2. Micro-extraction and Gas Chromatography with Selective Detectors

   The selective GC detectors in Methods 601 and 602 provide sensitivity that is 10–100 times greater than that required to detect the volatile analytes of interest. Some of this sensitivity can be used to substitute micro-extraction in place of purge-and-trap. The advantage of micro-extraction is that the pH of the water can be adjusted to attempt to keep the interferences in the water while the analytes of interest are extracted.[1]

3. Sample Dilution

   Methods 601 and 602 can achieve method detection limits of less than 1 µg/L (ppb) for all volatile analytes in the OCPSF regulation, and of less than 0.1 µg/L (ppb) for many of these analytes. The added sensitivity of the selective GC detectors can be used to overcome matrix problems by diluting the sample by a factor of 10–100. Even with this dilution, the pollutants can be detected at the levels required, and the effects of the interferences will be reduced or eliminated.

4. Isotope Dilution

   Method 1624 employs stable, isotopically labeled analogs of the pollutants as internal standards in the analysis. The use of these labeled compounds frequently permits the pollutant to be determined in the presence of interferences because the unique spectrum of the labeled compound can be located in the

[1] Rhodes, J.W., and Nulton, C.P., *J. Env. Sci. and Health*, vol. A15, no. 5, (1980).

presence of these interferences, and the pollutant can then be located by reference to the labeled compound.

Semivolatiles

1. Use of Selective GC Detectors

   Methods 604 through 612 employ gas chromatography with selective detectors and high-performance liquid chromatography with an ultraviolet (UV) or electrochemical detector to detect pollutants in the presence of interferences. In addition, Method 604 employs derivatization and a halogen-specific detector for the determination of phenols. As with volatiles, the added sensitivity of the selective detectors permits the sample to be diluted by a factor of 10–100 while allowing detection of the analytes at the effluent limits specified in the OCPSF regulation.

2. pH Change

   A very powerful means of separating the pollutants of interest from interferences is to adjust the pH of the sample to keep the interferences in solution while allowing the pollutants to be extracted in an organic solvent. For example, neutral pollutants can be extracted at either low or high pH. Therefore, if the main interferences are acidic, the pH can be adjusted to >13 and the acidic interferences will remain in the water in ionic form while the neutral pollutants are extracted using an organic solvent.

   Phenol and 2,4-dimethylphenol can be extracted at high pH (11–13) using continuous liquid/liquid extractors, as described in Method 1625. This permits phenol and 2,4-dimethylphenol to be extracted in the presence of other, stronger acids.[2] Continuous liquid-liquid extraction at low pH is also an effective means of extracting phenols and overcoming poor recovery "matrix interferences" caused by separatory funnel extraction.

   In a manner analogous to the pH change described above, the extract from the primary extraction can be back-extracted with water of the opposite pH to remove other interferences. To keep the organic pollutants in the extract, the water used for back-extraction can be saturated with salt (sodium sulfate and/or sodium chloride). Aqueous solutions containing 2% of each of these salts have been shown to be effective in keeping the pollutants of interest in the extract.

3. Gel-permeation (Size-exclusion) Chromatography (GPC)

   This technique is described in Revision C of Method 1625. The same technique is used in the Superfund Contract Laboratory Program (CLP) methods and SW-846 method 3640, and has been shown to be effective for removing lipids and high-molecular-weight interferences that can degrade GC and mass spectrometer performance.

4. Solid-phase Extraction (SPE)

   Although SPE has not been fully evaluated as a cleanup technique, SPE may be effective as a cleanup for acidic, basic and neutral organic species. It has been shown to be effective in removing interferences from extracts containing pesticides[3] and in its use for the extraction of pollutants from drinking water in

[2] Jackson, C.B. et. al., *J. Env. Sci. and Health*, vol. A15, no. 5, (1980).

[3] Tessari, J.D., *12th Annual EPA Conference on Analysis of Pollutants in the Environment,* Norfolk, Virginia, May 1989.

EPA Method 525. Method 525 is the drinking water analog to method 625. The principle has been extensively used to remove interferences in HPLC by adding a short C18 or other appropriate column as a guard column in front of the HPLC analytical column.

5. Florisil, Alumina, and Silica Gel Chromatography

   These adsorbents are effective in separating neutral species from polar interferences. For polar analytes of interest, the adsorbent should be evaluated to determine if the analyte will be recovered. The level of activation of the adsorbent plays a major role in this recovery process. Techniques can be found in SW-846 methods 3610, 3620 and 3630.

6. Isotope Dilution

   Method 1625 permits determination of pollutants in the presence of interferences in semivolatile samples in the same way described above for volatiles. In addition, the wide range of recovery of the labeled analogs permitted in the method allows good quantitation of the pollutant when interferences reduce the efficiency of the extraction.

Determination of Phenol as a Specific Example

Phenol is a commonly occurring pollutant in OCPSF wastewaters. The protocols below are suggested as approaches to the determination of phenol in a complex sample matrix. After a protocol has been found to be effective, the laboratory must demonstrate that the modification has equivalent performance to the original method. This demonstration involves the tests described in Chapter 3 of this document. The QC acceptance criteria in the approved method must be met before proceeding with analysis of a sample for compliance monitoring.

1. Base/neutral extraction, acid back extraction, and isotope dilution GC/MS (based on Method 1625)

1.1 Measure 1.0 L of well-mixed sample into a graduated cylinder and spike with labeled phenol per Section 10 of Method 1625. Stir and equilibrate per this method. Quantitatively transfer the sample to a continuous liquid/liquid extractor. Adjust the pH of the sample to 11–13 and extract with methylene chloride as described in the method.

1.2 Remove the extract from the extractor and place in a 1–2 L separatory funnel. Back-extract the extract sequentially three times with 500-mL portions of salt-saturated reagent water (pH <2), discarding the reagent water after each back-extraction.

1.3 Concentrate the extract to 10 mL and clean up using gel-permeation chromatography (GPC) per Section 10 of Method 1625.

1.4 After GPC, concentrate the extract to 0.5 mL and analyze by isotope dilution GC/MS, as described in Method 1625.

1.5 Calculate the recovery of labeled phenol and compare to the performance specifications in Method 1625.

2. Dilution, acid extraction, back-extraction with base, derivatization, silica gel cleanup, and gas chromatography with an electrolytic conductivity detector (based on Method 604)

2.1 Measure two 100-mL aliquots of well-mixed sample into 1000-mL graduated cylinders. Spike one of the aliquots with phenol at the level specified in Section 8 of Method 604. This aliquot serves as the matrix spike sample. Dilute both aliquots to 1.0 L with reagent water. Adjust the pH of each aliquot to less than 2 with HCl.

2.2 Pour each aliquot into a separate 1–2 L separatory funnel and sequentially extract three times with methylene chloride per Method 604. Discard the aqueous phase and return the extract to the separatory funnel. It is recommended that the use of continuous liquid-liquid extraction in place of separatory funnel extraction be used. The recoveries of the analyte of interest are usually better.

2.3 Back-extract the extract sequentially three times with salt-saturated reagent water, discarding the reagent water after each back extraction.

2.4 Concentrate, derivatize, and clean up the extract per Method 604.

2.5 Analyze using the electrolytic conductivity detector. This detector is less susceptible to interferences than the electron capture detector used in Method 604. Newer models have sensitivity nearly equivalent to the electron capture detector.

2.6 Calculate the recovery of phenol in the matrix spike aliquot and compare this recovery to the specifications in Method 604.

Chapter 7
Review of Data from Analysis of Samples

This chapter describes how a responsible party should review data submitted for compliance monitoring under the National Pollutant Discharge Elimination System (NPDES) and data submitted to EPA and State authorities under the Clean Water Act. This data should be maintained on file in an organized fashion available for inspection. The 1993 Pumpkin Book focused on the review of data for organic compounds regulated under the OCPSF Rule that was collected with the 600-series and 1600-Series wastewater methods. Although this revision of the Pumpkin Book now includes references to EPA documents for review of other data, the data from the 600- and 1600-Series methods has been described in this chapter so that the data reviewer can see details of the information reviewed. EPA uses these data reviews in data gathering to support development of effluent guidelines and standards under Sections 301, 304, 307, 308, 402, and 501 of the Clean Water Act and for other purposes. The principles of data review described in this Chapter would also be applicable to data from the 500-series drinking water methods, the SW-846 (RCRA) methods, and any method that contains the standardized quality control elements that are contained in these methods; e.g., recent ASTM International Committee D19 (Water) methods.

The following example is technically detailed and is intended for data reviewers familiar with the EPA methods and similar analytical methods. Reviewers unfamiliar with these methods should review the methods and the supporting background materials provided in the preamble to the promulgation of the 600- and 1600-Series methods for a full understanding of the philosophy behind these documents.

Standardized Quality Control

In developing methods for the determination of organic pollutants in wastewater, EPA sought scientific and technical advice from many sources, including EPA's Science Advisory Board, scientists at EPA's environmental research laboratories, scientists in industry and academia, and scientists, managers, and legal staff. The result of discussions held among these groups was the standardized quality assurance and quality control (QA/QC) approach that is an integral Part of the 600- and 1600-Series methods. This QA/QC takes the form of performance specifications for each method and contains the following elements:

- Purity and traceability of reference standards
- Number of calibration points
- Linearity of calibration
- Calibration verification
- Method detection limit (MDL) and minimum level of quantitation
- Initial precision and recovery
- Analysis of blanks
- Recovery of analytes spiked into the sample matrix (e.g., an matrix spike or laboratory-fortified matrix aliquot) or recovery of labeled compounds spiked into samples
- Statements of data quality for recovery of spikes of analytes or labeled compounds into samples
- Ongoing precision and recovery
- Statements of data quality for the laboratory

In reviewing data submitted for compliance, the permit writer or other individual or organization has the authority and responsibility to assure that the test data submitted contain the elements listed above. Otherwise, the data may be considered unacceptable for compliance monitoring.

Provision of QC Data

Dischargers and other organizations submitting test data under CWA or other acts may use their own laboratories or contract the testing to laboratories that meet the requirements specified in the methods. The permit writer may require that the supporting QA/QC data described above be submitted with results or that it be on record at the discharger's facility or at the testing laboratory.

EPA strongly recommends that the supporting QA/QC data be submitted along with the analytical results, so that the quality of the data can be evaluated directly, and so that these supporting data are not lost between the time of submission of the analytical results and the time that the QA/QC data are required.

In many of its early analytical programs, EPA relied upon laboratories to maintain records of QA/QC data. This practice was cumbersome for the laboratories, because many of the QA/QC data were common to the analytical results for a variety of clients. Retrieving these data from the laboratory to resolve questions of permit compliance was time-consuming for the discharger and the permit writer. More importantly, this practice occasionally resulted in unscrupulous laboratories failing to perform the necessary QA/QC testing, or performing the QA/QC testing "after the fact" to satisfy an audit or data submission request. In particular, many laboratories did not perform the initial precision and recovery test (the "start-up" test) prior to practice of the method and did not perform a spike of the analytes into the sample matrix to prove that the method would work on a particular sample. Therefore, while the data provided by those laboratories may have been compliant, there was no way to prove the data was acceptable for compliance purposes.

When collecting data for the development of a regulation, EPA requires that supporting QA/QC data be provided along with the results for the sample analyses. If an individual or organization submits analytical results for inclusion into EPA's regulations, EPA similarly requires submission of the QA/QC data. Sample results are evaluated relative to the QA/QC specifications in the method, and those results that pass the QA/QC requirements are included for consideration. Submission of QA/QC data at the time of submission of analytical results is essential to timely and effective evaluation of permit compliance issues.

Review of Data from the 600- and 1600-Series Methods

Details of the data review process depend to a great extent upon the specific analytical methods being employed for compliance monitoring. Even for data from the same methods, there are probably as many specific approaches as there are reviewers. However, given the standardized QA/QC requirements of the 600- and 1600-Series EPA methods, a number of basic concepts apply. The following sections provide the basic details for reviewing data submitted and provide some of EPA's rationale for the QA/QC tests.

1. Purity and Traceability of Reference Standards

The accuracy of any non-absolute empirical measurement is dependent on the reference for that measurement. In determining pollutants in water or other sample matrices, the analytical instrument and analytical process should be calibrated with a known reference material. The 600- and 1600-Series analytical methods, as well as other EPA methods, require that the standards used for calibration and other purposes be of known purity and traceable to a reliable reference source.

The ultimate source for reference materials is National Institute for Standards and Technology (NIST). Dischargers and their supporting laboratories submitting analytical data should be able to prove traceability of the reference standards used in the analysis to EPA or NIST. The proof of this traceability is a written certification from the supplier of the standard.

Documentation of the purity and traceability of the standards need not be provided with every sample analysis. Rather, it should be maintained on file at the laboratory and provided on request. When analyses are conducted in a contract laboratory, such documentation ought to be provided to the discharger the first time that a laboratory is employed for specific analyses and then updated as needed.

2. Number of Calibration Points

The 600-series methods specify a minimum of three calibration points. The lowest of these points is required to be near the MDL. The highest is required to be near the upper linear range of the analytical system, and the third point is approximately midway between the two. Some methods, such as Methods 1624 and 1625, require calibration at five specific concentrations for nearly all analytes, and three or four specific concentrations for the remaining analytes for which the methods are not as sensitive.

The lowest calibration point should be below the action level and the high standard should still be within the calibration range of the instrument.

The flexibility in selecting the levels of the calibration points in the 600-series methods has led to a wide variety of calibration ranges as each laboratory may determine its own calibration range. Some laboratories may establish a relatively narrow calibration range, for instance a five-fold concentration range such as 10 to 50 µg/L (ppb), because it makes it simpler to meet the linearity specifications of the 600-series methods. Other laboratories may choose wider calibration ranges, e.g., 10 to 200 µg/L (ppb), in order to minimize the number of samples that should be diluted and reanalyzed because the concentration of one or more analytes exceeds the calibration range.

The data reviewer will need to make certain that all measurements are within the calibration range of the instrument. Samples with analyte concentrations above the calibration range should have been diluted and reanalyzed. The diluted sample results need only apply to those analytes that were out of the calibration range in the initial analysis. In other words, it is acceptable to use results for different analytes from different levels of dilution within the same sample. Some flexibility may be exercised in acceptance of data that are only slightly above (<10%) the calibration range. Such data are generally acceptable as calculated.

If data from an analysis of the diluted sample are not provided, limited use should be made of the data that are above the calibration range (>10%). The response of the analytical instrument to concentrations of analytes will eventually level off at concentrations above the calibration range. While it is not possible to specify at what concentration this will occur from the calibration data provided, it is generally safe to assume that the reported concentration above the calibrated range is a lower limit of the actual concentration. Therefore, if concentration above the calibration range is also above a regulatory limit, it is highly likely that the actual concentration would also be above that limit.

3. Linearity of Calibration

The relationship between the response of an analytical instrument to the concentration or amount of an analyte introduced into the instrument is referred to as the “calibration curve.” An analytical instrument can be said to be calibrated in any instance in which an instrumental response can be related to a single concentration of an analyte. The response factor (GC/MS methods) or calibration factor (GC, HPLC methods) is the ratio of the response of the instrument to the concentration (or amount) of analyte introduced into the instrument. The response factor and calibration factor concepts are used in many methods for organic contaminants, while methods for metals and some other analytes may employ different concepts such as linear regressions.

While the shape of calibration curves can be modeled by quadratic equations or higher order mathematical functions, most analytical methods focus on a calibration range where the response is essentially a linear function of the concentration of the analyte. An advantage of linear calibration is that the response factor or calibration factor represents the slope of the calibration line and is relatively constant, simplifying the calculations and data interpretation. Whichever approach is used, all the 600- and 1600-Series methods specify some criterion for determining linearity of calibration. When this criterion is met, the calibration is sufficiently linear to permit the laboratory to use an average response factor or calibration factor, and it is assumed that the calibration is a straight line that passes through the zero/zero calibration point. Linearity is determined by calculating the relative standard deviation (RSD) of the response factor or calibration factor for each analyte and comparing this RSD to the limit specified in the method. If the RSD does not exceed the specification, linearity is assumed.

In the 600- and 1600-Series methods, the linearity specification varies from method to method, depending on the quantitation technique. The typical limits on the RSD are as follows:

- 15% for the gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods
- 20% for analytes determined by the internal standard technique in the gas chromatography/mass spectrometry (GC/MS) methods (624, 625, 1624, and 1625)
- 20% for analytes determined by isotope dilution in Methods 1613, 1624, and 1625
- 15% for mercury determined by atomic fluorescence in Method 1631

Metals methods that employ a linear regression specify a criterion for the correlation coefficient, r, such as 0.995.

If the calibration is *not* linear, as determined by the RSD of the response factor or calibration factor, a calibration curve should be used. This means that a regression line or other mathematical function should be employed to relate the instrument response to the concentration. However, properly maintained and operated lab instrumentation should have no difficulty in meeting linearity specifications for 600- and 1600-Series methods. Linear regression emphasizes the importance of higher concentration standards and that the correlation coefficient is little impacted by poor performance of calibration standards with low concentrations.

For determination of nearly all of the organic analytes using the 600- and 1600-Series methods, calibration curves are linear over a concentration range of 20–100 times the nominal concentration, depending on the detector being employed. Whatever calibration range is used, the laboratory should provide the RSD results by which one can judge linearity, even in instances where the laboratory is using a calibration curve. In instances where the laboratory employs a curve rather than an average response or calibration factor, the data reviewer should review each calibration point to assure that the response increases as the concentration increases. If it does not, the instrument is not operating properly, or the calibration curve is out of the range of that instrument, and data are not considered usable.

4. Calibration Verification

Calibration verification involves the analysis of a single standard, typically in the middle of the calibration range, at the beginning of each analytical shift. The concentration of each analyte in this standard is determined using the initial calibration data and compared to specifications in the method. If the results are within the specifications, the laboratory is allowed to proceed with analyses without recalibrating and to use the multi-point calibration data to quantify sample results. It is also recommended that a calibration verification at the action level is periodically analyzed.

Specifications for calibration verification are generally given as a range of concentrations, as a recovery range, or as a percentage difference from the test concentration. For the 600-series semivolatile GC and HPLC methods, the difference must be within 15%. For Method 625, the difference must be within 20%. The GC and GC/MS methods for volatiles and the 1600-Series methods specify a range of concentrations or recoveries for each analyte. These ranges are based on interlaboratory method validation studies.

If calibration cannot be verified, the laboratory may either recalibrate the instrument or prepare a fresh calibration standard and make a second attempt to verify calibration. If calibration cannot be verified with a fresh calibration standard, the instrument should be recalibrated. If calibration is not verified, subsequent data are considered to be invalid until the instrument is recalibrated.

5. Method Detection Limit or Minimum Level

Although this requirement is not explicitly stated in EPA wastewater methods (e.g., 600 and 1600-Series methods) we recommend use of the method detection limit (MDL) concept to establish detection capabilities. Detailed procedures for determining the MDL are provided at 40 CFR Part 136, Appendix B. Although exact frequencies vary by method, most methods require that, at a minimum, laboratories conduct an MDL study as part of their initial demonstration of capability and whenever a modification is made to the method that might affect the detection limit and amends thereafter. Data reviewers should consult the methods used for specific requirements, or the requirements of their customers, auditors, etc.

The Minimum Level (ML) is used as a quantitation level, and is defined in most of the 1600-Series methods as the lowest level at which the entire analytical system gives a recognizable signal and acceptable calibration point. Therefore, each 1600-Series method specifies that the calibration range for each analyte encompass the method-specified ML.

Many of the EPA wastewater methods provide specific requirements regarding reporting results that are below the ML or the method-specified quantitation limit when these data will be used for compliance monitoring. Since these requirements vary slightly, data reviewers should consult the specific method for details.

If the sample results are above the ML, but are below the facility's regulatory compliance level, then the laboratory should report the results to indicate that the pollutant has been detected but is compliant with a facility's permit, assuming all QC criteria are met. If sample results are above the regulatory compliance level, the data reviewer may wish to evaluate the laboratory QC sample results to verify that the reported concentration is not attributable to analytical bias. In addition, the data reviewer should evaluate all blank results to determine if the level of pollutant detected may be attributable to contamination.

6. Initial Precision and Recovery

Part 136 methods require this Initial Precision Recovery (IPR) test before use of a method. It is sometimes termed the "start-up test." The laboratory should demonstrate that it can meet the specifications in the method for the recovery of analytes spiked into a reference matrix (reagent water). EPA's experience has been that laboratories that have difficulty passing the start-up test have such marginal performance that they will have difficulty in the routine practice of the method.
The start-up test consists of spiking the analytes of interest into reagent water and analyzing four aliquots. The mean concentration and the standard deviation of the concentration are calculated for each analyte and compared to the specifications in each method. If the mean and standard deviation are

within the limits, the laboratory may use the method to analyze field samples. For some methods, a repeat test is allowed because of the large number of analytes being tested simultaneously.

If start-up test data fail to meet the specifications in the method, none of the data produced by that laboratory using that method should be considered usable. As with the documentation of the purity of the standards, the start-up test data need not be submitted with each set of sample results, but should be submitted the first time a laboratory is employed for analyses, and updated as changes to the method necessitate (see below) in order to allow the data reviewer to determine the adequacy of the laboratory's performance.

If the laboratory did not perform the start-up tests, the data should not be considered usable, unless all other QC criteria have been met and the laboratory has submitted IPR (and associated instrument QC) data that were generated after-the-fact on the same instrument. If these conditions are met, then the data reviewer may consider the data to be acceptable for most purposes.

Note: Discussion of this alternative should not in any way be construed as EPA approval of the practice of performing IPR analyses after the analysis of field samples. Rather, EPA regards the demonstration of laboratory capability prior to sample analysis as an essential QC component. This suggestion provides a tool to permitting authorities when data have already been collected without the required IPR samples. Once the missing IPR data has been identified as a problem, all responsible parties should implement corrective action necessary to ensure that it is not repeated.

It is important to remember that if a change is made to a method, the start-up test will need to be repeated with the change as an integral part of the method. Such changes may involve alternative extraction, concentration, or cleanup processes; alternative GC columns, GC conditions, or detectors; or other steps designed to address a particular matrix problem. If the start-up test is not repeated when these steps are modified or added, then laboratory data produced by the modified method should not be considered reliable and thus should not be used. Many laboratories report the configuration of their GCs (instrument number, column, detector) as a part of their report header. If a configuration change is made and new IPR data are not supplied, EPA recommends requesting the new IPR data from the laboratory.

7. Analysis of Blanks

Blanks should be analyzed on a routine basis, when any part of the analytical process has been changed, and when contamination of the analytical system is suspected. Most recent EPA methods require that a blank be prepared and analyzed with each batch (set) of samples. The size of a batch is usually limited to a maximum of 20 field samples. In practice, this means that on each day that a laboratory prepare samples, they should also prepare a blank, even if fewer than 20 samples are prepared. The purpose of analyzing a blank with each set of samples is to determine the extent of possible contamination of the samples while in the laboratory. If the blank is handled by the same analysts in the same way as the samples and the blank shows no contamination, it is likely that the samples will not have been contaminated. Analyzing a blank when the analytical process has been changed is consistent with requiring a repeat of the start-up tests, because the change introduces a new possibility for contamination of samples through the use of the new materials or procedures.
Contamination in the laboratory is a common problem, though there are many opinions on what constitutes contamination. In more recent EPA methods, a concentration above the minimum level of quantitation of the method is a cause for concern. In reality, it is not unusual to find low levels of common laboratory solvents, phthalates, and other ubiquitous compounds in laboratory blanks.

Controlling laboratory contamination is an important aspect of each laboratory's quality assurance plan. The laboratory should maintain records, typically in the form of control charts, of blank contaminants. These records should prompt corrective action by the laboratory, including reanalysis of any affected samples, when concentration of an analyte in a blank rises above a historical level. The reviewer in evaluating sample results may request control charts; however, they are not required in EPA methods and are not routinely submitted with sample data.

Unfortunately, by the time that results have been found to be contaminated, it is usually too late for corrective action. Therefore, the reviewer has several options in making use of the sample data. First, if a contaminant is present in a blank, but not present in a sample, then there is little need for concern about the sample result, though it may be useful to occasionally review the raw data for samples without the contaminant to ensure that the laboratory did not edit the results for this compound.

The second approach deals with instances where the contaminant is also reported in a sample. Some general guidance will help determine the degree to which the contaminant is affecting sample results:

- If the sample contains the contaminant at levels of at least 10 times that in the blank, then the likely contribution to the sample from the contaminant in the laboratory environment is at most 10%. Since most of the methods in question are no more accurate than that level, the possible contamination is negligible.

- If the sample contains the contaminant at levels of at least 5 times but less than 10 times the blank result, the compound is probably present in the sample, but the numerical result should be considered an upper limit of the true concentration.

- If the sample contains the contaminant at levels below 5 times the level in the blank, there is no adequate means by which to judge whether or not the sample result is attributable to laboratory contamination. The results for that compound in that sample should be considered unacceptable for compliance monitoring.

There are two difficulties in evaluating sample results relative to blank contamination. First, the reviewer should be able to associate the samples with the correct blanks. For analysis of volatiles by purge-and-trap techniques, where no sample extraction is required, the blanks and samples are associated by analysis date and time, and specific to the instrument as well. For methods involving the extraction of organic compounds from the samples, the blanks and samples are primarily associated by the date on which they were extracted, and by the batch of samples and associated lab equipment (glassware, reagents, cleanup media).

The second difficulty involves samples that have been diluted. Dilution of a sample with reagent water or dilution of an extract with solvent represents an additional potential source of contamination that will not be reflected in the results for the blank unless the blank was similarly diluted. Therefore, in applying the 10-times rule, the concentration of the sample is compared to the blank result multiplied by the dilution factor of the sample or sample extract. For instance, if 12 ppb of a contaminant are found in the blank, and the associated sample extract was diluted by a factor of 6 relative to the extract from the blank prior to analysis, then the sample result would have to be greater than 12x6x10, or 720 ppb, to be acceptable. Between 360 ppb and 720 ppb, the sample result would best be considered an upper limit of the actual concentration. Below 360 ppb, the sample result is not acceptable for compliance monitoring.

In general, practitioners of analytical methods do not subtract the concentration of the analyte in the blank from the concentration of the analyte in the sample to determine the true concentration of the

analyte in the sample. Experience indicates that this practice is not reliable. The obvious problem occurs when the blank concentration is higher than that in the sample, and subtraction would yield a negative concentration. Using the 10-times rule above provides a more appropriate means of evaluating the results and does not require that the reviewer alter the results reported by the laboratory.

8. Ongoing Precision and Recovery

The 1600-Series methods require that an "ongoing precision and recovery" (OPR) sample be analyzed with each sample set, and the results of this OPR sample should meet the acceptance criteria in the method prior to the analysis of blanks and samples. Most other methods approved at 40 CFR Part 136 contain a similar requirement, but may use different terminology, such as a laboratory control sample (LCS), laboratory fortified blank (LFB), or QC check sample. For this purposes of this discussion, all such samples are referred to as OPR samples.

The OPR samples are used to ensure that laboratory performance is in control during analysis of the associated batch of field samples. The data reviewer should determine if the OPR sample has been run with each sample set and if all criteria have been met. For methods that do not require sample digestion or extraction, such as volatile analyses by Method 1624, the OPR analysis is associated with the samples on the basis of the analysis date and time and the specific GC/MS system. For other analyses, such as semivolatile analyses by Method 1625, OPR results are associated with samples extracted (or digested) at the same time as the OPR. In addition to defining sample batches by date and time of extraction or analysis, each method specifies a maximum batch size (generally no more than 10 or 20 samples) that can be associated with a single OPR. The reviewer should verify that OPR samples were run at the proper frequency.

Because of the large number of compounds being tested simultaneously in the 600- and 1600-Series methods, there is a small probability that the OPR analysis will occasionally fail to meet the specifications. While the laboratory is supposed to correct any problems and analyze another OPR aliquot, it may still be possible to utilize the data associated with an OPR aliquot that does not meet all of the method specifications. The following guidelines may be useful to data reviewers when evaluating the usability of data:

- If the concentration of an analyte in the OPR is *above* the method specifications, but that compound is not detected in an associated sample, then it is unlikely that the sample result is affected by the failure in the OPR.

- If the concentration of the analyte in the OPR is *above* method specifications and the analyte *is* detected in the sample, then the numerical result may represent an upper limit of the true concentration, and data users should be cautioned when using the data for enforcement purposes.

- If the concentration in the OPR is *below* the method specifications, and that analyte is detected in an associated sample, then the sample result is likely a lower limit of the true concentration for that analyte.

- If the concentration of the analyte in the OPR is *below* method specification and that analyte is *not* detected in the associated sample, then the sample data are suspect and are not usable for regulatory compliance purposes because the analysis does not demonstrate the absence of the analyte.

If the OPR sample was not run, there is no way to verify that the laboratory processes were in control. In such cases, a data reviewer may be able to utilize the field sample data by examining labeled compound or matrix spike recovery results, the IPR results, OPR results from previous and subsequent batches, and any available historical data from both the laboratory and the sample site. If the matrix spike or labeled compound results associated with the sample batch do not meet the performance criteria in the methods, then the results for that set of samples cannot be considered usable.

If the laboratory's IPR results and the matrix spike or labeled compound results associated with the sample batch in question meet all applicable performance criteria in the methods, then the data reviewer may be reasonably confident that laboratory performance was in control during field sample analysis. This level of confidence may be further increased if there is a strong history of both laboratory performance with the method and method performance with the sample matrix in question, as indicated by additional OPR and matrix spike data collected from the laboratory and samples from the same site.

Note: The preceding discussion of maximizing use of failed OPR data is not an EPA endorsement of the practice of proceeding with uncontrolled laboratory analyses. Rather, laboratories failing to meet OPR specifications should identify and correct the problem and re-analyze affected samples whenever possible. This preceding discussion is provided only to describe a tool for permitting authorities when re-analysis is not possible due to sample holding times, insufficient sample volumes, or other reasons.

9. Recovery of Analyte Spiked into the Sample Matrix or Recovery of Labeled Compound Spiked into Samples

The majority of the 600- and 1600-Series methods were developed to analyze effluent samples, and may not be appropriate for in-process samples. While many of the methods were tested using effluents from a wide variety of industries, samples from some sources may not yield acceptable results. It is, therefore, important to evaluate method performance in the sample matrix of interest.

The non-isotope dilution wastewater methods require a spike of the analytes of interest into a second aliquot of the sample for analysis with the sample. The purpose of spiking the sample (often termed a "matrix spike (MS)") is to determine if the method is applicable to the sample in question. Most of these wastewater methods also require that laboratories prepare and analyze a duplicate aliquot of the matrix spike (often called a "matrix spike duplicate (MSD)"), or a duplicate aliquot of an unspiked field sample (usually called a "duplicate"). Generally, one matrix spike/matrix spike duplicate (MS/MSD) pair or one MS and one duplicate sample is required for every 10 or 20 field samples, depending on the requirements of the specific method being used.

In evaluating method performance in the sample matrix, data reviewers should examine both the precision and accuracy of the analysis. Precision is evaluated by comparing the relative percent difference (RPD) of results obtained from the MS/MSD pair or from the duplicate and its corresponding field sample. Accuracy is assessed by examining the recovery of compounds in the matrix spike sample (and if applicable, the matrix spike duplicate sample). In evaluating matrix spike results, the data reviewer should verify that:

- The unspiked sample has been analyzed.
- The spiked sample has been analyzed, and that the analytes were spiked at an appropriate concentration (generally 5 - 10 times the background concentration of the analyte in the sample or 1 - 5 times the regulatory compliance limit, whichever is greater). If the analytes are spiked too high or

too low, it is not usually possible to differentiate recovery of the spiked concentration from recovery of the analyte in the unspiked sample.
- The recovery of the spike is within the range specified.

If the RPD and recoveries of the MS/MSD or MS and duplicate samples are within the limits specified in the method, the method is judged to be applicable to that sample matrix. If, however, the RPD or recoveries in these samples are not within the range specified, either the method does not work on the sample, or the sample preparation process which includes sample collection is out of control.

If the method is not appropriate for the sample matrix, changes to the method or use of an alternative method would be needed. Matrix spike results are necessary in evaluating a modified method. If the analytical process is out of control, the laboratory should take immediate corrective action before any more samples are analyzed.

To separate indications of method performance from those of laboratory performance, the laboratory should prepare and analyze a quality control check standard (laboratory control sample) or an OPR sample, as described in Items 4 and 8 of this chapter. If the results for either of these analyses are not within the range specified, the analytical system or process should be repaired. After verifying the performance of the repaired system and processes through successful analysis of calibration verification and OPR samples, the sample and spiked sample analysis should be repeated. If recoveries and RPD of the repeated matrix spike and duplicate analyses are within the ranges specified, the analytical process is judged to be in control. If, however, the repeated analysis results are still outside the specified ranges, then sample results generally are not useful for regulatory compliance purposes because the matrix spike and duplicate results indicate that the method is not applicable to the sample.

In rare cases, it may be possible to make use of such data while efforts are being made to identify a method that works on the matrix in question. The following guidelines may be applicable as a temporary measure in such circumstances:

- If the recovery of the matrix spike and duplicate are *above* the method specifications, but the regulated analyte was not detected in the associated sample or was detected below the regulatory compliance limit, it is unlikely that the sample result was affected by the failure in the matrix spike because the factors that caused the analysis to over-estimate the concentration in the spiked sample would not likely have resulted in an under-estimate in the unspiked sample. In other words, it is likely that the sampled effluent is in compliance with the permit limit in such cases.

- If the recovery of the matrix spike and duplicate are *below* method specifications, but the regulated analyte was detected *above* the regulatory compliance limit in an associated sample, the sample result may represent the lower limit of the true concentration for that pollutant and it is likely that true concentration in the effluent is in violation of the permit limit.

- If the recovery of the matrix spike and duplicate are *above* the method specifications and the regulated pollutant was detected in an associated sample, the sample result may represent the upper limit of the true concentration and the data cannot be considered useable for regulatory compliance purposes.
- If the recovery of the matrix spike and duplicate are *below* the method specifications and the regulated pollutant was either *not detected* or was detected *below* the regulatory compliance limit, the sample result may represent a lower limit of the true concentration and cannot be considered usable for regulatory compliance purposes.

Note: The preceding discussion of maximizing use of failed matrix spike or duplicate data is not an EPA endorsement of the practice of using methods that do not work on the sample matrix. The preceding discussion is provided only to describe a tool for permitting authorities for use in evaluating compliance monitoring results pending the permittees' successful identification and use of an alternate method or method modifications such as those described in Chapter 6 of this document.

For isotope dilution analyses, data evaluation is simpler because isotopically labeled analogs of the pollutants are spiked into every sample. If recovery of a labeled compound spiked into a sample is *not* within the range specified in the method, and results of analysis of the ongoing precision and recovery standard *are* within the respective limits, sample results would be considered invalid. When labeled-compound recoveries are outside of method specifications, the problem may be related to the sample matrix. The isotope dilution methods specify that, in these instances, the sample should be diluted with reagent water and reanalyzed. If the labeled compound recoveries meet the method specifications after dilution of the sample, the sample results are acceptable, although the sensitivity of the analysis will be decreased by the dilution.

For some sample matrices, even dilution will not resolve the problem, and for other matrices, the loss of sensitivity precludes use of the results for determining compliance. In these instances, additional steps need to be taken to achieve acceptable results.

Steps that may be taken when the results of matrix-spike or labeled-compound recoveries are not within the limits specified in the methods are described in Chapter 6 of this document. These steps include suggestions for more extensive extraction and cleanup procedures, for sample dilution, and for other measures to overcome matrix interference problems.

10. Control limits for Recovery of Spiked Analytes or Labeled Compounds in Samples

The 600- and 1600-Series methods specify that after the analyses of five spiked samples, control limit is constructed for each analyte. The control limits for each analyte is computed as the mean percent recovery plus and minus two times the standard deviation of percent recovery for each analyte. The laboratory should then update their control limits after each five to ten subsequent spiked sample analyses.

For non-isotope dilution results, the control limits can be used to estimate the true value of a reported result and to construct confidence bounds around the result. For example, if the result reported for analysis of phenol is 25 µg/L (ppb), and the statement of data quality for phenol is 70% ± 30% (i.e., the mean recovery is 70% and the standard deviation of the recovery is 15%), the true value for phenol will be in the range of 28–43 µg/L (ppb), with 95% confidence. This range is derived as follows:

$$Lower\ limit = [(25 \div 0.7) - (25 \times 0.3)] = [35.7 - 7.5] = 28\ ug/L\ (ppb)$$

$$Upper\ limit = [(25 \div 0.7) + (25 \times 0.3)] = [35.7 + 7.5] = 43\ ug/L\ (ppb)$$

Many laboratories do not maintain or provide control limits with sample results, in which case a data reviewer should contact the laboratory to determine if the control limits are being maintained for each analyte. If necessary, the reviewer can construct a control limits from individual data points if the laboratory has records of recoveries for matrix spikes.

Statements of data quality for isotope dilution methods are based on the recoveries of the labeled compounds. Using an isotope dilution method, the sample result has already been corrected for the recovery of the labeled analog of the compound. Therefore, for a reported result for phenol of 25 µg/L where the standard deviation of the labeled phenol recovery is 15%, the true value for phenol will be in the range of 17–32 µg/L, with 95% confidence, derived as follows:

Lower limit = [25 – (25 x 0.3)] = 17 µg/L (ppb)

Upper limit = [25 + (25 x 0.3)] = 32 µg/L (ppb)

The lack of control limits does not invalidate results, but makes some compliance decisions more difficult. If the laboratory does not maintain control limits there may be increased concern about both specific sample results and the laboratory's overall quality assurance program.

11. Control limits for the Laboratory (Methods 1624 and 1625)

In addition to statements of data quality for results of analyses of the labeled compounds spiked into the samples, Methods 1624 and 1625 require that control limits be constructed from the initial and ongoing precision and recovery data. The purpose of the control limits is to assess laboratory performance in the practice of the method, as compared to the assessment of method performance made from the labeled compound results for the samples. Ideally, the two limits would be the same. Any difference is attributable to either random error or sample matrix effects.

If the laboratory is practicing isotope dilution methods, the data reviewer should review the control chart for the laboratory. If the laboratory does not make these statements available for the reviewer, they may be requested. If the laboratory still does not make them available, it does not necessarily invalidate any data, but indicates that the laboratory may not be following the method as written.

Chapter 8
When a Matrix Interference Is Demonstrated

The preceding chapters describe how to overcome matrix interference problems and case histories of matrix interference problems that were mitigated. This chapter describes help that may be available when all attempts at overcoming matrix interference problems have been exhausted.

Poor Recovery or Precision of Matrix Spikes

The most common indication of a matrix problem will be recovery or precision of the matrix spike and matrix spike duplicate (MS/MSD) outside of the QC acceptance criteria in the method or QC acceptance criteria suggested in the Streamlining Initiative (see Chapter 4 of this document). Once a matrix interference is demonstrated to be the cause of a laboratory's inability to meet the QC acceptance criteria, the laboratory should document the interference and attempt to overcome it using the procedures suggested in the analytical method, in Chapter 6 of this document, and other techniques in the test method or technical literature.

If an allowance for matrix effects is warranted or appropriate without a demonstration that a matrix interference exists and without an attempt to overcome the matrix interference, such an allowance provides a disincentive for addressing interferences that may be overcome using the procedures recommended in this document and in the method. The discharger should be familiar with its wastewater and thus able to find solutions to matrix interference problems. However, a site-specific or facility-specific allowance may be warranted after all efforts to remove the interference(s) have been exhausted, and should be handled on a case-by-case basis by the regulatory/control authority.

Inability to Meet the Method Detection Limit (MDL)

Another common indication of a matrix interference is that measurements cannot be made at low levels because interferences are present at these levels.

Statements of the performance in EPA methods, including estimates of MDLs, are estimates based on the Agency's evaluation of a method in various performance studies, and the method may not achieve all of the stated performance characteristics in all possible sample matrices. The Scope and Application section of most modern methods approved for use in EPA's wastewater programs states: “The detection limit and minimum level of quantitation in this method usually are dependent on the level of interferences rather than instrumental limitations.” Therefore, the MDL and minimum level of quantitation (ML) should be treated as “presumptive” performance characteristics. These characteristics may vary depending on the sample matrix and on the concentration of interest. The MDL issue has not been resolved and may change.

The MDL procedure at 40 CFR Part 136, Appendix B allows determination of an MDL in a matrix other than reagent water (see the Scope and Application section of the MDL procedure). A permit could specify a different detection or quantitation limit when a discharger demonstrates that a different limit is appropriate for its effluent based on the presumptive statement at the beginning of most modern EPA methods, and statements in the MDL procedure. After the discharger demonstrates that the approved test method cannot achieve the presumptive detection or quantitation limit on an effluent-specific basis, the discharger and regulatory authority should work cooperatively to establish the permit limit using a procedure such as the procedure given at 40 CFR Part 132, Appendix F, Procedure 8. This procedure, titled *Water Quality-based Effluent Limitations Below the Quantification Level* allows a discharger to establish an effluent-specific ML.

Although EPA has provided the procedure above to develop an effluent-specific ML, EPA recommends that the discharger attempt to achieve the MDL and ML stated in the approved method by using the interference-reducing procedures given in this document and the analytical method. Prior to allowing the adjustment of a permit limit because the discharger reports it is unable to achieve the MDL and ML the appropriate method approved at 40 CFR Part 136, the regulatory authority should review the steps taken by the discharger to reduce interferences to ensure that all reasonable efforts have been made to achieve the permit limit. It is critical that the permittee be able to measure and accurately report results at or above their permit limit and the achievement of method specified MDLs or MLs is of less importance.

Allowance for a Matrix Interference

Because every situation is different, EPA has not adopted a rigid protocol for obtaining data that demonstrate that a matrix interference exists, nor can a hard-and-fast rule be developed to state the conditions under which an allowance for a matrix interference should be granted. After all attempts at resolving the matrix interference are unsuccessful, the most common analytical solution to a matrix interference problem is to dilute the sample with reagent water until the precision and recovery are within normal levels. No more than the minimum amount of dilution should be used. The effect of this dilution will be to raise the MDL and ML and may necessitate development of an effluent-specific MDL and ML. Should this situation arise, EPA suggests that the regulatory/control authority solicit and evaluate the following information to demonstrate that an allowance for matrix interferences in the form of an effluent-specific MDL and ML may be appropriate:

- MDL, IPR, and blank data demonstrating that the laboratory can perform the method;
- Field, equipment, and reagent blank data demonstrating that the sampling and analysis systems are free from contamination at the levels required for reliable determination of the pollutant;
- MS/MSD data (where applicable) demonstrating that a potential matrix interference exists because the recovery and or precision is not within the QC acceptance criteria of the method;
- Confirmation of the out-of-specification MS/MSD recovery or precision by a second laboratory;
- Identification of the potential interferent(s);
- Steps taken to attempt to mitigate the interference (e.g., sample, extract, or digestate concentration; sample dilution; use of a larger sample size; use of cleanup procedures; use of pH change prior to extraction; use of a greater amount of a removal reagent; use of techniques to selectively remove the interferent; etc.); and
- Calculation of an effluent-specific MDL using the procedure at 40 CFR Part 136, Appendix B, and calculation of an ML using the procedure given at 40 CFR Part 132, Appendix F, Procedure 8;
- Other methods approved for NPDES compliance which utilize different approaches were tried.

Once the regulatory/control authority receives these data, the authority would make a determination that an effluent-specific MDL and ML are appropriate.

Chapter 9
Sources of Additional Help and Information

Following are several sources of information and EPA contacts related to the issues addressed in this document. Please visit the websites (listed below), and/or obtain a copy of the EPA CD-ROMs for reference information on analytical methods and topics discussed in this guidance.

Web Sites

EPA's home page	http://www.epa.gov
EPA's Office of Science and Technology's water science analytical methods pages	http://www.epa.gov/ost/methods or http://www.epa.gov/waterscience/methods
Effluent Guidelines	http://www.epa.gov/ost/guide

Method Indices

EPA Region 1 Library	http://www.epa.gov/epahome/index/
EPA Information Sources	http://www.epa.gov/epahome/index/key.htm
National Environmental Methods Index (NEMI)	http://www.nemi.gov

Office of Water CD-ROMs

Selected Office of Water Methods and Guidance, Version 5 (EPA 821-C-04-001; September 2004)

Water Docket

The Water Docket contains copies of materials that support our rules under the Safe Drinking Water Act (SDWA) and Clean Water Act (CWA). These materials include *Federal Register* notices, references cited in these notices; health criteria, analytical methods, treatment technology, and economic impact and environmental assessment data; development documents, public comments, and other background information.

US Environmental Protection Agency
EPA Docket Center (EPA/DC)
Public Reading Room
Room B102, EPA West Building
1301 Constitution Avenue, NW
Washington, DC 20460

The Docket is open to the public on all Federal government work days from 8:30 a.m. until 4:30 p.m. A reasonable fee may be charged for photocopying. On-line Docket searches may be performed at http://www.epa.gov/ow/docket.html.

Federal Register

The *Federal Register* page is at http://www.gpoaccess.gov/fr/index.html. All issues of the *Federal Register* from 1994 to the present are online. *Federal Register* notices prior to 1994 may be found at a library or through a search service. Search instructions for the *Federal Register* are at the Government Printing Office (GPO) web site at http://www.gpoaccess.gov.

Code of Federal Regulations

All issues from 1996 to the present are on line. The Code of Federal Regulations (CFR) is at http://www.gpoaccess.gov/cfr/index.html. CFRs prior to 1996 may be found at a library or through a search service.

Approval of an Alternate Test Procedure or Questions Specifically Related to this Guidance

Procedure for nationwide use (see the regulations at 40 CFR Parts 136.4 and 136.5)

Analytical Methods Staff (4303T)
U.S. EPA
Ariel Rios Building
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460
email: OSTCWAMethods@epa.gov

Procedure for use on a specific discharge (see the regulations at 40 CFR Parts 136.4 and 136.5)

Sources for Supporting Documents

Note: These sources are documents referenced in this document. For a more comprehensive list of guidance and other documents, see EPA's waterscience, yosemite, and other websites, or perform an online search for the document by title or subject.

Table 9-1 Sources for Supporting Documents

Subject	Title of Guidance	Document number	Date	Source
Methods Update Rule	*Guidelines Establishing Test Procedures for the Analysis of Pollutants Under the Clean Water Act; National Primary Drinking Water Regulations; and National Secondary Drinking Water Regulations; Analysis and Sampling Procedures; Final Rule*	EPA 821-F-06-005	March 12, 2007	72 FR 11200 http://www.epa.gov/waterscience/methods/update2003/index.html
Clean spaces guidance	*Guidance on Establishing Trace Metal Clean Rooms in Existing Facilities*	EPA 821B96001	January 1996	http://yosemite.epa.gov/water/owrccatalog.nsf
Cleanroom guidance	*Trace Metal Cleanroom*, prepared by the Research Triangle Institute	RTI/6302/04/02 F	October 1995	Research Triangle Institute
Mercury; Method 1631 guidance	*Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap and Cold Vapor Atomic Fluorescence Spectrometry*	EPA 821-R-02-019	August 2002	http://www.epa.gov/waterscience/methods/1631guid.pdf
Methods, wastewater and drinking water	*EPA Methods and Guidance for Analysis of Water*	EPA 821-C-99-004	June 1999	NTIS[1] PB99-500209
Methods, historical	*Methods for Chemical Analysis of Water and Wastes* (MCAWW)	EPA 600/4-79-020	March 1983	NTIS[1] PB84-128677
Methods and guidance	*Selected Office of Water Methods and Guidance, Version 5*	EPA 821-C-04-001	September 2004	
Metals sampling techniques evaluation	*Evaluating Field Techniques for Collecting Effluent Samples for Trace Metals Analysis*	EPA-821-R-98-008	June 1998	http://yosemite.epa.gov/water/owrccatalog.nsf

Table 9-1 Sources for Supporting Documents				
Subject	**Title of Guidance**	**Document number**	**Date**	**Source**
Metals sampling video	*Office of Water Methods and Guidance, Version 2.0 - Suite* (video and CD-ROM; includes methods on CD-ROM EPA 821-C-99-004)		2002	NTIS[1] PB2002-500076, includes video and methods on CD-ROMs
Metals data evaluation guidance	*Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring*	EPA 821-B-96-004	July 1996	http://yosemite.epa.gov/water/owrccatalog.nsf
Method flexibility	*Streamlining Initiative – Guide to Method Flexibility and Approval of EPA Water Methods*	EPA-821-D-96-006	December 1996	http://epa.gov/waterscience/methods/guide/flex.html
Oil and grease; Method 1664 guidance	*Analytical Method Guidance for EPA Method 1664A Implementation and Use (40 CFR Part 136)*	EPA 821-R-00-003	February 2000	http://www.epa.gov/waterscience/methods/1664guide.pdf
Radiochemistry method guidance	*Multi-Agency Radiological Laboratory Analytical Protocols Manual* (MARLAP Manual)	(EPA 402-B-04-001A to C (in three volumes)	December 2004	69 FR 77228
Sampling guidance	*Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels*	EPA 821-R-96-011	July 1996	
Whole-effluent toxicity (WET) testing guidance	*Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136)*	EPA 821-B-00-004	July 2000	http://www.epa.gov/waterscience/WET
Whole-effluent toxicity (WET) variability guidance	*Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System Program*	EPA 833-R-00-003	June 2000	http://www.epa.gov/waterscience/WET

[1] National Technical Information Service, http://www.ntis.gov

Appendix A

October 26, 1984 Federal Register Notice Preamble, pp. 43239 - 43243

compounds, they have been listed separately in Table ID rather than with the other organic parameters in Table IC because of the wide association between this subset of organic compounds and their end use. Sixteen of the 67 parameters are priority pollutants. Three additional pesticides were identified as priority pollutants under the consent decree. Table ID therefore now identifies 70 specific pesticides, of which 19 are priority pollutants. Methods 608 and 625, which were proposed for the priority organic toxic pollutants, were revised to incorporate substantive comments. All other references in Table ID have been updated, but the updated references do not require any substantive changes from previously approved test procedures.

Table IE now includes the five radiological test procedures approved in the 1976 Guidelines. All references have been updated, and an EPA reference has been added. There are no substantive textual changes in these updated test procedures.

B. GC, HPLC, and GC/MS Test Procedures

Analyses for organics depend upon a variety of chromatographic techniques. See subsection III–B above. EPA proposed and is approving two HPLC methods (605 and 610), 10 GC methods, and three GC/MS methods (613, 624, and 625). In addition, EPA has responded to critiques of Methods 624 and 625 by approving two GC/MS/isotope dilution variants (1624 and 1625). Each method is accompanied by a specific set of quality assurance (QA) procedures. The QA process relies on specific control limits calculated for each parameter for which the method can be used. The control limits indicate the outer range of precision and accuracy found in an extensive inter-laboratory study. The limits represent the minimum threshold of quality expected of competent laboratories: 95 percent confidence level per compound for the 600 series and the 99 percent confidence level across the set of compounds for the 1624 and 1625 methods. Most analyses should have far better precision and accuracy. The calculations of specific numerical control limits for the calibration and quality control sections of the GC, HPLC, and GC/MS test procedures is interim final. This means that they are legally effective, but that EPA will accept comments on their calculation. All other parts of these test procedures are finally approved for the analysis of the parameters which are indicated in Table IC and ID.

Each method is approved for specific organic compounds. In general, GC Methods 601–603 and GC/MS Methods 624 and 1624 are approved for the analyses of the purgeable priority pollutants. GC Methods 604 and 606–612 and GC/MS Methods 625 and 1625 are approved for the analysis of the non-purgeable, volatile priority pollutants, including, for Method 625 only, the priority pesticide pollutants. Method 625 is also approved for screening samples for 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), but only GC/MS Method 613 is approved for final qualitative confirmation or quantification of 2,3,7,8-TCDD in samples. HPLC Methods 605 and 610 are also approved for the analysis of the nonpurgeable volatiles (the benzidines and polynuclear aromatic hydrocarbons). Methods 1624 and 1625 are approved for use interchangeably with the other test procedures which are being approved for the analysis of the priority toxic organic pollutants. Their most significant difference from Methods 624 and 625 is the requirement that, where available, stable, isotopically-labeled analogs of the priority pollutants are to be used as method internal standards. Since Methods 624 and 625 do permit flexibility in the selection of internal calibration standards and surrogate standards, Methods 1624 and 1625 are, in essence, acceptable variants permitted by Methods 624 and 625. They improve on Methods 624 and 625 and are generally preferable. However, Methods 624 and 625 are also being approved because they are widely available, slightly less expensive, and they are of use when interference and recovery efficiency are not expected to be problems.

In general, both GC/MS and non-MS test procedures have been approved for each of the priority toxic pollutants. Most of the revisions of the proposed test procedures were made either for clarification or to give the analyst more flexibility to practice professional judgment. These procedures now contain a section on safety, cautioning analysts of the potential hazards associated with exposure to the chemical reagents required by the test procedures, or to the toxic chemicals being analyzed. Recommended and mandatory quality assurance practices are also given in each of the test procedures.

Methods 601–604, 606–609, 611–613, 624, 625, 1624, and 1625 include specifications for performing the tests. These specifications are based on a required primary GC column and specified detector. A primary HPLC column and specified detector are required for Methods 605 and 610 and specifications are provided. The primary column is also used to identify the pollutant. A secondary column and detector are also defined, but not required, for non-MS Methods 601–604 and 606–611. The secondary column and detector can be used for confirmation of priority pollutants identified by the primary column for unfamiliar (non-routine) samples (see sections 1.2 of the methods). The GC/MS test procedures are suggested as the confirmatory test for identifications made by Methods 605 and 612, and may also be used as the confirmatory test for identifications made by Methods 601–604 and 606–611. For example, an unfamiliar sample which would be likely to need confirmation would be a single sample taken for an NPDES application. See 40 CFR 122.21. In contrast, routine monitoring, such as that for discharge monitoring reports, would be less likely to require a secondary column for confirmation since the sample is more likely to be familiar to the analyst.

Methods 606, 609, 611 and 612 all use essentially the same procedure for sampling, sample extraction, and concentration. Thus a single sample may be used to measure the parameters within the scope of these methods.

Sample container materials, preservation techniques, and holding times are critical to the procedures and are specifically defined (Methods 601–613, 624, 625, 1624 and 1625). The design and operation of the purge-and-trap device in Methods 601–603, 624 and 1624, and the sample extraction procedures of Methods 604–613, 625 and 1625 are precisely defined as well.

In response to public comments, substantive revisions were made to allow more flexibility in the remaining parts of Methods 601–613, 624, 625, 1624 and 1625. In Methods 604–613, after the sample has been extracted, the analysts are now free to choose a technique to concentrate the extract. The same flexibility is provided for selecting the GC or HPLC configurations (column packings, operating conditions, and detectors). When analysts use concentration techniques or chromatographic configurations other than those described in the test procedures, their approaches must meet the performance criteria defined in the section of the procedures dealing with calibration and quality control.

The most difficult task in finalizing the methods for organic analyses was defining the relationship between desirable flexibility in the methods and

necessary quality control. The proposal specifically solicited comments on both issues and many comments were received on each. The final methods resolve the issue by allowing far greater method flexibility, but by establishing specific control limits as a mandatory part of the quality control procedure.

The proposal noted, and comments confirmed, that method flexibility should be inherent in the methods. Historically, rigid protocols have been a problem in organics analyses. For example, an analyst may be using a method, other than mass spectrometry, to identify a few specific components out of the several million known to exist. This requires that interferences be overcome and "canned" approaches may not effectively address interferences, particularly where matrices are variable or diverse. Thus, the Food and Drug Administration (FDA) and AOAC and other method standardization organizations have usually provided optional "clean-up" procedures for organics, for example, permitting analysts to use Florisil clean-up for pesticides. Further, the analyst may be interested in measuring only a few compounds, while the proposed method may be designed to measure large categories of compounds. For example, a particular industry may be regulated only for the compound that elutes from the gas chromatography after a long program temperature run. An inflexibile method might require the analyst to go through the entire temperature run to look for a single peak that elutes late in the chromatogram. This may be needlessly inefficient. For such reasons, EPA has decided to permit flexibility in chromatographic conditions.

Commentors also raised concerns about inflexibility in sample preparation. They objected to the Kuderna-Danish glassware concentration technique being the only approved approach for concentrating extracts. In fact, if the analysts are measuring only the less volatile compounds in a method category, it may not be necessary to require a rigid procedure for concentration. In this case, it may be appropriate to allow other procedures for concentrating extracts.

After considering these issues, the Agency has decided to allow limited flexibility within the methods. Specifically, chromatographic conditions, including column packings and detectors can be varied. This approach allows continued technical development of the methods. Thus EPA avoided a rigid prescription of technology that would soon be obsolete due to the rapid advances occurring in chromatography. However, the primary objective underlying this flexibility is to enhance precision and accuracy for each analysis. Flexibility should not be permitted if the altered technique would be *less* precise or *less* accurate than the standard approved analytical method. Thus, a corollary of increased flexibility was an increased need for a rigorous and unambiguous quality control procedure.

These basic decisions had become clear by the time of the second, reopened comment period. The comments received in the second comment period again supported the issue of quality control and requested that the criteria be specified more clearly. Another general comment was that the criteria should wait for the results of the inter-laboratory method validation studies and be based upon those results. Today's rulemaking reflects these comments, while specifying that EPA will accept further comments, limited specifically to the calculation of control limits from that new data base.

The quality control procedures now take two different forms. First, there is a "start-up test" to establish the laboratory's basic ability to set up and operate the analytical equipment and procedure. The purpose of the start-up test is two-fold; it establishes that analytical equipment has been properly set up, and it demonstrates the basic ability of the analyst to recognize the compounds of interest. It is required every time the method is changed. It requires the analysis of four spiked distilled water samples. The analyst compares his measures of precision and accuracy to establish criteria developed from the inter-laboratory method validation studies. Because of the basic threshold nature of the start-up test, the methods allow the test to be performed with reagent water.

If the analyst fails the criteria for accuracy or precision in the start-up test, the analyst is to repeat the test for any compound that fails a criterion. If the analyst is measuring, for example, eight compounds at once using Method 601, and fails the criteria for three of them, the analyst is required only to repeat the three that failed provided the method is not changed. It is not very difficult to meet the criteria for any individual compound. However, when one is analyzing for numerous compounds there is an accumulation of failure probabilities; that is, an increased likelihood that one of several parameters will fail for "statistical" reasons. Thus EPA allows a "second pass" opportunity to meet the criteria, as long as the method is not changed. Exhibit 1, below, offers some guidance as to when analysts may want to skip the "second pass" opportunity based on an excessive number of test criteria failures occurring on the first pass. An excessive number of failures should not occur if the system is operating properly. Thus, such a number of failed criteria may suggest poor operation to the analyst. In this case, the first pass criteria failures suggested the compound(s) tested would fail a second round. The analyst may wish to simply adjust the system and reinitiate the start-up test.

If the method is changed as a result of the initial test, the startup begins again. For example, if the start-up test indicates zero recovery of vinly chloride and a check reveals that the instrument trap was installed backwards, the operator must correct the problem and reinitiate the test for all compounds, since the method was just modified.

The second form of quality control is contained in the ongoing quality control program. Laboratories are required to analyze blank samples (e.g., reagent water) daily, and to analyze spiked wastewater samples periodically. Ten percent of all samples are to be spiked (five percent for Methods 624 and 625). The resulting accuracy of recovery must be compared to the established accuracy criteria for the method developed from the results of the inter-laboratory method studies.

If an analyst fails one or more accuracy criteria with the spiked wastewater, the analyst must analyze a check sample (e.g., spiked reagent water). The purpose of analyzing the check sample is to establish whether the inaccuracy is caused by matrix effects or by the laboratory operating improperly (i.e., out of control). Again, accuracy results are compared to the established accuracy criteria. The criteria for acceptable accuracy in these methods are based upon accuracy derived from testing reagent water. Use of check samples rather than spiked wastewater to verify the accuracy criteria for a laboratory is consistent with the fact that one set of regression equations in the inter-laboratory method study is derived from reagent water. That set of regression equations is the basis quality control criteria.

The decision to rely on spiked wastewater samples for the initial test is an alternative to requiring that analyses be conducted on ten percent spiked reagent water samples (to verify laboratory control) and ten percent of

spiked wastewater samples (to verify matrix effects). Accordingly, the need to also analyze a check sample is reduced to a second-tier requirement which is only mandated if accuracy criteria are not met with spiked wastewater.

The limits that are in the methods have been derived on a compound-by-compound and method-by-method basis. They are derived directly from the inter-laboratory method validation studies. The formal inter-laboratory validation studies for Methods 601–602, 604–613, 624, 625, and 1625 have been completed with 15 to 20 laboratories. These fifteen methods have been revised to include methods performance results derived from these studies.

Two methods (603 and 1624) have not been subject to an inter-laboratory validation study. A formal inter-laboratory validation study for Method 603 has not been completed due to an error in the draft method. Although the error was corrected, EPA was not able to perform an inter-laboratory validation study on the same scale as performed for the other methods. However, one commercial laboratory did validate the method and that validation was verified by EPA's laboratory. In addition, the method is similar to Methods 601 and 602 and the results from the validation are similar. EPA believes that the validation of 603 is adequate to establish that the method is appropriate. Therefore, Method 603 is being promulgated with warning limits based upon the best data now available.

Method 1624 was not formally validated through an inter-laboratory study. The specifications for Method 1624 were developed from Method 624 which was formally validated. In informal multi-laboratory and single-laboratory studies, Method 1624 has been shown to yield slightly better performance on treated effluents than Method 624, but this improvement is insufficient to warrant a separate inter-laboratory validation study.

The multi-laboratory validation studies were designed according to the method of W.J. Youden (Youden, W.J., "Statistical Technique for Collaborative Tests," Statistical Manual of the Association of Official Analytical Chemists, 1975) in which pairs of samples having slightly different spiked concentrations of the compound of interest are analyzed. Each collaborating analyst analyzes a sample only once and reports a single value. By having the analyst perform the analysis as he would have done for a normal routine sample, the Youden design helps to avoid accidental manipulation of data that can sometimes occur in a laboratory doing replicate determinations.

Each Youden sample pair for a given parameter is prepared so that the concentration of the pollutant of interest in one-half of the pair is similar to, but measureably different from, the concentration of the pollutant in the other half. Three Youden pairs were analyzed for each of the parameters. The mean values of each of the three pairs were designed to spread over a usable and realistic range of concentrations. The lowest concentration pair was prepared so that the concentration would be above the minimum detection concentration for the method.

The Youden pairs, prepared as concentrates, were spiked into six different water matrices: distilled water, municipal drinking water, a surface water vulnerable to synthetic chemical contaminants, and usually, three different industrial wastewaters from industries that normally would be regulated for the priority pollutants under study. The data were reduced to four statistical relationships related to the overall study: (1) Multi-laboratory mean recovery for each sample, (2) accuracy expressed as relative error or bias (the difference between the multi-laboratory mean recovery and the true value divided by the true value), (3) the multi-laboratory standard deviation of the spike recovery for each sample, and (4) the multi-laboratory relative standard deviation. In addition, two statistics were reduced from the raw data relating to the single-analyst performance: (1) Single-analyst standard deviation, and (2) single-analyst relative standard deviation.

The single-analyst standard deviations were calculated for each of the sample pairs according to the method of Youden by (1) calculating the difference for recoveries from each sample pair reported by each analyst, (2) calculating the average value of these differences across the entire study, (3) calculating a "sum-of-the-squares" by adding the square of the differences between each difference and mean difference, (4) dividing the "sum-of-the-squares" by the degrees of freedom to give the single-analyst variance, and (5) taking the square root of the variance to give the single-analyst standard deviation.

Fifteen to twenty-five percent of the data generated in the multi-laboratory validation studies were discarded as outliers, i.e., data too far from the vast majority of data to be acceptable. Outliers were determined based on widely accepted statistical tests prescribed by ASTM and AOAC.

There is an apparent linear relationship between the mean recovered spike values and the true spike values, overall standard deviation, and single-analyst standard deviation. These linear relationships have been expressed as regression equations over the concentration ranges studied in each matrix. Six different regression equations are derived for each of the six matrices for any given compound. In most cases the variations of the six lines do not appear to be statistically significant at the 5% significance level. The conclusions were reached for each water type by using the F-distribution to compare variance statistics of waste waters with those of distilled water. Mean recoveries were compared between wastewater and distilled water using paired t-test statistics.

EPA is aware that there are limits to the strength of these analyses. These comparisons assume independence among the observations and this was not exactly the case since the "spike" was made up of mixtures of all of the compounds under consideration in each method and hence there was an interdependence among compounds. Despite these limitations, the tests still provide strong evidence that water type generally had no statistically significant effect on the method's performance.

The multi-laboratory tests support an important conclusion. If a laboratory performs well with the methods using distilled water, it should be able to obtain good results with surface waters and industrial wastewaters. Based upon this conclusion, the multi-laboratory regression equations for accuracy and single-analyst overall precision for distilled or reagent water have been incorporated into the quality assurance and quality control provisions of the final texts of Methods 601, 602, 604–613, 624, and 625 to define method performance. The regression equations for the other matrices are also included in the texts of the methods.

The multi-laboratory validation of Method 1625 was performed at a single concentration in a reagent water matrix. Specifications were derived for linearity of calibration, for calibration verification, for retention time precision, for compound recovery from a reagent water matrix, and for precision and accuracy of analysis by isotope dilution and internal standard techniques. All specifications derived from the study are applied at the same level at which they were tested, and sample matrices which show labeled compound recoveries significantly different from

recoveries of these compounds from reagent water are diluted with reagent water to bring these recoveries into the expected range.

It is also important to note that the studies provide a strong basis for setting control limits which represent a range of acceptability. The studies show that most laboratories will do far better, especially on a single-operator, single-laboratory basis. Other performance studies, completed since the inter-laboratory analyses, incorporate too much flexibility to be directly analogous to EPA's collaborative test of the methods. However, they appear to confirm the assumption that most laboratories will exceed the minimum standards and indicate that method variability will be well within the range of the control limits.

The final specifications derived for all of the organics methods (except 603) were the result of a statistical analysis of the data from the multi-laboratory studies. These specifications adopt initial precision and accuracy for all methods. For start-up calibration verification, they specify control limits for Methods 601, 602, 624, 1624, 625 and 1625. For on-going accuracy, they specify control limits for recovery of pollutant spikes for Methods 601–613, 624, and 625, and for recovery of labeled compound spikes for Methods 1624 and 1625. The methods allow for simultaneous testing of all the parameters listed in each method.

In theory, a problem could arise from simultaneous tests for numerous compounds. The control limits have been calculated to allow only a 5% likelihood that a result that exceeds the limits for each compound is merely a statistical fluctuation (rather than actual error). However, the chance of "statistical error" rises with the number of compounds being tested.

EPA has corrected for this possibility in several ways. First, most users will not apply each analysis to all parameters simultaneously; thus they will have a greater chance of passing all test criteria. Second, in order to allow for simultaneous testing of all parameters in a given method, the specifications for accuracy and precision have either been broadened, or a re-test has been allowed, or both. The technique of using a re-test was chosen because a one-test-only specification which allowed for simultaneous testing of a large number of parameters would be so broad as to have little meaning. The provision for a re-test preserved a meaningful specification while allowing for simultaneous testing of all parameters. If a laboratory fails the re-test as well as the initial test, the likelihood of "statistical error" is extremely low (5% times 5%, i.e., .0025 for a given compound). Third, when a re-test is required, it need only be performed on the particular compounds which failed the initial test. Finally, the control criteria for Methods 1624 and 1625—those most likely to be simultaneously used on many compounds—were determined based on the 99% confidence level.

As a voluntary guide to laboratories practicing a given method, the following Exhibit 1 gives suggested numbers of first pass test criteria failures which are unlikely if the laboratory is satisfying the probability based quality control specifications. It assumes all parameters in a given method are tested simultaneously. The Exhibit indicates the maximum number of parameters for which each method can be used simultaneously. The two right-hand columns indicate a certain number of unacceptable results. If the analyst finds that number, or a greater number, of unacceptable results, he may conclude that the entire analysis is flawed. If so, it may be more efficient to repeat the entire analysis than to re-examine only the compounds which exceed the control limits.

EXHIBIT 1.—SUGGESTED MAXIMUM NUMBER OF TEST CRITERIA FAILURES WHICH JUSTIFY REPEATING ENTIRE ANALYSIS

Method	Number of simultaneous parameters	Number of test startup [1]	Criteria failures on-going [2]
601	29	7	4
602	7	3	2
603/605	2	2	2
604	11	4	3
606	6	3	2
607	3	2	2
608	25	6	4
609	4	3	2
610	16	5	3
611	5	3	2
612	9	4	3
613	1	2	1
624	31	7	5
625	61	11	7
1624	66	12	7
1625	151	7	5

[1] Based on twice the number of parameters being tested since both accuracy and precision are being evaluated.
[2] Based on the number of parameters being tested.

Section 8 of each method defines acceptable analytical performance limits for the GC, HPLC, and GC/MS test procedures (Methods 601–613, 624, 625, 1624, and 1625). These acceptable performance limits are also specified in Footnote 7 to Table IC, "List of Approved Test Procedures for Non-Pesticide Organic Compounds," and Footnote 7 to Table ID, "List of Approved Test Procedures for Pesticides." System performance is acceptable only when the average recoveries and standard deviations of spikes of the pollutants of interest into reagent water meet these performance standards. Where large numbers of parameters are being analyzed (see Exhibit 1 above), there is an increased chance that at least one parameter will fail for either average recovery or standard deviation limits based purely on chance. Where such failure occurs, the spiking and recoveries must be repeated, but only for the failed parameters. Repeated failure confirms a general problem with the analytical measurement system. When such failed recoveries are experienced the system is judged to be out-of-control for the failed parameter. Thus, the results for the failed parameters in unspiked samples are suspect and cannot be reported to show regulatory compliance.

The acceptance criteria for spikes into samples for each parameter were calculated to include both an allowance for error in prior measurement of the background and another allowance for error in prior measurement of spike concentrations. The calculation assumed a spike-to-background ratio of 5 to 1. Thus such error will be accounted for to the extent the analysts' spike-to-background ratio approaches 5 to 1. In many cases this allows analysts a greater margin of error than should actually be expected. This is because the calculation assumes that two prior errors are cumulative, ignoring the degree to which they actually cancel each other out.

Today's final test procedures represent an effort to provide the maximum uniformity that is practical for a wide cross-section of classes of chemical compounds. They will be continually reevaluated for their general applicability to complex wastewater matrices.

The substantive revisions made in the GC, HPLC, and GC/MS methods in response to comments are discussed in the public participation section of this preamble. Three of the most significant changes include: (1) Addition of a confirmatory column to Method 602; (2) deletion (from 613) of the gas chromatographic/electron capture (GC/EC) test procedure for screening for 2,3,7,8-TCDD, and (3) revision of Methods 613 and 625 to show that Method 625 may be used whenever screening for 2,3,7,8-TCDD is required. The full text of the approved GC, HPLC, and GC/MS test procedures are being printed in Appendix A of this regulation.

The GC, HPLC, and GC/MS test procedures are now cited in the regulations in the new Table IC, "List of

Approved Test Procedures for Non-Pesticide Organic Compounds," and Table ID, "List of Approved Test Procedures for Pesticides."

C. ICP Test Procedure

The ICP test procedure is cited in the regulation as an additional analytical option for trace metal analysis in the new Table IB, "List of Approved Inorganic Test Procedures."

The ICP test procedure, Method 200.7, has been changed only slightly from the version proposed on December 3, 1979. EPA proposed that lithium and strontium be analyzed using the ICP test procedure, since these parameters could be analyzed using this method. Because EPA did not propose or develop accuracy or precision criteria for these parameters, EPA is unable to approve the ICP test procedure for them. EPA is considering the ICP and other alternative test procedures in a separate rulemaking. In light of additional information received in the public comments showing good recoveries for antimony and adequate recoveries for thallium by the proposed test procedure, both of these metals have been added to the scope of the ICP test procedure. Also in response to public comments the detection limit for silica has been doubled and the wavelengths of the metal are now given to the third decimal. In section 3 of the ICP test procedure a new definition for "Quality Control Sample" has been provided for clarification, and a new section on safety has been added to alert the analyst to the hazards of the toxic reagents and pollutants involved. Other revisions made in response to comments are discussed in the public participation section of this preamble. The full text of the ICP procedure is printed as Appendix C to this regulation.

D. $CBOD_5$ Test Procedure

The final test procedure for $CBOD_5$ is essentially the same as that proposed. See Section III-D, above. EPA's proposed test procedure was taken from a draft *Standard Methods* test procedure for $CBOD_5$.

The final method language is the same as the language now included in the 15th edition of *Standard Methods*. This has required minor changes from the wording of the proposal, but no substantive changes were required.

E. Table II: Required Containers, Preservation Techniques, and Holding Times

Table II in Section 136.3(e) now restricts the materials of which sample containers can be made, and specifies the procedures by which samples are to be preserved. Table II also limits the maximum time for which samples may be held from the the time of sampling until they are analyzed. Table II has been restructured in this final regulation to correlate with the parameters in the new Tables IA, IB, IC, ID, and IE in Section 136.3(a). Table II allows cross-reference between the container, preservative, and holding times and the individual parameters in Tables IA, to IE.

In response to comments, several changes were made in Table II of the final regulations for-prescribed container materials, preservation requirements, and holding times of wastewater samples. Where supported by comments, changes were made primarily in holding times. In response to comments, EPA has adopted the requirement that some samples be analyzed immediately, to avoid sample degradation. This would be as soon as the sample is collected and labelled, generally within 15 minutes. Longer holding times are generally not appropriate where the sample may quickly degrade. However, a longer time period may be justified under the variance procedure. Exhibits 3 and 4, below, show that for organic compounds and pesticides, the holding times were generally extended from 30 days after extraction to 40 days after extraction. Changes were also made to enable a single sample to be used for analyses of extractable organics and of pesticides. This was a step towards the goal of uniformity, sought by EPA and by the commenters.

Table II as promulgated also allows a variance to holding times under § 136.3(e). Analysts may exceed the holding times if they have data on file to show that the specific types of samples are stable for a longer time and if they receive a variance from the Regional Administrator.

No changes were made for container materials, preservation requirements, or holding times in final Table II from the proposed requirements for the biological parameters listed in Table IA, or the radiological parameters listed in Table IE. Changes which were made in Table II for inorganic parameters listed in Table IB, organic parameters listed in Table IC, and pesticide parameters listed in Table ID are summarized in the following Exhibits 2, 3, and 4, of this preamble. Proposed and final container materials, preservation requirements, and holding times in Exhibits in 2, 3, and 4 are given only for the affected pollutant parameters in Tables IB, IC and ID of the regulation.

EXHIBIT 2.—CHANGES MADE IN TABLE II FOR TABLE IB PARAMETERS

Parameter	Requirement	Change	
		From (proposed)	To (final)
Chlorine residual	Holding time	2 hours	Analyze immediately.
Cyanide	Preservative	$Na_2S_2O_3$	Ascorbic acid.
			Add: Remove sulfide as cadmium sulfide.
pH	Holding time	2 hours	Analyze immediately.
Chromium VI	Holding time	48 hours	24 hours.
Mercury	Preservative	0.05% $K_2Cr_2O_7$	Delete.
Organic carbon	Preservative	None	Add: HCl or H_2SO_4 to pH<2.
Dissolved oxygen probe	Holding time	1 hour	Analyze immediately.
Winkler	Preservative		Add: Store in dark.
Phenols	Container	P and G	G only.
Residue, total	Holding time	14 days	7 days.
Residue, filterable	Holding time	14 days	7 days.
Residue, settleable	Holding time	7 days	48 hours.
Sulfide	Preservative		Add: NaOH to pH>9.
	Holding time	28 days	7 days.
Sulfite	Holding time	48 hours	Analyze immediately.
	Preservative	Cool to 4 °C	None required.

Appendix B

Text of the March 12, 2007 *Federal Register* Notice Preamble introducing the new method flexibility language at Section 136.6 (page 11203) and Section 136.6 (pp. 11239-11241)

When this document was reformatted in 2014, the scanned image of the prepublication text originally used for this appendix was replaced with an excerpt from the actual *Federal Register* Notice.

9. The rule replaces EPA Method 180.1 (1978) for determination of turbidity with EPA Method 180.1 (Revision 2.0, 1993).

10. The rule replaces EPA Method 200.7 (1990) for determination of elements by ICP–AES with EPA Method 200.7 (Revision 4.4, 1994).

11. The rule replaces EPA Method 245.1 (1974) for determination of mercury with EPA Method 245.1 (Revision 3.0, 1994).

12. The rule replaces EPA Method 335.3 (1978) for determination of total cyanide with EPA Method 335.4 (Revision 1.0, 1993) with a footnote to clarify the proper procedure for removing sulfide interferences.

13. The rule replaces EPA Method 350.1 (1978) for determination of ammonia with EPA Method 350.1 (Revision 2.0, 1993).

14. The rule replaces EPA Method 351.2 (1978) for determination of total Kjeldahl nitrogen (TKN) with EPA Method 351.2 (Revision 2.0 1993).

15. The rule replaces EPA Method 353.2 (1978) for determination of nitrate-nitrite with EPA Method 353.2 (Revision 2.0, 1993).

16. The rule replaces EPA Method 365.1 (1978) for determination of phosphorus (all forms) with EPA Method 365.1 (Revision 2.0, 1993).

17. The rule replaces EPA Method 375.2 (1978) for determination of sulfate with EPA Method 375.2 (Revision 2.0, 1993).

18. The rule replaces EPA Method 410.4 (1978) for determination of chemical oxygen demand (COD) with EPA Method 410.4 (Revision 2.0, 1993).

19. The rule replaces EPA Method 420.2 (1974) for determination of total phenols with EPA Method 420.4 (Revision 1.0, 1993).

20. The rule approves a new method for the determination of mercury, EPA Method 245.7 ''Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry'' [Revision 2.0, 2005] (EPA–821–R–05–001).

21. The rule approves a new method for determination of available cyanide by ligand exchange followed by flow injection analysis, ASTM D6888–04.

22. The rule approves a new method for determination of cations by ion chromatography, ASTM D6919–03.

23. The rule approves a new method for determination of chloride by potentiometry, SM 4500–Cl–D [18th, 19th, 20th Editions] and SM 4500–Cl–D (2000).

24. The rule approves a new method for determination of chloride by ion selective electrode, ASTM D512–89 (1999).

25. The rule approves two new methods for determination of total cyanide by ion selective electrode, SM 4500–CN–F [18th, 19th, 20th Editions] and SM 4500–CN–F (2000), and ASTM D2036–98 A.

26. The rule approves two new methods for determination of sulfide by ion selective electrode, SM 4500–S_2–G [18th, 19th, 20th Editions] and ASTM D4658–03 (1996).

27. The rule approves a new method for determination of nitrate by ion selective electrode, SM 4500–NO_3–[18th, 19th, 20th Editions] and SM 4500–NO_3–(2000).

28. The rule approves an errata sheet to correct typographical errors in the following methods manuals, ''Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms,'' Fourth Edition, U.S. Environmental Protection Agency, Office of Water, Washington DC, EPA/821/R–02/013 (the ''freshwater chronic manual''), and ''Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms,'' Fifth Edition, U.S. Environmental Protection Agency, Office of Water, Washington DC, EPA/821/R–02/012 (the ''freshwater acute manual'').

29. The rule approves the use of newer versions of 74 methods published by ASTM International. The new versions are published in the 1994, 1996, and 1999 Annual Book of ASTM Standards Vols. 11.01 and 11.02, in the 2000 Annual Book of ASTM Standards, Vol. 11.02 and in individual standards published after 2000.

30. The rule approves the use of newer versions of 88 methods published by the Standard Methods Committee and adopts a new numbering system to track the approved versions of Standard Methods. The new versions are published in Standard Methods Online (APHA 2003).

31. The rule approves the use of newer versions of 19 methods published by AOAC–International. The new versions of these methods are published in Official Methods of Analysis of AOAC–International, 16th Edition, 1995.

32. The rule approves the replacement of the mercuric sulfate catalyst with copper sulfate in methods approved for the determination of total Kjeldahl nitrogen (TKN).

33. The rule approves the use of styrene divinyl benzene beads and stabilized formazin as alternatives to the presently approved formazin standard for determination of turbidity.

34. As described in the preamble to the April 2004 proposed rule (69 FR 18213), EPA is adopting a new § 136.6 to introduce greater flexibility in the use of approved methods. The section describes the circumstances in which approved methods may be modified and the requirements that analysts must meet to use these modified methods in required measurements without prior EPA approval. The rule also includes language at § 136.6(c) to clarify that analysts need only meet method performance requirements for target analytes (those analytes being measured for NPDES reporting) when using multi-analyte methods for compliance monitoring purposes. The rule also includes the language at § 136.6(d) to allow explicitly the use of capillary (open tubular) GC columns with EPA Methods 601–613, 624, 625, and 1624B as alternatives to the packed GC columns specified in those methods, provided that analysts generate new retention time tables with capillary columns to be kept on file with other information for review by auditors.

35. The rule withdraws 109 methods contained in EPA's ''Methods for the Chemical Analysis of Water and Wastes'' for which approved alternatives published by voluntary consensus standards bodies (*e.g.*, ASTM and Standard Methods) are available.

36. The rule withdraws liquid-liquid extraction (LLE) methods, including EPA Methods 612 and 625, as approved procedures for determination of 1,2-dichlorobenzene, 1,3-dichlorobenzene, and 1,4-dichlorobenzene.

37. The rule withdraws approval of all oil and grease methods that use chlorofluorocarbon-113 (CFC–113; Freon–113) as an extraction solvent.

38. The rule revises Table II (Required Containers, Preservation Techniques, and Holding Times) and the footnotes to the table at 40 CFR 136.3(e). The table and footnotes specify approved sampling, preservation, and holding time requirements for the methods approved for compliance monitoring to reduce confusion, resolve any conflicts with instructions in the underlying compliance monitoring method, and reflect current understanding of sample preservation requirements. The most significant of the changes are those made to Footnote 6, which addresses the preservation of samples to be analyzed for cyanide. Based on information gathered during the development of new cyanide methods approved in this rulemaking, and information collated from various commenters and experts in cyanide analyses, EPA revised footnote 6 to Table II by adding text that describes procedures that are recommended for removal or suppression of cyanide interferences, including interferences

(4) Aldehyde: If formaldehyde, acetaldehyde, or another water-soluble aldehyde is known or suspected to be present, treat the sample with 20 mL of 3.5% ethylenediamine solution per liter of sample.

(5) Carbonate: Carbonate interference is evidenced by noticeable effervescence upon acidification in the distillation flask, a reduction in the pH of the absorber solution, and incomplete cyanide spike recovery. When significant carbonate is present, adjust the pH to ≥ 12 using calcium hydroxide instead of sodium hydroxide. Allow the precipitate to settle and decant or filter the sample prior to analysis (also see Standard Method 4500-CN.B.3.d).

(6) Chlorine, hypochlorite, or other oxidant: Treat a sample known or suspected to contain chlorine, hypochlorite, or other oxidant as directed in footnote 5.

[7] For dissolved metals, filter grab samples within 15 minutes of collection and before adding preservatives. For a composite sample collected with an automated sampler (e.g., using a 24-hour composite sampler; see 40 CFR 122.21(g)(7)(i) or 40 CFR Part 403, Appendix E), filter the sample within 15 minutes after completion of collection and before adding preservatives. If it is known or suspected that dissolved sample integrity will be compromised during collection of a composite sample collected automatically over time (e.g., by interchange of a metal between dissolved and suspended forms), collect and filter grab samples to be composited (footnote 2) in place of a composite sample collected automatically.

[8] Guidance applies to samples to be analyzed by GC, LC, or GC/MS for specific compounds.

[9] If the sample is not adjusted to pH 2, then the sample must be analyzed within seven days of sampling.

[10] The pH adjustment is not required if acrolein will not be measured. Samples for acrolein receiving no pH adjustment must be analyzed within 3 days of sampling.

[11] When the extractable analytes of concern fall within a single chemical category, the specified preservative and maximum holding times should be observed for optimum safeguard of sample integrity (i.e., use all necessary preservatives and hold for the shortest time listed). When the analytes of concern fall within two or more chemical categories, the sample may be preserved by cooling to ≤6 °C, reducing residual chlorine with 0.008% sodium thiosulfate, storing in the dark, and adjusting the pH to 6-9; samples preserved in this manner may be held for seven days before extraction and for forty days after extraction. Exceptions to this optional preservation and holding time procedure are noted in footnote 5 (regarding the requirement for thiosulfate reduction), and footnotes 12, 13 (regarding the analysis of benzidine).

[12] If 1,2-diphenylhydrazine is likely to be present, adjust the pH of the sample to 4.0 ± 0.2 to prevent rearrangement to benzidine.

[13] Extracts may be stored up to 30 days at <0 °C.

[14] For the analysis of diphenylnitrosamine, add 0.008% $Na_2S_2O_3$ and adjust pH to 7–10 with NaOH within 24 hours of sampling.

[15] The pH adjustment may be performed upon receipt at the laboratory and may be omitted if the samples are extracted within 72 hours of collection. For the analysis of aldrin, add 0.008% $Na_2S_2O_3$.

[16] Sufficient ice should be placed with the samples in the shipping container to ensure that ice is still present when the samples arrive at the laboratory. However, even if ice is present when the samples arrive, it is necessary to immediately measure the temperature of the samples and confirm that the preservation temperature maximum has not been exceeded. In the isolated cases where it can be documented that this holding temperature cannot be met, the permittee can be given the option of on-site testing or can request a variance. The request for a variance should include supportive data which show that the toxicity of the effluent samples is not reduced because of the increased holding temperature.

[17] Samples collected for the determination of trace level mercury (<100 ng/L) using EPA Method 1631 must be collected in tightly-capped fluoropolymer or glass bottles and preserved with BrCl or HCl solution within 48 hours of sample collection. The time to preservation may be extended to 28 days if a sample is oxidized in the sample bottle. A sample collected for dissolved trace level mercury should be filtered in the laboratory within 24 hours of the time of collection. However, if circumstances preclude overnight shipment, the sample should be filtered in a designated clean area in the field in accordance with procedures given in Method 1669. If sample integrity will not be maintained by shipment to and filtration in the laboratory, the sample must be filtered in a designated clean area in the field within the time period necessary to maintain sample integrity. A sample that has been collected for determination of total or dissolved trace level mercury must be analyzed within 90 days of sample collection.

[18] Aqueous samples must be preserved at ≤6 °C, and should not be frozen unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted as valid by the regulatory authority. Also, for purposes of NPDES monitoring, the specification of "≤ °C" is used in place of the "4 °C" and "<4 °C" sample temperature requirements listed in some methods. It is not necessary to measure the sample temperature to three significant figures (1/100th of 1 degree); rather, three significant figures are specified so that rounding down to 6 °C may not be used to meet the ≤6 °C requirement. The preservation temperature does not apply to samples that are analyzed immediately (less than 15 minutes).

[19] An aqueous sample may be collected and shipped without acid preservation. However, acid must be added at least 24 hours before analysis to dissolve any metals that adsorb to the container walls. If the sample must be analyzed within 24 hours of collection, add the acid immediately (see footnote 2). Soil and sediment samples do not need to be preserved with acid. The allowances in this footnote supersede the preservation and holding time requirements in the approved metals methods.

[20] To achieve the 28-day holding time, use the ammonium sulfate buffer solution specified in EPA Method 218.6. The allowance in this footnote supersedes preservation and holding time requirements in the approved hexavalent chromium methods, unless this supersession would compromise the measurement, in which case requirements in the method must be followed.

[21] Holding time is calculated from time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

■ 8. Section 136.4 is amended by revising the first sentence of paragraph (d) introductory text to read as follows:

§ 136.4 Application for alternate test procedures.

* * * * *

(d) An application for approval of an alternate test procedure for nationwide use may be made by letter in triplicate to the Alternate Test Procedure Program Coordinator, Office of Science and Technology (4303), Office of Water, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460. * * *

* * * * *

■ 9. Section 136.5 is amended as follows:

■ a. In paragraph (b) by revising the last sentence.

■ b. By revising paragraph (c).

■ c. In paragraph (d) by revising the second and third sentences.

■ d. By revising paragraphs (e)(1) and (e)(2).

§ 136.5 Approval of alternate test procedures.

* * * * *

(b) * * * Where the Director recommends rejection of the application for scientific and technical reasons which he provides, the Regional Administrator shall deny the application and shall forward this decision to the Director of the State Permit Program and to the Alternate Test Procedure Program Coordinator, Office of Science and Technology (4303), Office of Water, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460.

(c) Before approving any application for an alternate test procedure proposed by the responsible person or firm making the discharge, the Regional Administrator shall forward a copy of the application to the Alternate Test Procedure Program Coordinator, Office of Science and Technology (4303), Office of Water, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460.

(d) * * * Prior to the expiration of such ninety day period, a recommendation providing the scientific and other technical basis for acceptance or rejection will be forwarded to the Regional Administrator by the Alternate Test Procedure Program Coordinator, Washington, DC. A copy of all approval and rejection notifications will be forwarded to the Alternate Test Procedure Program Coordinator, Office of Science and Technology (4303), Office of Water, U.S. Environmental Protection Agency, 1200 Pennsylvania Ave., NW., Washington, DC 20460, for the purposes of national coordination.

(e) *Approval for nationwide use.* (1) As expeditiously as is practicable after receipt by the Alternate Test Procedure Program Coordinator, Washington, DC, of an application for an alternate test procedure for nationwide use, the Alternate Test Procedure Program Coordinator, Washington, DC, shall notify the applicant in writing whether the application is complete. If the application is incomplete, the applicant shall be informed of the information necessary to make the application complete.

(2) As expeditiously as is practicable after receipt of a complete package, the Alternate Test Procedure Program Coordinator shall perform any analysis necessary to determine whether the alternate test procedure satisfies the applicable requirements of this part, and the Alternate Test Procedure Program Coordinator shall recommend to the Administrator that he/she approve or reject the application and shall also notify the application of the recommendation.

* * * * *

■ 10. Section 136.6 is added to Part 136 to read as follows:

§ 136.6 Method Modifications and Analytical Requirements.

(a) Definitions of terms used in this Section.

(1) *Analyst* means the person or laboratory using a test procedure (analytical method) in this Part.

(2) *Chemistry of the Method* means the reagents and reactions used in a test procedure that allow determination of the analyte(s) of interest in an environmental sample.

(3) *Determinative Technique* means the way in which an analyte is identified and quantified (*e.g.,* colorimetry, mass spectrometry).

(4) *Equivalent Performance* means that the modified method produces results that meet the QC acceptance

criteria of the approved method at this part.

(5) *Method-defined Analyte* means an analyte defined solely by the method used to determine the analyte. Such an analyte may be a physical parameter, a parameter that is not a specific chemical, or a parameter that may be comprised of a number of substances. Examples of such analytes include temperature, oil and grease, total suspended solids, total phenolics, turbidity, chemical oxygen demand, and biochemical oxygen demand.

(6) *QC* means ''quality control.''

(b) *Method Modifications.*

(1) *Allowable Changes.* Except as set forth in paragraph (b)(3) of this section, an analyst may modify an approved test procedure (analytical method) provided that the chemistry of the method or the determinative technique is not changed, and provided that the requirements of paragraph (b)(2) of this section are met.

(i) Potentially acceptable modifications regardless of current method performance include changes between automated and manual discrete instrumentation; changes in the calibration range (provided that the modified range covers any relevant regulatory limit); changes in equipment such as using similar equipment from a vendor other than that mentioned in the method (*e.g.*, a purge-and-trap device from OIA rather than Tekmar), changes in equipment operating parameters such as changing the monitoring wavelength of a colorimeter or modifying the temperature program for a specific GC column; changes to chromatographic columns (treated in greater detail in paragraph (d) of this section); and increases in purge-and-trap sample volumes (provided specifications in paragraph (e) of this section are met). The changes are only allowed provided that all the requirements of paragraph (b)(2) of this section are met.

(ii) If the characteristics of a wastewater matrix prevent efficient recovery of organic pollutants and prevent the method from meeting QC requirements, the analyst may attempt to resolve the issue by using salts as specified in *Guidance on Evaluation, Resolution, and Documentation of Analytical Problems Associated with Compliance Monitoring* (EPA 821–B–93–001, June 1993), provided that such salts do not react with or introduce the target pollutant into the sample (as evidenced by the analysis of method blanks, laboratory control samples, and spiked samples that also contain such salts) and that all requirements of paragraph (b)(2) of this section are met. Chlorinated samples must be dechlorinated prior to the addition of such salts.

(iii) If the characteristics of a wastewater matrix result in poor sample dispersion or reagent deposition on equipment and prevents the analyst from meeting QC requirements, the analysts may attempt to resolve the issue by adding an inert surfactant (*i.e.* a surfactant that will not affect the chemistry of the method), which may include Brij-35 or sodium dodecyl sulfate (SDS), provided that such surfactant does not react with or introduce the target pollutant into the sample (as evidenced by the analysis of method blanks, laboratory control samples, and spiked samples that also contain such surfactant) and that all requirements of paragraph (b)(2) of this section are met. Chlorinated samples must be dechlorinated prior to the addition of such surfactant.

(2) *Requirements.* A modified method must produce equivalent performance to the approved methods for the analyte(s) of interest, and the equivalent performance must be documented.

(i) *Requirements for Establishing Equivalent Performance*

(A) If the approved method contains QC tests and QC acceptance criteria, the modified method must use these QC tests and the modified method must meet the QC acceptance criteria. The Analyst may only rely on QC tests and QC acceptance criteria in a method if it includes wastewater matrix QC tests and QC acceptance criteria (e.g., as matrix spikes) and both initial (start-up) and ongoing QC tests and QC acceptance criteria.

(B) If the approved method does not contain QC tests and QC acceptance criteria, or if the QC tests and QC acceptance criteria in the method do not meet the requirements of paragraph (b)(2)(i)(A) of this section, the analyst must employ QC tests specified in *Protocol for EPA Approval of Alternate Test Procedures for Organic and Inorganic Analytes in Wastewater and Drinking Water* (EPA–821–B–98–002, March 1999) and meet the QC provisions specified therein. In addition, the Analyst must perform on-going QC tests, including assessment of performance of the modified method on the sample matrix (*e.g.*, analysis of a matrix spike/matrix spike duplicate pair for every twenty samples of a discharge analyzed), and analysis of an ongoing precision and recovery sample and a blank with each batch of 20 or fewer samples.

(C) Calibration must be performed using the modified method and the modified method must be tested with every wastewater matrix to which it will be applied (up to nine distinct matrices; as described in the ATP Protocol, after validation in nine distinct matrices, the method may be applied to all wastewater matrices), in addition to any and all reagent water tests. If the performance in the wastewater matrix or reagent water does not meet the QC acceptance criteria the method modification may not be used.

(D) Analysts must test representative effluents with the modified method, and demonstrate that the results are equivalent or superior to results with the unmodified method.

(ii) *Requirements for Documentation.* The modified method must be documented in a method write-up or an addendum that describes the modification(s) to the approved method. The write-up or addendum must include a reference number (*e.g.*, method number), revision number, and revision date so that it may be referenced accurately. In addition, the organization that uses the modified method must document the results of QC tests and keep these records, along with a copy of the method write-up or addendum, for review by an auditor.

(3) *Restrictions.* An analyst may not modify an approved analytical method for a method-defined analyte. In addition, an analyst may not modify an approved method if the modification would result in measurement of a different form or species of an analyte (*e.g.*, a change to a metals digestion or total cyanide distillation). An analyst may also may not modify any sample preservation and/or holding time requirements of an approved method.

(c) *Analytical Requirements for Multi-analyte Methods (Target Analytes).* For the purpose of NPDES reporting, the discharger or permittee must meet QC requirements only for the analyte(s) being measured and reported under the NPDES permit.

(d) The following modifications to approved methods are authorized in the circumstances described below:

(1) *Capillary Column.* Use of a capillary (open tubular) GC column rather than a packed column is allowed with EPA Methods 601–613, 624, 625, and 1624B in Appendix A to this part, provided that all QC tests for the approved method are performed and all QC acceptance criteria are met. When changing from a packed column to a capillary column, retention times will change. Analysts are not required to meet retention time specified in the approved method when this change is made. Instead, analysts must generate new retention time tables with capillary columns to be kept on file along with

other startup test and ongoing QC data, for review by auditors.

(2) *Increased sample volume in purge and trap methodology.* Use of increased sample volumes, up to a maximum of 25 mL, is allowed for an approved method, provided that the height of the water column in the purge vessel is at least 5 cm. The analyst should also use one or more surrogate analytes that are chemically similar to the analytes of interest in order to demonstrate that the increased sample volume does not adversely affect the analytical results.

PART 141—NATIONAL PRIMARY DRINKING WATER REGULATIONS

■ 11. The authority citation for part 141 continues to read as follows:

Authority: 42 U.S.C. 300f, 300g–1, 300g–2, 300g–3, 300g–4, 300g–5, 300g–6, 300j–4, 300j–9, and 300j–11.

■ 12. Section 141.21 is amended by adding four sentences to the end of footnote 1 to the Table in paragraph (f)(3) to read as follows:

§ 141.21 Coliform sampling.

* * * * *

(f) * * *

(3) * * *

1* * * In addition, the following online versions may also be used: 9221 A, B, D–99, 9222 A, B, C–97, and 9223 B–97. Standard Methods Online are available at *http://www.standardmethods.org.* The year in which each method was approved by the Standard Methods Committee is designated by the last two digits in the method number. The methods listed are the only Online versions that may be used.

* * * * *

■ 13. Section 141.23 is amended as follows:

■ a. In paragraph (a)(4)(i) by revising the table entries for ''Cyanide,'' ''Nitrate,'' and ''Nitrite''.

■ b. In paragraph (k)(1) by revising the table.

§ 141.23 Inorganic chemical sampling and analytical requirements.

* * * * *

(a) * * *

(4) * * *

(i) * * *

DETECTION LIMITS FOR INORGANIC CONTAMINANTS

Contaminant	MCL (mg/L)	Methodology	Detection Limit (mg/L)
*	*	* * * * *	
Cyanide	0.2	Distillation, Spectrophotometric[3]	0.02
		Distillation, Automated, Spectrophotometric[3]	0.005
		Distillation, Amenable, Spectrophotometric[4]	0.02
		Distillation, Selective Electrode[3, 4]	0.05
		UV, Distillation, Spectrophotometric[9]	0.0005
		Micro Distillation, Flow Injection, Spectrophotometric[3]	0.0006
		Ligand Exchange with Amperometry[4]	0.0005
*	*	* * * * *	
Nitrate	10 (as N)	Manual Cadmium Reduction	0.01
		Automated Hydrazine Reduction	0.01
		Automated Cadmium Reduction	0.05
		Ion Selective Electrode	1
		Ion Chromatography	0.01
		Capillary Ion Electrophoresis	0.076
Nitrite	1 (as N)	Spectrophotometric	0.01
		Automated Cadmium Reduction	0.05
		Manual Cadmium Reduction	0.01
		Ion Chromatography	0.004
		Capillary Ion Electrophoresis	0.103
*	*	* * * * *	

* * * * *

[3] Screening method for total cyanides.

[4] Measures ''free'' cyanides when distillation, digestion, or ligand exchange is omitted.

* * * * *

[9] Measures total cyanides when UV-digestor is used, and ''free'' cyanides when UV-digestor is bypassed.

* * * * *

(k) * * *

(l) * * *

Contaminant	Methodology [13]	EPA	ASTM [3]	SM [4] (18th, 19th ed.)	SM [4] (20th ed.)	SM Online [22]	Other
1. Alkalinity	Titrimetric		D1067–92, 02 B	2320 B	2320 B	2320 B–97	
	Electrometric titration					I–1030–85 [5].	
2. Antimony	Inductively Coupled Plasma (ICP)—Mass Spectrometry.	200.8 [2]					
	Hydride-Atomic Absorption.		D3697–92, 02.				
	Atomic Absorption; Platform.	200.9 [2]					
	Atomic Absorption; Furnace.			3113 B		3113 B–99	
3. Arsenic [14]	Inductively Coupled Plasma [15].	200.7 [2]		3120 B	3120 B	3120 B–99.	
	ICP-Mass Spectrometry	200.8 [2]					

Appendix C

November 1, 2006 EPA memorandum regarding Recommended Approved Modifications to EPA Method 625

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MEMORANDUM

OFFICE OF WATER

SUBJECT: **Recommended Approved Modifications to EPA Method 625**

FROM: Richard Reding, Chief
Engineering & Analytical Support Branch, EAD, OST

TO: Quality Assurance Managers
ATP Coordinators
NPDES Coordinators

DATE: November 1, 2006

The 304(h) methods branch recommends allowing several modifications to EPA Method 625 for environmental permitting and compliance monitoring under the EPA's Clean Water Act (CWA) programs. This memorandum does not address laboratory certification requirements that states have mandated.

The text in "Protocol for EPA Approval of Alternate Test Procedures for Organic and Inorganic Analytes in Wastewater and Drinking Water" Section 1.3.2 allows flexibility in the modification of "front end techniques" of the test method provided all criteria in this section and **all QC in the method are** met and documented. This protocol can be downloaded at http://www.epa.gov/waterscience/methods.

Recommendations on Method Modifications to EPA Method 625 when Capillary Columns are used:

1. **Combining sample extracts before analysis**

If the analytes can be reliably identified and quantified in the combined extracts, the extracts may be combined. If, however, the identification and quantitation of any analyte is adversely affected by another analyte, a surrogate, or an interferant, the extracts must be analyzed separately. If there is ambiguity, the extracts must be analyzed separately.

2. **Reverse order of pH extraction**

The pH extraction sequence may be reversed to better separate acid and neutral components. Neutral components may be extracted with either acid or base components.

Previously, neither of these modifications has been used with Method 625 primarily because of limitations of the resolving power of the packed columns used. In 1985, EPA Region 3 Central Regional Lab requested a modification to method 625 as an alternate test procedure (ATP). Although the approval was for limit use by EPA's Region 3, Central Regional Laboratory only, this modification has come to be used throughout the laboratory community (see attached memo).

Why allow these modifications? Following the base-neutral than acid extraction sequence of method 625 in some cases demonstrated the decomposition of some analytes under basic conditions. Organochlorine pesticides may dechlorinate; phthalate esters may exchange; phenols may react to form tannates. These reactions increase with increasing pH. Reversing the extraction pH sequence may better separate acid and neutral waste components.

Other Recommended Modifications to Method 625

A smaller sample volume may be used to minimize matrix interferences provided matrix interferences are demonstrated and documented.

Alternate surrogate and internal standard concentrations other than those specified in the method are acceptable provided that method performance is not degraded;

An alternate calibration curve and a calibration check other than those specified in the method;

A different solvent for the calibration standards to match the solvent of the final extract.

Other Method Flexibility News

We are revising the "Guidance on Evaluation, Resolution, and Documentation of Analytical Problems Associated with Compliance Monitoring" often referred to as the "Pumpkin Book". Many of the recommendations in the revised "Pumpkin Book" cover ways to mitigate matrix effects.

More explicit flexibility to make changes in approved methods without prior EPA approval is now described at 40 CFR Part 136.6. Such changes are only allowed if the modified method produces equivalent performance for the analyte(s) of interest, and the equivalent performance is documented. It is essential to consult the full text at 40 CFR 136.6 before undertaking method modifications.

Please feel free to forward this information. If you have any questions regarding this memorandum, please contact Lemuel Walker of EASB/EAD/OST by email at walker.lemuel@epa.gov.

cc Lemuel Walker
ATP Coordinator