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Data Validation Guidance Manual for Selected Sediment Variables (QA-2)

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Edited Draft Report

Data Validation Guidance Manual for Selected Sediment Variables

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CALL NUMBER: DVG 001

For

Washington Department of Ecology
Olympia, Washington

Ecology Contract C0089018

June 1989 Draft

PTI Environmental Services
15375 SE 30th Place
Suite 250
Bellevue, Washington 98007

EDITED DRAFT REPORT

DATA VALIDATION GUIDANCE MANUAL FOR SELECTED SEDIMENT VARIABLES

For

Washington Department of Ecology
Sediment Management Unit
Mail Stop PV-11
Olympia, Washington

Ecology Contract C0089018
PTI Contract C901-02

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1. INTRODUCTION

1.1 OBJECTIVE

Data validation is the process by which a sample, measurement method, or data point is deemed useful for a specific purpose. The objective of the *Data Validation Guidance Manual for Selected Sediment Variables* is to provide a thorough description of the data quality review process, and a standardized format for assessing data accuracy, precision, completeness, and usability. This document is designed to be used by Washington Department of Ecology (Ecology) staff to assess the quality of sediment data collected throughout Puget Sound to determine if they are suitable for inclusion in Ecology's sediment quality values database. ~~This document is designed to be used by Washington Department of Ecology (Ecology) staff to assess the quality of sediment data collected through Puget Sound to determine if they are acceptable for inclusion into Ecology's sediment quality value database.~~ Environmental variables in Puget Sound are measured by a wide variety of organizations, including government agencies, universities, and private institutions. However, comparisons of results from different studies frequently are limited because different methods are used to measure the same variable(s). The ability to compare data among different studies is highly desirable for developing a comprehensive management strategy for Puget Sound. A standardized, rigorous review process is essential to ensuring the quality and integrity of the sediment quality values database.

1.2 BACKGROUND

A joint effort to develop sediment quality values for Puget Sound was undertaken during 1986 and 1987 by Ecology, the U.S. Army Corps of Engineers Seattle Office, U.S. Environmental Protection Agency (EPA) Region 10 - Office of Puget Sound, and the Washington Department of Natural Resources. The goal of this project was to identify the concentrations of chemicals in sediments that are expected (based on field evidence or theoretical predictions) to be associated with adverse biological effects. The specific objectives of this study were to compile synoptic biological and chemical data from Puget Sound, to evaluate techniques that could be used to develop chemical-specific sediment quality values, and to evaluate the reliability (i.e., ability to correctly identify sites with known biological effects) of the values generated using the different techniques. Details of this project are described in Tetra Tech (1986). These data have been incorporated into a Puget Sound sediment quality values database (SEDQUAL), which can be accessed by a menu-driven program. A detailed description of the database and of the menu-driven features is given in the SEDQUAL users manual (PTI 1988b). ~~Details of this project are described in Barrick et al. (1988). These data have been incorporated into a Puget Sound sediment quality values database, which can be accessed by a menu-driven program (SEDQUAL). A detailed description of the database and menu-driven features is given in the SEDQUAL users manual (PTI 1988b).~~ Because the chemical concentrations associated with biological effects are relatively low (relative to routine analytical detection limits), the data quality objectives for the sediment quality values database are stringent.

Prior to inclusion in the database, data are subjected to a thorough quality assurance/quality control (QA/QC) review. The guidelines and control limits by which the data are judged were developed as an offshoot of the Puget Sound Estuary Program [(PSEP) 1986]. PSEP was formed in 1985 by a variety of agencies [i.e., Puget Sound Water Quality Authority (PSWQA), EPA, Ecology] with regulatory, resource management, and resource responsibilities in response to widespread concern over the environmental health of Puget Sound. As part of this program, regional experts met to discuss the analytical techniques appropriate to the assessment of environmental data in Puget Sound. As a result, *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound* (PSEP 1986) was produced.

Prior to the publication of the *Data Validation Guidance Manual for Selected Sediment Variables*, the process for data validation had not been formally documented. However, the process was guided by PSEP (1986) control limits, EPA Contract Laboratory Program (CLP) procedures (U.S. EPA 1985, 1987, 1988), and the best professional judgment of the individuals conducting the data validation.

1.3 APPROACH

The approach to the data validation process presented in this guidance manual reflects the data quality objectives of Ecology's Sediment Management Unit, which is responsible for maintaining the sediment quality values database. These data quality objectives are relatively rigorous because the data may ultimately be used to generate data quality values which, in turn, will be used as a basis for developing and/or revising sediment cleanup standards. The data validation process that was applied to the Phase I baseline survey for Puget Sound Dredged Disposal Analysis (PSDDA) was used as a general guide for level of effort and level of review detail (PTI 1988a). Many of the examples provided in this report were taken directly from the data review that accompanied the PSDDA Phase I survey.

The approach to describing the data review process is organized by the major categories of analytes [i.e., conventional variables, metals, semivolatile organic compounds and volatile organic compounds (VOC)~~, semivolatile organic compounds and polychlorinated biphenyls (PCB)/pesticides, volatile organic compounds (VOC),~~ bioaccumulation, bioassays, and benthic infauna]. These major categories represent classes of chemical or biological variables that have similar analytical requirements~~. These major categories represent chemical groups or classes that have similar analytical requirements.~~ Only chemical and biological variables commonly used to characterize the quality of Puget Sound sediments are included in the guidance manual. The discussion of the data validation process is further focused by emphasizing the analytical techniques that best address the data quality requirements of the database or that have been recommended by the major programs that address data quality in Puget Sound [PSDDA~~, The discussion of the data validation process is further focused by emphasizing the analytical techniques that best address the data quality requirements of the database, or that have been recommended by the major programs that address data quality in Puget Sound (PSDDA,~~ PSEP, Puget Sound Ambient Monitoring Program (PSAMP)]~~, PSWQA).~~ Screening techniques, which generally have higher detection limits and less stringent precision and accuracy requirements, are not addressed in this manual~~, are generally not addressed in this manual.~~

1.4 REPORT OVERVIEW

General guidance on QA during field sampling is provided in Section 2. Section 3 summarizes general data collection and reporting requirements. Sections 4 through 10 contain specific QA/QC requirements, evaluation procedures, and recommended actions for data on conventional variables, metals, semivolatile organic compounds, VOC, bioaccumulation, bioassays, and benthic community structure. A list of acronyms and a glossary are included in Appendix A to clarify terms used throughout the manual.

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2. GENERAL GUIDANCE ON FIELD SAMPLING QUALITY ASSURANCE

In this section, general guidance on QA procedures associated with the collection of sediment and benthic community samples is provided. ~~general guidance on the QA associated with the collection of sediment and benthic community samples is provided. The QA review of sampling procedures is not generally conducted during data validation.~~ The guidance provided here is intended to provide perspective on appropriate field sampling precautions and the problems that may arise if the precautions are not observed. For example, the uncertainty in the sampling site coordinates is a concern for data that will be mapped, particularly if the site will be reoccupied (i.e., it is a permanent monitoring station). Sampling strategy may also become an issue if the validation process indicates that chronic field contamination may have occurred during a particular field survey (e.g., contamination of a VOC used during equipment cleaning), or some other sampling precaution was not observed (e.g., elevated levels of VOC were expected based on the results of previous studies, but chronic losses seem to have occurred in the field study under review).

2.1 POSITIONING

Accurate navigation is essential to ensuring stations can be plotted and reoccupied with a high degree of certainty. Although several navigation or position fixing systems are currently available, factors such as price and accuracy vary considerably among them. The position fixing system selected for a given survey should be able to meet all study design requirements for accuracy and should, at a minimum, provide a high degree of precision (i.e., repeatable measurements). Positioning systems that are precise but lack a high degree of accuracy may be used after actual station locations are determined by accurate, independent means (i.e., "ground-truthed"). For bottom-related samples, all positioning systems should be used in conjunction with a fathometer to ensure sampling occurs at the proper water depth (allowing for tidal stage and any fathometer corrections). Protocols for station positioning are provided in PSEP (1986).

The ability of a positioning method to achieve its highest projected accuracy depends, in part, on site-specific conditions. A preferred method may not be usable or sufficiently accurate at all locations. For example, Loran-C cannot be used in some parts of Puget Sound, and the accuracy of visual sighting methods decreases with distance from shore. Thus, the location (or the combination of locations) of the study is a principal determinant in the usefulness of a specified positioning method.

Weather, currents, and other physical factors may also reduce the achievable accuracy of a positioning method. For example, the relative drift of the sampling equipment away from the boat under strong currents or winds can increase with depth. Resulting positioning errors in sample location (as opposed to boat location) may exceed acceptable limits for the study if effects of site location on positioning accuracy are not considered during design of the sampling program.

Different levels of accuracy are required for different sampling. Water column sampling generally does not require a precisely known station location because the water column is relatively homogeneous compared with sediments. Trawling transects do not require high positioning accuracies because the sampled area is large and because the precise location of the net at any specified moment is uncertain. Accuracy is much more important for sampling conducted with equipment that penetrates or rests on the bottom (e.g., cores, grabs). Heavier equipment will usually reduce wire angles and the area in which the sampler was probably located. Sampling of point sources generally requires both high absolute accuracy for exact location of sources and high relative accuracy for proper definition of the spatial distribution of sediment pollutant concentrations.

The chemical or statistical analyses to which the collected samples are subjected should also be considered in determining the required navigational accuracy. If a gradient of environmental effects is suspected, but the analytical technique cannot measure small differences in the value of a specified variable, sampling stations can be located farther apart and a relatively less accurate positioning method can be used. However, within-station variability may be more difficult to discern using a less accurate positioning method. For variables with a patchy distribution, the patch size could be smaller than the area defined by the repeatable accuracy of the positioning method, resulting in replicates sampled across community or physical boundaries. These conditions may not be noticed in the field and could prevent correct interpretation of the data. Statistical comparisons with replicate samples (e.g., "synoptic" data, field replicates, time-series samples) from heterogeneous stations deserve special attention. The effects of navigational positioning accuracy and the associated probable sampling area (i.e., area from which samples could have been collected) on statistical comparisons of data should be considered in the study design.

Generally, it is sufficient to calculate probable sampling areas at three levels of accuracy: ±2, 20, and 100 meters to determine the accuracy required for the survey. Both absolute and repeatable accuracies of positioning methods can be divided into these groupings. Each positioning method will provide accuracies that could fall anywhere within a certain range depending on site-specific conditions. The ±2, 20, and 100-meter accuracy levels are generally representative of the highest accuracies achievable under ideal conditions within the ranges of the various positioning methods. Candidate positioning methods can be evaluated by accuracy limitations to identify the most appropriate method. However, state agency or contractually required accuracies should never be exceeded. Having established accuracy requirements and survey area characteristics, the planner can then proceed with a detailed review of available systems.

Once a positioning method that is adequate for the specific sampling objective has been selected, the proper setup, calibration, and operational procedures must be followed to achieve projected accuracies. If the appropriate equipment is already on board the vessel or the positioning task is hired out, the responsible party of the cruise should be sure at least one member of the field crew is familiar with the positioning method. If the scientific team is supplying the equipment, appropriate training or experienced personnel should be provided to ensure proper equipment operation and documentation of positioning data. A backup method should be available on short notice to avoid loss of ship time if the primary method fails. To ensure station locations are accurately occupied regardless of method and adequate documentation is available for other parties, recordkeeping requirements should be established, as described in Section 2.3.

2.2 SEDIMENT SAMPLE COLLECTION AND STORAGE

In Puget Sound, the most common sampling device for subtidal surficial sediments is the modified van Veen bottom grab. However, several coring devices are also used. The primary criterion for an adequate sampler is that it consistently collect undisturbed samples to the required depth below the sediment surface without contaminating the samples. An additional criterion is that the sampler can be handled properly on board the survey vessel. An otherwise acceptable sampler may yield inadequate sediment samples if it is too large, heavy, or awkward to be handled properly.

Collection of undisturbed sediment requires that the sampler:

- Create a minimal bow wake when descending
- Form a leakproof seal when the sediment sample is taken
- Prevent winnowing and excessive sample disturbance when ascending
- Allow easy access to the sample surface.

Most modified van Veen grabs have open upper faces that are fitted with rubber flaps. Upon descent, the flaps are forced open to minimize the bow wake, whereas upon ascent the flaps are

forced closed to prevent sample winnowing. Some box corers have solid flaps that are clipped open upon descent and snap shut after the corer is triggered. Although most samplers seal adequately when purchased, the wear and tear of repeated field use eventually reduces this sealing ability. A sampler should therefore be monitored constantly for sample leakage. If unacceptable leakage occurs, the sampler should be repaired or replaced. If a sampler is borrowed or leased for a project, its sealing ability should be confirmed prior to sampling. Also, it is prudent to have a backup sampler on board the survey vessel in case the primary sampler begins leaking during a cruise.

For characterizing surficial sediments in Puget Sound, the upper 2 cm of the sediment column should be evaluated. When collecting the upper 2 cm of sediment, a minimum penetration depth of 4-5 cm should be achieved for each acceptable sample.

Although the 2-cm specification is arbitrary, it will ensure that relatively recent sediments are sampled, adequate volumes of sediments can be obtained readily for laboratory analyses, and data from different studies can be compared. Sampling depths other than 2 cm may be appropriate for specific purposes. For example, the upper 1 cm of sediment may be required to determine the age of the most recently deposited sediments. By contrast, a sample depth much greater than 2 cm may be required to evaluate the vertical profile of sediment characteristics or to determine depth-averaged characteristics prior to dredging. If a sampling depth other than 2 cm is used, comparisons with data from 2-cm deep samples may be questionable.

After the sampler is secured on deck, the sediment sample should be inspected carefully before being accepted. The following acceptability criteria should be satisfied:

- The sampler is not over-filled with sample so that the sediment surface is pressed against the top of the sampler
- Overlying water is present (indicates minimal leakage)
- Overlying water is not excessively turbid (indicates minimal sample disturbance)
- Sediment surface is relatively flat (indicates minimal disturbance or winnowing)
- Desired penetration depth is achieved (i.e., 4-5 cm for a 2-cm deep surficial sample).

If a sample does not meet all criteria, it should be rejected.

Before subsamples of the surficial sediments are taken, the overlying water must be removed. The preferred method of removing this water is by slowly siphoning it off near one side of the sampler. Methods such as decanting the water or slightly cracking the grab to let the water run out are not recommended, because these methods may result in unacceptable disturbance or loss of fine-grained surficial sediment and organic matter.

Once the overlying water has been removed, the surficial sediment can be subsampled. It is recommended that subsamples be taken using a flat scoop shaped like a coal shovel. The shoulders of the scoop should be 2 cm high. This device will allow a relatively large subsample to be taken accurately to a depth of 2 cm. Coring devices are not recommended because generally they collect small amounts of surficial sediment, and therefore require repeated extractions to obtain a sufficient volume of material for analysis of conventional sediment variables. A curved scoop is not recommended because it does not sample a uniform depth. Because accurate and consistent subsampling requires practice, it is advisable that an experienced person perform this task.

If samples are to be analyzed for trace metals or priority pollutant organic compounds, sample contamination during collection must be avoided. All sampling equipment (i.e., siphon hoses, scoops, containers) should be made of noncontaminating material and should be cleaned appropriately before use. Samples should not be touched with ungloved fingers. In addition, potential airborne contamination (e.g., stack gases, cigarette smoke) should be avoided. More detailed guidance for preventing sample contamination is provided in the QA guidance for metals and organic compounds in other sections of this report and in PSEP (1986).

Samples should be placed in precleaned containers, stored on ice, and later transferred to refrigerators or freezers (see Table 2-1 for preservation conditions for each type of sample). Guidelines for maximum holding times are also listed in Table 2-1.

2.3 SAMPLE HANDLING AND DOCUMENT CONTROL

After sample collection, proper sample handling minimizes changes in the constituents of interest and potential errors when shipping and analyzing samples. All stages of sample handling should be documented adequately. Consistency and thoroughness are best maintained by following detailed, written standard operating procedures (SOPs). Documentation ensures all sample handling requirements were performed and provides proof that handling was conducted properly if questions arise later.

It is important throughout any sampling and analysis program to maintain integrity of the sample from the time of collection to the point of data reporting. Proper chain-of-custody procedures allow the possession and handling of samples to be traced from collection to final disposition. The following documents are needed to maintain proper chain-of-custody:

- Field logbook—All pertinent information on field activities and sampling efforts should be recorded in a bound logbook. The field supervisor should be responsible for ensuring that sufficient detail is recorded in the logbook. The logbook should enable someone else to completely reconstruct the field activity without relying on the memory of the field crew. All entries should be made in indelible ink, with each page signed and dated by the author, and a line drawn through the remainder of any page. All corrections should consist of permanent line-out deletions that are initialed. At a minimum, entries in a logbook should include:

  - Date and time of starting work
  - Names of field supervisor and team members
  - Purpose of proposed sampling effort
  - Description of sampling site, including information on any photographs that may be taken
  - Location of sampling site
  - Details of actual sampling effort, particularly deviations from standard operating procedures
  - Field observations
  - Field measurements performed (e.g., pH, temperature, flow)
  - Field laboratory analytical results
  - Sample identification
  - Type and number of sample bottles collected
  - Sample handling, packaging, labeling, and shipping information (including destination).

  Chain-of-custody procedures should be maintained with the field logbook. While being used in the field, the logbook should remain with the field team at all times. Upon completion of the sampling effort, the logbook should be kept in a secure area.

- Sample labels—Sample labels must be waterproof and must be securely fastened to the outside or placed inside each sample container (depending on the kind of sample) to prevent misidentification of samples. Labels must contain at least the sample number, preservation technique, date and time of collection, location of collection,

TABLE 2-1. RECOMMENDED SAMPLE SIZES, CONTAINERS, PRESERVATION TECHNIQUES, AND HOLDING TIMES FOR SEDIMENT CONVENTIONAL VARIABLES

Variable	Minimum Sample Size (grams)[c]	Container[b]	Preservation	Maximum Holding Time
Particle size	100-150[c]	P,G	Cool, 4° C	6 months[d]
Total solids	50	P,G	Freeze	6 months[d]
Total volatile solids	50	P,G	Freeze	6 months[d]
Total organic carbon	25	P,G	Freeze	6 months[d]
~~Oil and grease~~	~~100~~	~~G only~~	~~Cool, 4° C, HCl;~~ ~~Freeze~~	~~28 days[d]~~ ~~6 months[d]~~
Total sulfides	50	P,G	Cool, 4° C, 1N zinc acetate	7 days[d]
~~Total nitrogen~~	~~25~~	~~P,G~~	~~Freeze~~	~~6 months[d]~~
~~Biochemical oxygen demand~~	~~50~~	~~P,G~~	~~Cool, 4° C~~	~~7 days[e]~~
~~Chemical oxygen demand~~	~~50~~	~~P,G~~	~~Cool, 4° C~~	~~7 days[e]~~
Ammonia	20	P,G	Cool, 4° C (minimize air contact, keep field moist)	7 days[e]

Adapted from Tetra Tech (1986).

[a] Recommended field sample sizes for one laboratory analysis. If additional laboratory analyses are required (e.g., replicates), the field sample size should be adjusted accordingly.

[b] P = polyethylene; G = glass.

[c] ~~Sandier sediments require larger sample sizes than do muddier sediments.~~ Sandy sediments require larger sample sizes than do muddy sediments.

[d] This is a suggested holding time. No EPA criteria exist for the preservation of this variable.

[e] This holding time is recommended by Plumb (1981).

and signature of the collector. Labels should be marked with indelible ink. Abbreviated labels may also be placed on the cap of each jar to facilitate sample identification.

- Chain-of-custody records—A chain-of-custody record must accompany every sample. Each person who has custody of the sample must sign the form and ensure that the samples are not left unattended unless secured properly.

- Custody seals—Custody seals are used to detect unauthorized tampering with the samples. Sampling personnel should attach seals to all shipping containers sent to the laboratory by common carrier. Gummed paper seals or custody tape should be used so the seal must be broken when opening the sample container.

For further information regarding proper chain-of-custody procedures, consult the policies and procedures manual for the National Enforcement Investigations Center (U.S. EPA 1978).

2.4 REFERENCES

Plumb, R.H. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. U.S. Army Corps of Engineers, Vicksburg, MS.

PSEP. 1986. Recommended protocols for measuring selected environmental variables in Puget Sound. Final Report. Prepared for Puget Sound Estuary Program. Tetra Tech, Inc., Bellevue, WA.

U.S. EPA. 1978 (revised 1983). NEIC policies and procedures. EPA-330/9-78-001. National Enforcement Investigations Center, Denver, CO.

3. GENERAL DATA REPORTING REQUIREMENTS

Considerable documentation must be obtained from laboratories for a complete data review. The following lists, excerpted from PSEP protocols, specify documentation that should be included in laboratory data packages for chemical analysis. The items listed below include some, but not all, of the standard documentation required by EPA/CLP. The documentation below is required for independent QA review of the data and should always be specified in the original statement of work (SOW).

3.1 CONVENTIONAL VARIABLES

3.1.1 Particle Size, Total Solids, and Total Volatile Solids

The weight of each sediment fraction should be reported to the nearest 0.0001 gram dry weight. The laboratory should report the results of all samples analyzed (including QA replicates) and should note any problems that may have influenced data quality.

Total solids (TS) should be reported as a percentage of the wet weight of the sample to the nearest 0.1 unit. The laboratory should report the results of all samples analyzed (including QA replicates) and should note any problems that may have influenced sample quality.

Total volatile solids (TVS) should be reported as a percentage of the dry weight of the uncombusted sample to the nearest 0.1 unit. The laboratory should report the results of all samples analyzed (including QA replicates) and should note any problems that may have influenced data quality.

3.1.2 Total Organic Carbon

Total organic carbon (TOC) should be reported as a percentage of the dry weight of the unacidified sample to the nearest 0.1 unit. The laboratory should report the results of all samples (including QA replicates, method blanks, and standard reference measurements) and should note any problems that may have influenced sample quality. The laboratory should also provide a summary of the calibration procedure and results (e.g., range covered, regression equation, coefficient of determination).

3.1.3 Total Sulfides

Total sulfides should be reported as mg/kg of sediment dry weight to the nearest 0.1 unit. The laboratory should report the results of all samples (including QA replicates) and should note any problems that may have influenced sample quality. The laboratory should also describe the calibration curve used to determine total sulfide concentrations.

3.2 METALS

For metals, the data report package for analyses of each sample should include the following:

- Tabulated results in units as specified for each matrix in the analytical protocols, validated and signed in original by the laboratory manager

- Any data qualifications and explanation for any variance from the analytical protocols
- Results for all of the QA/QC checks initiated by the laboratory
- Tabulation of instrument detection limits (IDL) and method detection limits (MDL).

All contract laboratories are required to submit metals results that are supported by sufficient backup data and QA results to enable independent QA reviewers to conclusively determine the quality of the data. The laboratories should be able to supply legible photocopies of original data sheets with sufficient information to unequivocally identify:

- Calibration results
- Calibration and preparation blanks
- Samples and dilutions
- Duplicates and spikes
- Any anomalies in instrument performance or unusual instrumental adjustments.

3.3 ORGANIC COMPOUNDS

The following documentation is needed for organic compounds:

- A cover letter referencing or describing the procedure used and discussing any analytical problems
- Reconstructed ion chromatograms for gas chromatography/mass spectrometry (GC/MS) analyses for each sample
- Mass spectra of detected target compounds (GC/MS) for each sample and associated library spectra
- Gas chromatography/electron capture detection (GC/ECD) and/or gas chromatography/flame ionization detection (GD/FID) chromatograms for each sample
- Raw data quantification reports for each sample
- A calibration data summary reporting calibration range used [and decafluorotriphenylphosphine (DFTPP) and bromofluorobenzene (BFB) spectra and quantification report for GC/MS analyses]
- Final dilution volumes, sample size, wet-to-dry ratios, and instrument detection limit (IDL)
- Analyte concentrations with reporting units identified (to two significant figures unless otherwise justified)
- Quantification of all analytes in method blanks (ng/sample)
- Method blanks associated with each sample
- Recovery assessments and a replicate sample summary (laboratories should report all surrogate spike recovery data for each sample; a statement of the range of recoveries should be included in reports using these data)
- Data qualification codes and their definitions.

3.4 BIOASSAYS

3.4.1 Amphipod Mortality Test

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements during testing [e.g., dissolved oxygen (DO), temperature, salinity, pH]
- Daily emergence for each beaker and the 10-day mean and standard deviation (SD) for each treatment
- 10-day survival (no. survivors) in each beaker and the mean and SD for each treatment
- Salinity of interstitial water in each sediment sample
- 96-hour LC_{50} values and 95 percent confidence intervals for reference toxicants
- ~~10-day survival in each beaker and the mean and SD for each treatment~~
- ~~Interstitial salinity values of test sediments~~
- ~~96-hour LC50 values with reference toxicants~~
- Any problems that may have influenced data quality.

3.4.2 Juvenile Infauna ~~Bivalve~~ Mortality Test

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements during testing (e.g., DO, temperature, salinity, pH)
- 10-day survival (no. survivors) in each beaker and the mean and SD for each treatment
- 96-hour LC_{50} values and 95 percent confidence intervals for reference toxicants
- ~~10-day survival in each beaker and the mean and SD for each treatment~~
- ~~96-hour LC50 values with reference toxicants~~
- Any problems that may have influenced data quality.

3.4.3 Juvenile *Neanthes* Growth~~/Mortality~~ Test

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements during testing (e.g., DO, temperature, salinity, pH)
- 20-day biomass (mg dry weight) in each beaker and the mean and SD for each treatment
- 20-day survival (no. survivors) in each beaker and the mean and SD for each treatment
- 96-hour LC_{50} and EC_{50} values and 95 percent confidence intervals for reference toxicants
- ~~10-day survival in each beaker and the mean and SD for each treatment~~

- ~~96-hour LC_{50} values with reference toxicants~~
- Any problems that may have influenced data quality.

3.4.4 Bivalve Larvae Abnormality Test (Whole Sediment)

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements at the beginning and end of testing (e.g., DO, temperature, salinity, pH)
- Individual replicate and mean and SD data for larval survival after 48 hours
- Individual replicate and mean and SD data for larval abnormalities after 48 hours
- 48-hour LC_{50} and EC_{50} values and 95 percent confidence intervals for reference toxicants
- ~~48-hour LC_{50} and EC_{50} with reference toxicants~~
- Any problems that may have influenced data quality.

3.4.5 Bivalve Larvae Abnormality Test (Suspended Phase)~~(Elutriate)~~

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements at the beginning and end of testing (e.g., DO, temperature, salinity, pH)
- Individual replicate and mean and SD data for larval survival after 48 hours
- Individual replicate and mean and SD data for larval abnormalities after 48 hours
- 48-hour LC_{50} and EC_{50} values and 95 percent confidence intervals for reference toxicants
- ~~48-hour LC_{50} and EC_{50} values with reference toxicants~~
- Any problems that may have influenced data quality.

3.4.6 Echinoderm Embryo Abnormality Test

The following data should be reported by all laboratories performing this bioassay:

- Water quality measurements at the beginning and end of testing (e.g., DO, temperature, salinity, pH)
- Individual replicate and mean and SD data for embryo survival after 48 hours
- Individual replicate and mean and SD data for embryo abnormalities after 48 hours
- 48-hour LC_{50} and EC_{50} values and 95 percent confidence intervals for reference toxicants
- ~~48-hour LC_{50} and EC_{50} values with reference toxicants~~
- Any problems that may have influenced data quality.

3.4.7 Microtox™ Test (Saline Extract)

The following data should be reported by all laboratories performing this bioassay:

- Range-finding assay results, if available
- Percent decrease in luminescence for each concentration of supernatant (e.g., saline sediment extract) tested, including blanks
- Determination of a significant dose-response relationship by least-squares regression of percent decrease in luminescence on the logarithm of sample dilution
- Determination of EC_{50} values and 95 percent confidence limits for the reference toxicant
- Any problems that may have influenced data quality.

3.4.8 Microtox™ Test (Organic Extract)

The following data should be reported by all laboratories performing this bioassay:

- Range-finding assay results
- Raw light emission data for each test series
- 15-minute EC_{50} data and 95 percent confidence intervals for each test series and for controls
- Any problems that may have influenced data quality.

3.5 BENTHIC INFAUNA

A sample sorting efficiency of 95 percent of total number of individuals generally is considered acceptable. That is, no more than 5 percent of the organisms in a given sample are missed by the sorter. Similarly, species identifications by each taxonomist can be expected to be accurate for at least 95 percent of the total number of species. Unless otherwise specified, all organisms should be identified to the lowest possible taxon and to species level whenever possible. In cases where the identity of a species is uncertain, a species number will suffice (e.g., *Macoma* sp. 1, *Macoma* sp. 2). Numerical designations must be consistent throughout each study. To facilitate comparability among different studies, the distinguishing characteristics of each unidentified species should be recorded. Data for each replicate sample should be reported as number of individuals per sample for each species and as biomass (nearest 0.1 gram wet weight per sample) for each major taxonomic group.

4. QUALITY ASSURANCE FOR CONVENTIONAL VARIABLES IN SEDIMENT

4.1 INTRODUCTION

Guidelines for evaluating the following conventional sediment variables are provided in this section:

- Particle size
- Total solids
- Total volatile solids
- Total organic carbon
- Total sulfide
- Ammonia.

The QA guidelines for evaluating these analyses were developed to standardize Puget Sound data for comparability among studies. Laboratory procedures for conventional variables are described in *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound* (PSEP 1986).

QA reporting requirements for conventional analyses under PSEP protocols (PSEP 1986) should include the following:

- Sample analytical results
- QA replicates
- Summary of problems influencing data quality.

Additional reporting requirements for TOC include method blanks, certified reference material (CRM) results, ~~standard reference material (SRM) results,~~ and a description of calibration procedures (e.g., range covered, regression equation, coefficient of determination). For total sulfides and ammonia, method blanks and calibration descriptions should be included in the reporting package.

QA measures for sediment conventional variables are less extensive than those for specific chemical and biological variables. PSEP protocols specify frequencies of QA analyses but do not specify control limits. Because no control limits are described, the data reviewer should apply best professional judgment when assessing the data. Reasonably achievable control limits are provided in Table 4-1 as an aid in the review process.

Triplicate analyses at a frequency of 5 percent are required for all sediment conventional variables. The failure of the laboratory to perform this QA requirement could indicate problems in the analytical system. Data usability is highly questionable if no replicates are performed.

Laboratory QA requirements and data validation and assessment for conventional analyses are discussed in the following three sections: 1) Particle Size, Total Solids, and Total Volatile Solids; 2) Total Organic Carbon; 3) Total Sulfide and Ammonia. In each section, the definition and general use of each type of variable are described, followed by the data validation process (i.e., requirements, evaluation procedure, action).

TABLE 4-1. RECOMMENDED FREQUENCIES AND CONTROL LIMITS FOR CONVENTIONAL QA SAMPLES

Analysis Type	Parameter[a]	Frequency of Analysis[b]	Control Limit[c]
Method blanks	TOC[d]	5% or one per batch, whichever is more frequent	<1/10 sample concentration
	Ammonia	~~One per batch~~5% or one per batch, whichever is more frequent	<1/10 sample concentration
	Total sulfide	~~One per batch~~5% or one per batch, whichever is more frequent	<1/10 sample concentration
Analytical ~~replicates~~ triplicates	All parameters	5% or one per batch, whichever is more frequent	±20% ~~RPD or~~ CV
~~Standard~~ Certified reference materials	TOC	1 per major survey, if available	Within 95% confidence limit
Matrix spikes	None required[e]	NA[f]	NA

[a] Only applicable parameters are listed.

[b] Frequencies listed are minimums; some programs may require higher levels of effort.

[c] Not a PSEP protocol control limit. Control limits are recommendations to aid in data review.

[d] TOC = Total organic carbon.

[e] Matrix spike may be analyzed for TOC, total sulfide, and ammonia.

[f] NA = Not applicable.

4.2 PARTICLE SIZE, TOTAL SOLIDS, AND TOTAL VOLATILE SOLIDS

In this section, guidance is provided for the evaluation of particle size data, TS data, and TVS data. Particle size is used to characterize the physical characteristics of sediments. Because particle size influences both chemical and biological variables, it can be used to normalize chemical concentrations according to sediment characteristics and to account for some of the variability found in biological assemblages.

TS are the organic and inorganic material remaining after a sample has been completely dried. This variable is commonly used to convert sediment concentrations of substances from a wet-weight to a dry-weight basis. It is typically measured in conjunction with other variables. TVS represents the fraction of TS that are lost on ignition at a higher temperature than is used to determine TS. TVS is used as a crude estimate of the amount of organic matter in TS. Both TS and TVS are operationally defined by the temperature of drying or ignition.

4.2.1 Requirements

The following laboratory QA checks are required for particle size, TS, and TVS analyses:

- Analyze one sample in triplicate for every 20 samples, or one per batch, whichever is more frequent.

4.2.2 Evaluation Procedure

During data review, the reviewer should perform the following:

- Verify that triplicate analyses were performed at the proper frequency.
- Spot check sample calculations for particle size and TVS at a frequency of 10 percent. If any calculation errors are found, all samples must be recalculated. TS calculations should be 100 percent validated because calculation errors in TS can affect other variables (i.e., chemical concentrations).

4.2.3 Action

The assessment of precision for all three parameters is performed by calculating the SD and percent coefficient of variation (CV) for the replicate analyses. The calculations are as follows:

$$SD = \sum_{i=1}^{n} \sqrt{\frac{(C_i - \overline{C})_2}{(n-1)}}$$

where:

$\overline{C}$ = The mean of the replicate measurements, C_i ~~Ci~~

n = Number of replicate measurements

$$\text{Percent CV} = 100 \times \frac{SD}{C}$$

There are no control limits specified in PSEP protocols, but the CV should be less than 20 percent. Best professional judgment should be used if only a few of the particle size fractions or the solids determinations are outside this limit. Sample heterogeneity may be the problem and not laboratory technique. The laboratory data summary should discuss problems associated with the samples and their analyses.

Overall sample recovery for particle size is assessed by comparing the sum of the fraction weights with the calculated dry weight of the initial sample aliquot. PSEP protocol recommends losses assessed by this method be less than 5 percent (or >95 percent recovery). If samples have recoveries less than 95 percent but greater than 90 percent, these low recoveries would probably not significantly change the size fractions. For recoveries less than 90 percent, the data need to be reviewed as to the possible effect the low recoveries might have on the size fractions reported.

Figure 4-1 demonstrates size fraction calculations, CV calculations, and comparison of the fraction weights to the calculated dry weight.

TS calculation errors affect all sample values calculated on a dry-weight basis. After correcting the TS result, further corrections are necessary for affected data (e.g., each metal analyte). Figure 4-2 demonstrates TS and TVS calculations.

4.3 TOTAL ORGANIC CARBON

In this section, guidance is provided for the evaluation of TOC data. TOC is a measure of the total amount of nonvolatile, partially volatile, volatile, and particulate organic material in a sample. TOC is independent of the oxidation of organic carbon. Because inorganic carbon (e.g., carbonates, bicarbonates, free carbon dioxide) will interfere with TOC determinations, samples are treated to remove inorganic carbon before analysis.

4.3.1 Requirements

The following laboratory QA checks are required for TOC analyses:

- The laboratory should provide a summary of the calibration procedure and results
- One method blank must be analyzed for every 20 samples, or one per batch, whichever is more frequent
- The laboratory should analyze one sample in triplicate for every 20 samples, or one per batch, whichever is more frequent
- A CRM ~~SRM~~ should be analyzed at least once for each major survey.

4.3.2 Evaluation Procedure

During data review, the reviewer should perform the following:

- Verify that instrument calibration procedures were properly followed
- Verify that triplicate analyses, method blanks, and CRM ~~SRM~~ were performed at the proper frequency
- Spot check sample calculations at a frequency of 10 percent.

4.3.3 Action

No control limits are specified for TOC calibration under PSEP. The data reviewer should review the raw data sheets to check the frequency of calibration verification checks. Potassium acid phthalate is commonly used to verify instrument calibration, but other standards may also be used.

Sample Name
Lab Code: 444-63

Sample Preparation
(g) air-dried sample: 108.74
(g) retained on #10: 15.320
(g) passing #10: 93.420

Hydrometer Analysis
(g) air-dried sample: 83.212
(g) oven dry sample: 82.213
% passing #10: 85.91
Mass (W) sample: 95.70

Hygroscopic Moisture
(g) air-dried sample + tare: 10.294
(g) tare: 0.916
(g) oven-dried sample: 10.186
Hygroscopic Moisture Factor: 0.988

Start Time	Minutes	Hyd. Reading	Comp. Corr. Factor	H	°C	L	K	% Remaining = H/W ×100	Diameter D = K√L/T
	0								
	2	37	6	31	19	11.2	.0139	~~11.7~~ 32.3	0.0329
	5	28	6	26	19	12		~~12.5~~ 27.2	0.0219
	15	23	6	16	19	13.7		~~14.3~~ 16.7	0.0133
	30	20	6	14	19	14.0		14.6	0.0095
	60	17	6	11	19	14.5		~~15.1~~ 11.4	0.0068
	250	13	7	6	21	15.3		~~16.0~~ 6.3	0.0034
	1440	9	7	2	20	16.0		~~16.7~~ 2.1	0.0014

Sieve Analysis, Coarse Fraction

(g) on		% passing
#10		
3/4	0	
3/8	5.922	94.5
#4	3.706	91.1
#10	5.622	
pan		

Sieve Analysis, #200 Fraction
Mass retained on #10 (g): _____
Mass passing #200: 42.57

(g) on		(g) Passing	% Passing
#10	15.320	80.380	84.0
#20	4.804	75.576	79.0
#40	5.350	70.226	73.4
#60	4.867	65.359	68.30
#140	14.295	51.064	53.4
#200	8.495	42.57	44.4
-#200	0.644		
g total (Σ #10 thru #200)	53.13		

Figure 4-1. Example of laboratory raw data sheet for particle size analysis

SAMPLE NUMBER(S)	SERVICE REQUEST NUMBER 88453
ANAYLSIS FOR: TOTAL SOLIDS	METHOD NUMBER PSEP

DATA

PAN #	SAMPLE	WET WEIGHT (g)	TARE (g)	TARE + DRY WT. (g)	DRY WEIGHT (g)	% TOTAL SOLIDS
30	453-12	25.790	49.378	59.488	10.110	39.2
135	453-13	25.062	49.417	59.067	9.650	38.6
18	453-14	25.826	55.053	65.344	10.291	39.8
3L	453-15	25.272	69.729	78.464	8.735	34.6
17L	453-16	27.131	64.050	76.418	12.368	45.6
14L	453-17	27.486	67.405	77.080	9.675	35.2
6L	453-17	25.065	68.209	77.340	9.131	36.4
8L	453-17	25.757	68.054	77.337	9.283	36.0

Avg 35.8 CY

$$\% \text{ Solids} = \frac{(A-B)(100)}{C} \text{ or } \frac{\text{dry wt.}(100)}{\text{wet wt.}}$$

Sample no: 453-12

$$\% \text{ solids} = \frac{59.488 - 49.378(100)}{25.790}$$

$$= 39.2\ \%$$

COMMENTS:

ANALYST	DATE: 5/31/88	NB Number
WITNESS	DATE: 7/12	PAGE NUMBER

Computer File Name is: totsldwr.snt

Figure 4-2a. Example of laboratory raw data sheet and calculations for total solids (Figure 4-2a) and total volatile solids (Figure 4-2b)

SAMPLE NUMBER(S)	SERVICE REQUEST NUMBER 88453
ANAYLSIS FOR: Total Volatile Solids (TVS)	METHOD NUMBER

DATA

PAN #	SAMPLE	Dry WEIGHT (g)	TARE (g)	TARE + Ash WT. (g)	Ash WEIGHT (g)	% TVS
30	453-12	10.110	49.378	58.791	9.413	6.89
135	453-13	9.650	49.417	58.390	8.973	7.02
18	453-14	10.291	55.053	~~77.766~~ 64.654	9.601	6.70 ✓
3"	453-15	8.735	69.729	77.766	8.037	7.99 ✓
17"	453-16	12.368	64.050	75.647	11.597	6.23
14"	453-17	9.675	67.405	76.299	8.894	8.07 ✓
6"	453-17	9.131	68.209	76.609	8.400	8.01
8"	453-17	9.283	68.054	76.575	8.521	8.21 ✓
						($\bar{x}$ = 8.09

$$\% \text{ TVS} = \frac{A-B(100)}{A} \text{ or } \frac{\text{dry wt.} - \text{ash wt.}(100)}{\text{dry wt.}}$$

Sample no: 453-12

$$\% \text{TVS} = \frac{10.110 - 9.413(100)}{10.110}$$

$$\% \text{TVS} = 6.89\ \%$$

COMMENTS:

ANALYST	DATE: 5/31/88	NB Number
WITNESS MW	DATE: 7/10/88	PAGE NUMBER

Computer File Name is: totsldwr.sht

Figure 4-2b. Example of laboratory raw data sheet and calculations for total solids (Figure 4-2a) and total volatile solids (Figure 4-2b)

A calibration verification standard should be analyzed at the beginning of each analytical run. Most instruments are precalibrated, and the calibration verification standard is necessary to verify that the instrument is functioning properly and is able to produce accurate data. The percent recovery should be between 90-110 percent, which is routinely achievable in most laboratories. Calibration verification standards with percent recoveries outside this range could indicate sample values are over- or under-estimates, depending on the direction of the discrepancy. If the standard has a recovery of less than 80 percent or greater than 120 percent, the associated data should be considered estimates and assigned an E qualifier.

Method blanks are to be analyzed at a frequency of 5 percent. The blanks used for sediment analyses are usually 1 to 2 orders of magnitude lower than the sample concentrations. If the blank is less than 1/10 the ~~sample~~ concentration of the lowest concentration sample, no further action is necessary. If the blank is greater than 1/10, the reviewer should blank-correct the data for all samples with concentrations ≤10 times the blank concentration, if not already done by the laboratory, and assign corrected results a B qualifier if the data have been blank-corrected down to the detection limit or a Z qualifier if the blank-corrected value exceeds the detection limit.

The method precision is measured by triplicate analyses. The SD and CV are calculated as previously described. The CV should be less than 20 percent. If the CV for the replicate analysis is greater than 20 percent, the samples should be considered estimates and assigned an E qualifier.

Sediment CRM are ~~SRM is~~ not commonly available for TOC. If one is analyzed, CRM ~~SRM~~ recovery should be within 80-120 percent. An example of a laboratory raw data sheet is shown in Figure 4-3 to illustrate blank analyses, calibration standards, and triplicate analyses.

4.4 TOTAL SULFIDE AND AMMONIA

In this section, guidance is provided for the evaluation of total sulfide and ammonia data. Total sulfide represents the amount of acid-soluble hydrogen sulfide (H_2S), bisulfide (HS^-), and sulfide (S^{2-}) in a sample. Sulfides are measured because they may be toxic and may create unaesthetic conditions (i.e., rotten odor). Special precautions must be taken during sulfide sampling to minimize losses due to volatilization of H_2S or oxidation by DO.

Ammonia is the most reduced form of inorganic nitrogen found in sediments. It is an essential nutrient that is produced during organic matter degradation. Elevated levels of ammonia commonly co-occur with dissolved or total sulfide.

4.4.1 Requirements

The following QA checks are required under PSEP protocols for total sulfide and ammonia analyses:

- The samples must be analyzed within specified holding times
- The laboratory should provide a summary of calibration procedures
- One triplicate analysis should be analyzed for every 20 samples, or one per batch, whichever is more frequent.

ANALYST

WITNESS

DATE: 6-14-88

NB Number

DATE:

PAGE NUMBER

Computer File Name is: blkwrk.sht

509-1 → 10

Work Order: 495-7 → 14

Material: Sediment

Analysis For: Total Organic Carbon, TOC

Method: Combustion/Coulometric
EPA 415 1 Modified

DATA

	blank	Standard				triplicates	
Sample Number	Blank 1	~~EPA-QC~~	495-7	495-8	495-9	495-10	495-10D
~~mLS~~ mg Sample Injected		.006	.0170	.0166	.0257	.0147	.0174
Sample Reading, ug C	5.6	2795.2	510.1	421.3	526.9	298.3	349.9
Blank Reading, ug C	5.6	5.6	5.6	5.6	5.6	5.6	5.6
Net ug C	0.0	2789.6	504.5	415.7	521.3	292.7	344.3
TOC, mg/~~L~~kg		464,933.3	29,676.5	25,042.2	20,284	19,911.6	19,787.4
% Carbon		46.5%	2.96%	2.50%	2.03%	1.99%	1.98%

Ave TOC ug/L = 19754.4
blank carbon = 1.98%

Sample Number	495-10DD	495-11	495-12	495-13	495-14	Blank 2	509-1
~~mLS~~ mg Sample Injected	.0234	.0230	.0298	.0270	.0239		.0543
Sample Reading, ug C	463.4	475.8	614.2	589.3	540.1	5.7	372.4
Blank Reading, ug C	5.7	5.6	5.6	5.6	5.6	5.7	5.7
Net ug C	457.7	470.2	608.6	583.7	434.5	0.0	366.7
TOC, mg/~~L~~kg	19,559.8	20,443.5	20,422.8	21,618.5	22,364.4		6753.2
% Carbon	1.96%	2.04%	2.04%	2.16%	2.24%		.67%

Sample Number	509-2	509-3	509-4	509-5	509-5D	509-5DD	509-6
~~mLS~~ mg Sample Injected	.0391	.0260	.0233	.0256	.0204	.0277	.0252
Sample Reading, ug C	563.1	392.9	407.9	399.8	322.7	419.3	415.3
Blank Reading, ug C	5.7	5.7	5.7	5.7	5.7	5.7	5.7
Net ug C	557.4	387.2	403.2	394.1	317.0	413.6	409.6
TOC, mg/~~L~~kg	14,255.8	14,892.3	17,304.7	15,394.5	15,539.2	14,931.4	16,253.9
% Carbon	1.43%	1.49%	1.73%	1.54%	1.55%	1.49%	1.63%

Ave TOC ug/L = 15,288.4, Ave % Carbon = 1.53%

, minutes: 35 min.

COMMENTS:

Figure 4-3. Example of laboratory raw data sheet for total organic carbon

4.4.2 Evaluation Procedures

During data review, the reviewer should perform the following:

- Verify that analyses were performed within the specified holding time.
- Verify that a blank and at least three standards were used to develop the calibration curve. Fresh standards should be used for each analytical run.
- Verify that triplicate analyses were performed at the proper frequency.

4.4.3 Action

Holding time is very critical for sulfide analyses. The PSEP-recommended holding time is 7 days. Samples analyzed past the holding time should be considered estimates and assigned either an E or a G qualifier. While no holding time is described for ammonia, the EPA-recommended holding time for ammonia in preserved water samples is 28 days.

The determination of the calibration curve for total sulfide should include a reagent blank and at least three standards. The correlation coefficient for the least squares fit of the data should be equal to or greater than 0.995.

PSEP protocols do not specify a method for the analysis of ammonia in sediments. Calibration procedures will vary depending on the analytical method chosen by the laboratory. The two most common methods of determining ammonia after extraction are by colorimetric or selective ion electrode methods. In either case, to determine the calibration curve, a minimum analysis of one blank and three standards is required. Colorimetric procedures should produce a linear standard curve, with a correlation coefficient equal to or greater than 0.995. Selective ion electrode methods produce a logarithmic curve which is plotted on linear-log paper; therefore, an r-value should be reported. ~~Select ion electrode methods produce a logarithmic curve.~~

Triplicate analyses are required for total sulfide and ammonia. The SD and CV should be calculated, and the percent CV for each triplicate should be less than 20 percent. If no triplicates were analyzed or if the CV was greater than 20 percent, flag the data as estimates and assign an E qualifier. Laboratory raw data sheets for ammonia and total sulfide are shown in Figure 4-4 to demonstrate calibration information the laboratory should provide.

4.5 REFERENCES

PSEP. 1986. Recommended protocols for measuring selected environmental variables in Puget Sound. Final Report. Prepared for Puget Sound Estuary Program. Tetra Tech, Inc., Bellevue, WA.

SAMPLE NUMBER(S): 88453

SERVICE REQUEST NUMBER: 88432

ANALYSIS FOR: Ammonia Nitrogen in Water Orion 901

METHOD NUMBER: EPA 350.3, Known Additions, Selective Ion Electrode

DATA

Sample Number	BLK	Wp987	432-16	432-16	
Initial mV Reading		-87	-92	-86	
Initial Spike Level, mg/L					
KA Setting on 901	1	1	1	1	
Known Addition Spike, mL	0.5	0.5	0.5	0.5	
Known Addition Spike, mg/L	10	100	100	100	
Sample Aliquot, mL	50	50	40.685g → 250mL	40.685g → 250mL	
Final Volume, mL	50	50	50	50	
mg/L in Sample (less initial spike)	.030	2.06	2.92 / 17.94 mg/kg	2.77 / 17.0 mg/kg	$\bar{x}$ = 17.5

Sample Number	432-26 Triplicate		453-8	453-8	
Initial mV Reading	-49		-76.7	-74.3	
Initial Spike Level, mg/L					
KA Setting on 901	1		1	1	
Known Addition Spike, mL	0.5		0.5	0.5	
Known Addition Spike, mg/L	100		100	100	
Sample Aliquot, mL	40.222g → 250mL		43.769g → 250mL	43.769 → 250	
Final Volume, mL	50		50	50	
mg/L in Sample (less initial spike)	.747 / 4.64 mg/kg		2.82 / 16.1 mg/kg	2.57 / 14.7 mg/kg	$\bar{x}$ = 15.4

Sample Number	453-17	453-17 dup	453-17 dup		
Initial mV Reading	-40	-34	-35		
Initial Spike Level, mg/L					
KA Setting on 901	5	5	5		
Known Addition Spike, mL	2.5	2.5	2.5		
Known Addition Spike, mg/L	10	10	10		
Final Volume, mL	42.676g → 250	41.356g → 250	41.356g → 250		
~~mg/L in Sample (less initial spike)~~ mg/kg	.343 / 2.64	.347 / 2.11	.345 / 2.07		($\bar{x}$ = 2.08)

1.0 ppm = -69.3 10 ppm = -127.4

0.1 ppm = -11.2 100 ppm = -185.5

slope = 58.1

ANALYST: DATE: 7/8/88 SWV 7/8/88 HB NUMBER:

WITNESS: DATE: PAGE NUMBER:

True value of Wp987 = 2.0

Recovery = 103%

VG 11/0011

Figure 4-4a. Example of laboratory raw data sheets for ammonia (Figure 4-4a) and total sulfide (Figure 4-4b)

Material: sediment

Analysis For: S=

WORK ORDER # 432

Method

DATA

Sample ID	Blank	.1 ppm	.2 ppm	.5 ppm	1 ppm	2 ppm	432-6 as received	432-7 as received
Dilution Factor							5.07g → 50ml	5.35g → 50ml
absorbance	.004	.047	.077	.197	.395	.805	.012	.242
Blank correction	.004	.004	.004	.004	.004	.004	.004	.004
net absorbance	.000	.043	.073	.193	.391	.801	.008	.238
conc. (from graph)							.030 ~~.02~~	.604
actual conc (mg/L)							.30	5.64

Sample ID	432-8	432-9	432-10	432-11	432-12	432-13	432-~~16~~ 16	432-~~17~~ 17
Dilution Factor	5.06g → 50ml	5.41g → 50ml	5.26g → 50ml	5.13g → 50ml	5.09g → 50ml	5.47g → 50ml	5.69g → 50ml	5.75g → 50ml
absorbance	.032	.154	.020	.649	.354	arr 1.0	over	over
Blank correction	.004	.004	.004	.004	.004	repeat	repeat	repeat
net absorbance	.028	.150	.016	.645	.350			
conc (from graph)	.079	.384	.049	1.621	.884			
actual conc (mg/L)	.78	3.55	.47	15.80	8.68			

Sample ID	432-18A	432-18B	432-18C	432-19	432-20	432-21	432-22	432-23
Dilution Factor	5.13g → 50ml	5.31g → 50ml	5.62g → 50ml	5.37g → 50ml	5.05g → 50ml	5.16g → 50ml	5.66g → 50ml	5.25g → 50ml
absorbance	.236	.227	.273	.718	.606	.611	over	over
Blank correction	.004	.004	.004	.004	.004	.004	repeat	repeat
net absorbance	.232	.223	.269	.714	.602	.605		
conc (from graph)	.589	.567	.682	1.793	1.514	1.521		
actual conc (mg/L)	5.74	5.34	6.07	16.7	15.0	14.74		

Comments: r = .99984 c_v = -0.004 93% slope = .4002 105.7%

weights as received

ANALYST

WITNESS

DATE 5/20/88

DATE 7/14/88

NB NUMBER

PAGE NUMBER

Figure 4-4b. Example of laboratory raw data sheets for ammonia (Figure 4-4a) and total sulfide (Figure 4-4b)

5. QUALITY ASSURANCE FOR METALS IN SEDIMENT

5.1 INTRODUCTION

Metals analysis includes a variety of metals and metal species. There are also a variety of acceptable digestion procedures (e.g., strong acid, total acid) and analytical methods [e.g., graphite furnace atomic absorption (GFAA) analysis, inductively-coupled plasma (ICP) emission spectroscopy] that can be applied to metals analysis. In this section, emphasis is placed on the methods that are recommended by PSEP (1986) or PSDDA (U.S. COE 1988) programs.

The development of QA guidelines for evaluating metals data to be included in the Puget Sound database was based on the QA requirements of the EPA/CLP program. CLP protocols were developed to provide the highest quality data possible for evaluating samples most likely to contain high contaminant concentrations from hazardous waste sites. The QA criteria laboratories must meet under CLP guidelines are rigid yet reasonably achievable because of the high detection limits required. Implementation of these same criteria for data acceptance under PSEP protocols, which require lower limits of detection (LOD) and a different sample digestion matrix, increases the challenge to produce acceptable data. In many cases, PSEP protocols require LOD approaching or below the detection limits of the instrumentation, making accuracy and precision requirements difficult to achieve.

In the step-by-step process of review, the reviewer should keep in mind the protocol restrictions described above and make QA assessments based on these factors. Each section below discusses problems that could be encountered based on the methodology required under PSEP and PSDDA. Because QA criteria under PSEP were adapted from EPA/CLP, the review guidelines describe below are based on *Laboratory Data Validation Functional Guidelines for Evaluating Inorganics Analyses* (U.S. EPA 1985). Table 5-1 lists the recommended frequencies and control limits for QA samples. A series of worksheets is provided in Appendix B to aid the reviewer in the step by step review process for metals data.

5.2 UNIQUE SAMPLING REQUIREMENTS FOR METAL ANALYSIS

Sample collection for metals must be conducted in a manner that ensures that samples are free from metals contamination. In the field, sources of contamination include sampling gear, lubrication and oils, engine exhaust, airborne dust, insufficiently cleaned or inappropriate storage containers, and ice used for cooling samples.

Exposure to airborne dust can be minimized by using capped containers and by keeping physical sample handling to a minimum. The best containers for the collection and storage of trace metal sediment samples are made of linear polyethylene or polypropylene, with a polyethylene cap. Borosilicate glass may also be used, but lids must not have aluminum or cardboard liners.

Prior to use, containers and any glass or plastic parts associated with the sampling equipment should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked 24 hours at 70° C in an acid solution of 1:1 deionized water:HNO_3 or 1:1 deionized water:HCl, then rinsed with deionized water. Chromic acid should not be used for cleaning purposes.

5.3 DATA COMPLETENESS AND FORMAT

The first step in the QA review process is to determine if the laboratory has provided results for all samples and required QA/QC information. Incomplete data sets can result by the

TABLE 5-1. RECOMMENDED FREQUENCIES AND CONTROL LIMITS FOR METALS QA SAMPLES

Analysis Type	Frequency of Analysis[a,b]	Control Limit
Preparation blanks	5% or one per batch, whichever is more frequent	Low level - ≤2xIDL High level - <IDL
~~Standard~~ Certified reference materials[c]	5% or one per batch, whichever is more frequent	80-120% recovery
Matrix spikes	5% or one per batch, whichever is more frequent	75-125% recovery
Analytical replicates	5% or one per batch, whichever	±20% RPD

[a] Frequencies listed are minimums; some programs may require higher levels of effort.

[b] For batches of five samples or less, the minimum QA checks should be a method blank and the analysis of ~~an SRM~~a CRM. If an analyte is not in the ~~SRM~~ CRM, a matrix spike must be analyzed for that particular analyte. In general, for small batches (i.e., ≤5 samples), the priority of QC checks should be: ~~SRM~~ CRM > analytical duplicates > matrix spikes. If several batches of the same matrix are analyzed sequentially (i.e., for several small projects), a ~~SRM~~ CRM can be analyzed at a frequency of 5% overall, with at least one sample duplicate analyzed per individual batch.

[c] Certified values not available for all elements (e.g., silver).

laboratory's inadvertent omission of information from a data package or when required information may not have been requested by the contractor. Section 3.2 lists required laboratory deliverables.

~~The following laboratory information is required to evaluate sample data for inclusion in the Puget Sound database:~~

- ~~Tabulated results in units as specified for each matrix in the analytical protocols, validated and signed in original by the laboratory manager~~
- ~~Any data qualifications and explanations for any variance from the analytical protocols~~
- ~~Results for all of the QA/QC checks initiated by the laboratory~~
- ~~Tabulation of IDL and MDL.~~

~~All contract laboratories are required to submit results supported by sufficient backup and QA data to enable independent QA reviewers to conclusively determine data quality. The laboratories should supply legible photocopies or original data sheets with sufficient information to unequivocally identify the following:~~

- ~~Calibration results~~
- ~~Calibration and preparation blanks~~
- ~~Samples and dilutions~~
- ~~Duplicates and spikes~~
- ~~Any anomalies in instrument performance or unusual instrumental adjustments.~~

In some cases, the sample data may be acceptable if a limited amount of information is missing. For example, if no field replicates or field blanks were analyzed, but all other QA/QC requirements were met, the data set is complete enough for review. If no matrix spikes or CRM ~~SRM~~ were analyzed, analyzed, the data set would be rejected because of insufficient QA documentation. The data reviewer should assess the extent and severity of omitted QA data.

Data reporting by the laboratory should conform to standard format for concentration units, number of significant figures, data flags, ~~data qualifiers,~~ and detection limits. For comparison to screening levels and existing Puget Sound data, concentration units for metals data should be in ppm (mg/kg) dry weight for sediment and ppm (mg/kg) wet weight for tissue. In accord with EPA/CLP guidelines, the number of significant figures reported by the laboratory should be dependent on the sample concentration. For sample values less than 10 ppm, two significant figures are sufficient. For sample values greater than 10 ppm, three significant figures are appropriate. If a laboratory reports excessive significant figures than described above, the entire data package should be questioned.

Data flags ~~qualifiers~~ should be clearly defined in the laboratory report sheets. For example, undetected compounds are indicated differently (e.g., < vs. U) by different laboratories. Laboratory qualifiers employed by the EPA/CLP in Table 5-2 may be used by laboratories when reporting data. Laboratory flags ~~qualifiers~~ are not to be confused with data review qualifiers. Samples not flagged by the laboratory may be assigned qualifier codes after data review. ~~Samples may not need to be qualified by the laboratory, but after review are assigned review qualifier codes.~~ Table 5-3 lists the qualifier codes used by SEDQUAL in reviewing data. An explanation of the meaning and use of SEDQUAL data qualifiers is described in Appendix C.

Detection limits are a critical aspect of data quality control. For sediment quality management, it is necessary to have detection limits considerably lower than the established sediment

TABLE 5-2. EPA/CLP ~~QUALIFIER CODES~~ LABORATORY FLAGS

~~C (Concentration) Qualifier Codes~~
C (Concentration) Flags

B	The reported value is less than the contract-required detection limit (CRDL), but greater than the instrument detection limit (IDL)
U	The analyte was analyzed for, but not detected

~~Q (Questionable) Qualifier Codes~~
Q (QA/QC Requirements) Flags

E	The reported value is estimated because of the presence of interference; an explanatory note must be included under comments on the cover page
M	Duplicate injection precision not met
N	Spiked sample recovery not within control limits
S	The reported value was determined by the method of standard additions (MSA)
W	Post-digestion spike for furnace AA analysis is out of control limits (85-115%), while sample absorbance is less than 50% of spike absorbance
*	Duplicate analysis not within control limits
+	Correlation coefficient for the MSA is less than 0.995

Note: Entering "S", "W", or "+" is mutually exclusive; no combination of these qualifiers can appear in the same field for an analyte

~~M (Method) Qualifier Codes~~
M (Method) Flags

P	ICP analysis
A	Flame AA analysis
F	Furnace AA analysis
CV	Manual cold vapor AA analysis
AV	Automated cold vapor AA analysis
AS	Semi-automated spectrophotometric analysis
C	Manual spectrophotometric analysis
T	Titrimetric analysis
NR	The analyte is not required to be analyzed

TABLE 5-3. SEDQUAL DATA ASSESSMENT QUALIFIER CODES

Qualifier Code	Description
B	Blank-corrected down to detection limit
C	Combined with unresolved substances
E	Estimate
G	Estimate is greater than value shown
K	Detected at less than detection limit shown
L	Value is less than the maximum shown
M	Value is a mean
Q	Questionable value
T	Detected below quantification limit shown
U	Undetected at the detection limit shown
X	Recovery less than 10 percent
Z	Blank-corrected, still above detection limit

quality against which they are tested. PSEP protocols have adopted the American Chemical Society's Committee on Environmental Improvements (CEI) definition for reporting detection limits. Throughout this document, LOD will be used when discussing detection limits. CEI defines the LOD as the lowest level that can be determined to be statistically different from the blank. A further discussion of detection limits during sample data review will be discussed in Section 5.4.2.

5.4 DATA VALIDATION AND ASSESSMENT

Data review can be divided into two sections: 1) validation of the data package, and 2) assessing the data to determine if data objectives of the project have been met. Data validation is the process of reviewing the laboratory reports and raw data to determine if all QA/QC requirements, either written into the contract or implied under PSEP criteria, have been performed. After the data package has been validated, the laboratory and field QC (blanks, spikes, etc.) are assessed and the quality of the data is then determined.

PSEP guidelines (PSEP 1986) lists the specific requirements the laboratory must meet to produce a valid data package. The reviewer performing data validation must verify the following areas:

- Data completeness and format
- Holding times
- Instrument calibration verification
- Laboratory blank analysis
- Detection limits
- Specific instrument QA requirements
- Certified reference material results
- Matrix spike percent recovery results
- Laboratory precision evaluation
- Calculations.

Data assessment is performed using the quality control information generated during data validation. The reviewer performing data assessment determines if the data quality objectives are met for the project by reviewing both laboratory and field quality control results. ~~Data validation includes QA/QC checks to assess the performance of analytical methods, and to determine the accuracy and precision of the measurements. Analytical methods are evaluated by reviewing instrument calibration, field blanks, method blanks, and detection limits. Accuracy is assessed through the analysis of SRM and matrix spikes. Precision is determined by replicate analysis. In the following section, each of these QA/QC checks is described.~~

5.4.1 Instrument Calibration

Objective—The objective for requiring satisfactory instrument calibration is to ensure the instrument is capable of producing acceptable quantitative data. This objective is satisfied using three separate measures: initial calibration, calibration verification, and continuing calibration standards.

Requirements—The following QA calibration checks are required:

- Instruments must be calibrated daily, and each time a new analytical run is set up.
- Calibration verification should be performed by the analysis of an EPA control solution or an independent standard at a concentration other than that used for calibration. For both standards, the concentration must fall within the calibration range.
- Continuing calibration must be performed at a minimum frequency of 10 percent or every 2 hours during an analysis run, whichever is more frequent, and after the last sample is analyzed.
- Continuing calibration checks must be either an EPA QC standard or an independent standard from a different source than that used for the initial calibration standards.
- Continuing calibration verification must occur at or near the mid-range level of the calibration.
- For ICP and atomic absorption (AA) analysis, the calibration verification and continuing calibration results must fall within the control limits of 90-110 percent of the true value. For mercury, the control limits are 80-120 percent.
- For ICP analysis, a calibration blank and at least one standard must be analyzed to establish the analytical curve.
- For AA analysis, a calibration blank and at least three standards must be analyzed to establish the analytical curve.

Evaluation Procedure—During data review, the reviewer should perform the following:

- Verify that the instrument was calibrated at the proper frequency using the correct number of standards and calibration blanks for the method used.
- Verify that the analytical curve for AA contained one standard at the required LOD, and the sample values were bracketed within the standards use.
- Verify that the calibration verification and continuing calibration sources used met contract requirements. The laboratory should clearly state sources of standards.
- Verify that continuous calibration checks were analyzed at the proper frequency.
- Spot check calibration verification checks by recalculating the percent recovery from the raw data using the following equation:

$$\% \text{ Recovery} = \frac{\text{standard value}}{\text{true value}} \times 100$$

Action—Failure of the laboratory to perform acceptably on the calibration criteria indicates serious problems in the analytical system. Until these problems are resolved, any data generated under such conditions should be considered suspect. If contract criteria were not met or if improper calibration procedures were used, all data associated with that calibration should be reanalyzed.

The QA reviewer should review the raw data (e.g., instrument printouts, laboratory worksheets) to verify proper instrument calibration. Figure 5-1 provides a schematic approach for reviewing instrument calibration. Examples of acceptable and unacceptable data are provided below. The examples use the following EPA/CLP terminology:

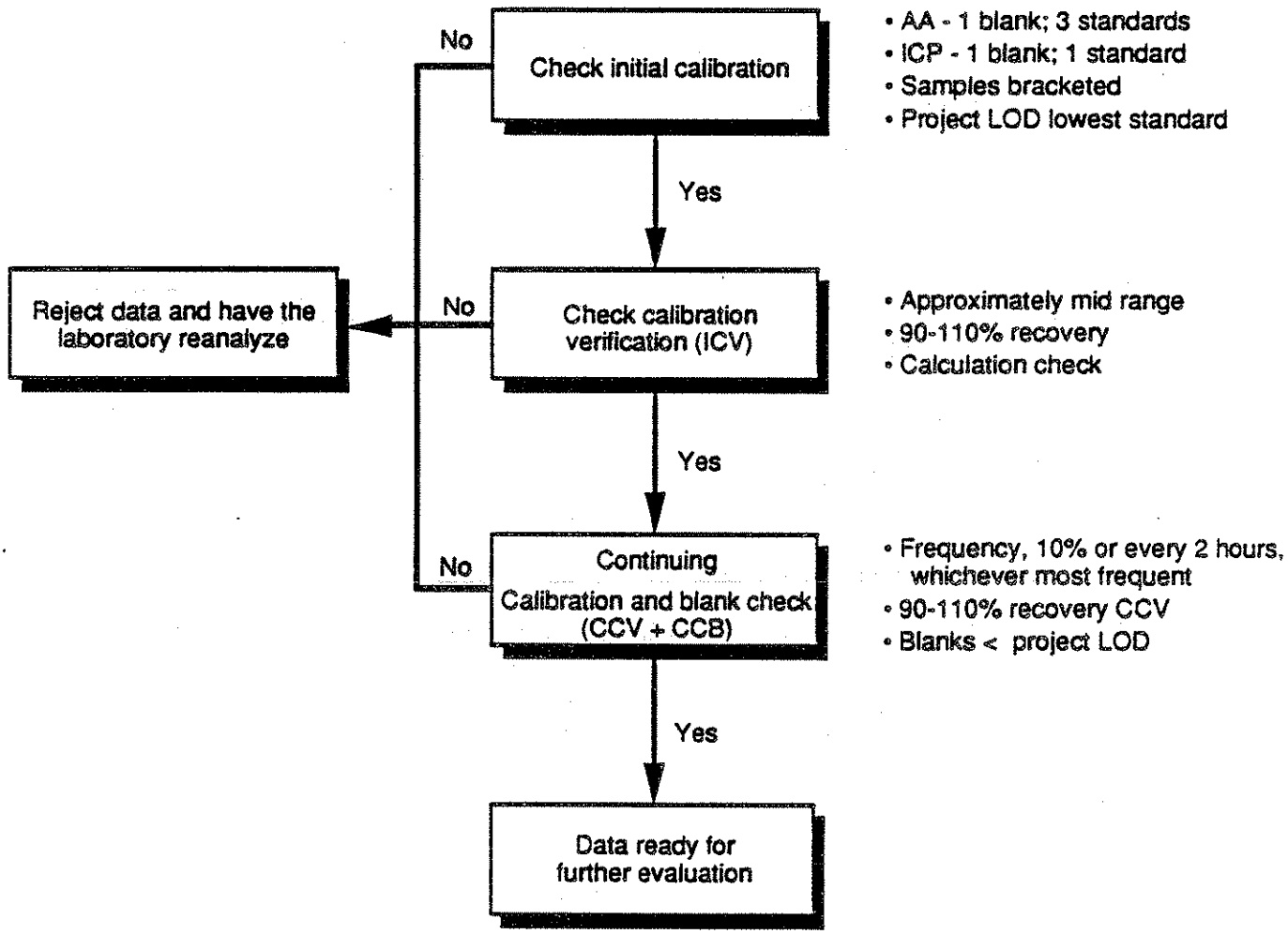

Figure 5-1. Schematic approach for reviewing instrument calibration

ICV - Initial calibration verification standard
ICB - Initial calibration blank
CCV - Continuing calibration verification standard
CCB - Continuing calibration blank.

Laboratories not using EPA/CLP terminology should label the calibration standards clearly so the data reviewer can assess calibration data.

Examples of accepted and rejected calibration data are illustrated in Figure 5-2. In Example 1, the data are acceptable because the blank and three standards were used for the initial calibration, the lowest standard was at the Project LOD, and all sample concentrations were within the instrument calibration range. In Example 2, the data are unacceptable because there is no verification of the analytical curve at low analyte concentrations. The sample values were not bracketed within the standards used to establish the analytical curve. PSEP protocols require analysis of a standard at the Project LOD. In this case, the laboratory would be requested to reanalyze the samples using a calibration curve that bracketed the sample concentration. In Example 3, the data are acceptable because calibration standards included Project LOD and bracketed sample concentrations, percent recovery of ICV and CCV analysis were within the 90-110 percent control limit, and the CCB values all were less than the Project LOD. In Example 4, the data would be rejected on the basis of the CCV. The initial CCV standard was acceptable, but subsequent CCV standards indicated severe analytical drift. ~~but subsequent CCV standards indicated poor analyte recovery.~~ These results demonstrate a change in instrument conditions that make any data associated with this analytical run suspect.

5.4.2 Detection Limits

Objective—The evaluation of detection limits is a major component in reviewing data for submission to the Puget Sound database. As previously discussed, the detection limits required under PSEP protocols approach the IDL; therefore, low readings are susceptible to instrument noise and minor traces of contaminants from field or laboratory procedures. For further discussion, the following definitions prepared by the American Chemical Society's CEI will apply (Keith et al. 1983):

- Instrument detection limit (IDL)—The smallest signal above background noise an instrument can detect reliably.
- Limit of detection (LOD)—The lowest concentration level that can be statistically different from the blank. The recommended value for LOD is 3σ, where σ is the SD of the blank in replicate analyses.
- Limit of quantitation (LOQ)—The level above which quantitative results may be obtained with a specified degree of confidence. The recommended value for LOQ is 10σ, where σ is the SD of blanks in replicate analyses.
- Method detection limit (MDL)—The minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is determined from seven replicate analyses of a sample of a given matrix containing the analyte (Glaser et al. 1981).

The LOD recommended under PSEP are not strictly based on the CEI definition, but are considered typically obtainable values based on instrument sensitivity, blank contamination, matrix interferences, and reasonable levels of laboratory analytical effort (PSEP 1986). The LOD developed for PSEP fall between the IDL and the MDL.

REVIEW OF INITIAL CALIBRATION

Example 1: Acceptable Data for AA

Instrument calibration standards: 0, 10, 50, 100 ppb
Project LOD: 10 ppb
Sample values: 25-83 ppb

Example 2: Unacceptable Data for AA

Instrument calibration standards: 0, 50, 100, 150 ppb
Project LOD: 10 ppb
Sample values: 15-60 ppb

REVIEW OF CALIBRATION VERIFICATION AND CONTINUING CALIBRATION CHECKS

Example 3: Acceptable Data

Instrument calibration standards: 0, 10, 50, 100 ppb
ICV true value: 36 ppb
ICV value: 38 ppb
Project LOD: 10 ppb
CCV true value: 55 ppb
CCV values: 57, 55, 54, 55 ppb
CCB: <10, <10, <10, <10 ppb
Sample values: 25-83 ppb

Example 4: Unacceptable Data

Instrument Calibration Standards: 0, 10, 50, 100 ppb
ICV true value: 36 ppb
ICV value: 38 ppb
Project LOD: 10 ppb
CCV true value: 55 ppb
CCV values: 56, 30, 28, 29 ppb
CCB: <10, <10, <10, <10 ppb
Sample values: 25-83 ppb

Figure 5-2a. Examples of acceptable and unacceptable calibration, calibration verification and continuing calibration checks (Figure 5-2a), laboratory QA/QC report (Figure 5-2b), and data review sheet (Figure 5-2c)

QA/QC Report
Concentration Units: mg/L

Initial and continuing calibration verification

	Initial Calibration			Continuing Calibration					
Analyte	True	Found	%R	True	Found	%R	Found	%R	M
Antimony	1.01	.986	97.6	.0400	.0386	96.5	.0396	99.0	F
Arsenic	.047	.0493	104.9	.0400	.0411	102.8	.0408	102.0	F
Cadmium	.00984	.00959	97.5	.01000	.00958	98.8	.01031	103.1	F
Copper	.52	.53	101.9	1.000	.996	99.6	1.022	102.2	A
Lead	4.96	4.97	100.2	3.00	2.93	97.7	2.75	91.7	A
Mercury	.0049	.0050	102.0	.0030	.0030	100.0	.0030	100.0	CV
Nickel	2.40	2.58	107.5	5.00	5.13	102.6	5.1	102.0	A
Silver	.48	.50	104.2	.0050	.0051	102.0	.0048	96.0	F
Zinc	2.92	3.04	104.1	5.00	5.00	100.0	4.86	97.2	A

Figure 5-2b. Examples of acceptable and unacceptable initial calibration, calibration verification and continuing calibration checks (Figure 5-2a), laboratory QA/QC report (Figure 5-2b), and data review sheet (Figure 5-2c)

III. Initial and Continuing Calibration Verification

✓ Calibrations were performed at the beginning of sample analysis and at a minimum frequency of ten percent or every two hours during the analysis, and met PSEP criteria.

_____ Calibrations were not performed as specified and/or did not meet PSEP-specified windows. Action: The sample set for all analytes affected should be rejected and all associated data assigned an R qualifier. Failure to meet calibration criteria is an indication of serious problems in the analytical system.

Remarks: frequency ok – no sample calibration data outside control limits.

Figure 5-2c. Examples of acceptable and unacceptable calibration, calibration verification and continuing calibration checks (Figure 5-2a), laboratory QA/QC report (Figure 5-2b), and data review sheet (Figure 5-2c)

Requirements—Table 5-4 lists LOD for sediment and tissue matrices by instrument as presented in PSEP protocols (PSEP 1986). The LOD for sediment were developed using the strong acid digestion (SAD) method with a sediment sample size of 5 grams wet weight diluted to a 100-mL final volume. In comparison, the total acid digestion (TAD) method, strongly recommended under the PSDDA program, uses a sample size for digestion of 0.2 gram diluted to a final volume of 25 mL. Use of the TAD method presents several problems in achieving the PSEP LOD. The TAD method introduces considerable matrix interference due to the high concentrations and types of reagents employed, and often requires further dilution prior to instrument analysis. The result is a LOD in solution approaching the IDL to meet the final detection limit required. Table 5-5 is a comparison of common instrument IDL with the in-solution LOD required to meet the PSEP-recommended LOD and the routinely achievable LOD for PSDDA. Note how the required LOD for TAD approaches or is below routine IDL for some elements.

Evaluation Procedure and Action—Validation of detection limits requires a review of the instrument calibration raw data. The instrument printout should contain all calibration standards and respective absorbance values. Instrument noise and sensitivity varies depending on the analyte. As a general rule, the absorbance of the LOD standard should be at least 0.010 absorbance units above the calibration blank absorbance value. If the difference between absorbance values is less, the reported LOD for that element may not be significantly different from the blank and reevaluation of the reported LOD is required. If the LOD is in question, the laboratory should be requested to provide a statistical justification for the stated LOD (i.e., replicate analysis of a standard at the LOD). Depending on the laboratory results, the LOD can then be adjusted or the samples reanalyzed.

5.4.3 Blank Analysis

Objective—~~Blanks are used to assess sample contamination. Field blanks (e.g., transfer, transport, and rinsate), used to assess contamination associated with the sampling method, are discussed under field procedures.~~—Method and calibration blanks are used to assess laboratory contamination during sample preparation and analysis. Method blanks represent the net contamination of all stages of preparation and analysis and are used to detect. ~~Blank contamination is assessed to avoid possible~~ false positives (i.e., erroneous reports of the metal present in the sample) and over-estimates of sample concentrations. Assessment of blank contamination is important under PSEP guidelines because of the low levels of detection required. The guidelines described under PSEP protocols (PSEP 1986) were modeled after the EPA/CLP. CLP LOD are considerably higher than those of PSEP for most elements of concern (Table 5-6); therefore, the CLP LOD is much higher than the IDL. Because PSEP LOD are very near the IDL and because sample concentrations are basically low, results can be significantly impacted by instrument background noise and trace amounts of laboratory contamination. Therefore, it is important to carefully evaluate any blank data above detection limits. ~~Because PSEP LOD are nearer the IDL than CLP LOD, reported concentrations can be influenced more by instrument background noise and trace amounts of laboratory contamination. It is important to carefully evaluate blank data because PSEP-required LOD are close to the IDL, and the blank values can become very significant relative to sample concentrations.~~

Requirements—The criteria for evaluating blanks apply to continuing calibration blanks, method blanks, and field blanks. If problems with any blank occur, all data associated with the data set must be carefully evaluated to determine inherent variability in the data or if the problem is an isolated case.

Requirements for calibration blanks include the following:

- A calibration blank is to be analyzed each time the instrument is set up, at the beginning and end of the run, and at a frequency of 10 percent during the run

TABLE 5-4. LIMITS OF DETECTION FOR SEDIMENT AND TISSUE MATRICES BY INSTRUMENT

	Sediment[a]		Tissue[b]		
	ICP	GFAA	ICP	FAA	GFAA
Antimony	3.2	0.1	1.0	--	0.02
Arsenic	--	0.1	3.0	--	0.02
Cadmium	4.0	0.1	0.4	0.1	0.01
Copper	0.6	0.1	0.5	0.1	0.01
Iron	0.7	--	NA[c]	NA	NA
Lead	4.2	0.1	4.0	1.0	0.03
Mercury	0.01 (CVAA)	--	0.01 (CVAA)	--	--
Manganese	2.0	--	NA	NA	NA
Nickel	1.5	0.1	1.0	0.5	0.02
Silver	0.7	0.1	0.7	0.1	0.01
Zinc	0.2	0.2	0.2	0.1	0.2

[a] ICP data from Tetra Tech 1984; GFAA and CVAA data are detection limits that can be reasonably attained by various laboratories. Under strict conditions these limits can be lowered (e.g., Battelle 1985). Values are mg/kg dry weight for 5-gram (wet) sediment in a 100-mL digest.

[b] PSEP 1986. Values are μg/g wet weight for 5-gram tissue in a 50-mL digest.

[c] NA = Not applicable. Iron and manganese used as natural tracers for sediments only.

Reference: PSEP 1986.

TABLE 5-5. COMPARISON OF ROUTINELY ACHIEVABLE DETECTION LIMITS WITH REQUIRED IN-SOLUTION VALUES FOR SAD AND TAD TO MEET PSEP LIMITS OF DETECTION

	IDL[a] (μg/L)	SAD[b] (μg/L)	TAD[c] (μg/L)
Antimony	1	2.5	0.8
Arsenic	1	2.5	0.8
Cadmium	0.5	2.5	0.8
Copper	3[d]	2.5	0.8
Lead	1	2.5	0.8
Nickel	1	2.5	0.8
Silver	0.5	2.5	0.8
Zinc	4[d]	5.0	1.6

[a] Routinely achievable instrument detection limit (IDL).

[b] 5.0-gram wet sample in 100 mL, 50 percent solids.

[c] 0.2-gram dry sample in 25 mL.

[d] Detection limit affected by blank contamination.

TABLE 5-6. DETECTION LIMITS FOR TISSUE AND SEDIMENT MATRICES

	PSEP Tissue LOD[a]	PSEP Sediment LOD[b]	Routinely Achievable PSDDA Sediment LOD[b]	EPA CLP CRDL[a]
Antimony	0.02	0.1	1.0	2.0
Arsenic	0.02	0.1	2.5	2.0
Cadmium	0.01	0.1	0.25	1.0
Copper	0.01	0.1	1.0	5.0
Iron	NA[c]	0.7	0.7	20.0
Lead	0.03	0.1	0.7	1.0
Mercury	0.01	0.01	0.01	0.2
Manganese	NA	2.0	2.0	3.0
Nickel	0.2	0.1	0.5	8.0
Silver	0.01	0.1	0.15	2.0
Zinc	0.2	0.2	1.0	4.0

[a] mg/kg wet weight.

[b] mg/kg dry weight.

[c] NA = Not applicable. Iron and manganese used as natural tracers for sediments only.

- Blanks are to be reported down to the IDL
- If the concentration of the calibration blank is greater than the Project LOD, the laboratory is required to terminate analysis, correct the problem, and recalibrate.

Requirements for method blanks include the following:

- One reagent blank, taken through all sample preparation procedures, is to be analyzed for every 20 samples, or one per batch of samples digested, whichever is more frequent. Each matrix and digestion procedure must meet this requirement.
- If the concentration of the method blank is less than the Project LOD, no corrective action is necessary by the laboratory.
- Under PSEP guidelines, the laboratory is required to redigest and reanalyze any samples less than 10 times the blank concentration for the samples associated with that particular contaminated blank. The laboratory may still report samples associated with a contaminated blank, but they are not to blank-correct the data. The reviewer will assess the level of contamination, determine the usability of the data and correct if necessary.

During data review, the reviewer should perform the following:

- Review the results reported (and raw data) and verify the correct number of blanks analyzed and accuracy of reported results
- If any blanks were reported above the Project LOD, determine if redigestion and reanalysis were required and performed.

~~Evaluating blank contamination under PSEP guidelines is critical because the LOD required are at or near the IDL. For many elements, blank contamination will not be a problem, but for some elements (e.g., cadmium, copper, and zinc) even meticulous laboratory practices cannot produce blanks without low-level contamination. Low-level contamination could significantly impact the samples with concentrations at or near the project LOD.~~

~~**Evaluation Procedure**—The review process for blanks is divided into two parts, depending on the sample concentrations. Low-level samples, for this application, are those samples and analytes analyzed by GFAA. High-level samples are analyzed by flame atomic absorption (FAA) or ICP. Table 5-7 provides review guidelines for low-level samples.~~

Low-Level Sample Evaluation and Action—Low-level samples, for this application, are those samples and analytes analyzed by GFAA. The evaluation of low-level samples is performed by reviewing the raw data sheets and comparing the in-solution result of the blank to the IDL and sample concentrations. Differences between the blank and samples at this level may be only a few parts per billion. The reviewer should note the IDL reported by the laboratory and determine the blank action level in relation to the IDL. The blank action level (μg/L) is 2 times the IDL (μg/L). The appropriate category from Table 5-7 is selected and the samples treated accordingly. ~~The appropriate category from the table is selected and the samples treated accordingly.~~ If the samples need blank correction, this step is performed prior to converting the in-solution μg/L result to the final mg/kg result.

The following equations are used to calculate the corrected digest sample result and to calculate the blank-corrected sample concentration for both sediment and tissue analyses: ~~The following equations are used to calculate the corrected sample concentration and recalculate the actual sample concentration for both sediment and tissue analyses:~~

TABLE 5-7. BLANK CONTAMINATION REVIEW GUIDELINES FOR LOW-LEVEL SAMPLES (GFAA ANALYSES)

Blank Action Level	Sample Concentration	Action	Qualifier
Blank<2xIDL	Sample (U)[a]	Accept	None
	IDL<sample<PLOD[b]	Correct	B or Z
	Sample>PLOD	Accept	None
	Sample>10xIDL	Accept	None
Blank>2xIDL	Sample (U)	Accept	None
	IDL<sample<PLOD	Correct	B or Z
	Sample>PLOD	Correct	B or Z
	Sample>10xIDL	Correct	B or Z
Blank>10xIDL	Sample (U)	Accept	None
	Sample<10xIDL	Correct	B or Z and E
	Sample>10xIDL	Correct	B or Z

[a] U = Nondetected elements.

[b] PLOD = Project limit of detection.

$$\text{Corrected digest sample result } (\mu g/L) = \text{digest sample result } (\mu g/L) - \text{digest blank result } (\mu g/L)$$

Equation for TAD, SAD, and tissue digestion determinations:

$$\text{mg/kg} = \text{corrected digest sample result} \times \frac{\text{volume diluted (mL)}}{\text{weight digested (g)}} \times \frac{1\ \text{L}}{1{,}000\ \text{mL}} \times \frac{1{,}000\ \text{g}}{1\ \text{kg}} \times \frac{1\ \text{mg}}{1{,}000\ \mu\text{g}}$$

In addition, SAD results must be converted to dry weight, as follows:

$$\text{Final concentration (mg/kg dry weight)} = \frac{\text{wet weight sample result (mg/kg)}}{\%\ \text{solids}} \times 100$$

~~TAD is performed on a dried sample and tissue results are reported on a wet-weight basis; no further corrections are necessary.~~

~~High-Level Sample Evaluation and Action—Action—~~If the laboratory reports a concentration greater than the IDL for high-level analyses (FAA and ICP), samples with analyte concentrations less than 5 times the concentration in the highest associated blank should be considered suspect. Action levels are determined by multiplying the highest concentration of contamination determined in the method or calibration blank by five. The action level for samples that have been diluted should be multiplied by the dilution factor. Prior to applying action values to sediment and tissue samples, it is necessary to convert the blank result (μg/L) to mg/kg for each sample with the following calculations:

$$\text{Blank action level } (\mu g/L) = 5\times \text{ highest blank result } (\mu g/L)$$

SAD sample action value (mg/kg) =

$$\frac{100\times \text{blank action level } (\mu g/L)}{\%\ \text{solids}} \times \frac{\text{volume diluted (mL)}}{\text{weight digested (g)}} \times \frac{1\ \text{L}}{1{,}000\ \text{mL}} \times \frac{1{,}000\ \text{g}}{1\ \text{kg}} \times \frac{1\ \text{mg}}{1{,}000\ \mu\text{g}}$$

$$\cancel{\frac{\text{blank } 100\times \text{ action level } (\mu g/L)}{\%\ \text{solids}}}$$

TAD and tissue sample action value (mg/kg) =

$$\text{blank action level } (\mu g/L) \times \frac{\text{volume diluted (mL)}}{\text{weight digested (g)}} \times \frac{1\ \text{L}}{1{,}000\ \text{mL}} \times \frac{1{,}000\ \text{g}}{1\ \text{kg}} \times \frac{1\ \text{mg}}{1{,}000\ \mu\text{g}}$$

To simplify the review process, perform the above calculation on the sample with the lowest percent solids. This calculation will give the maximum action level, and any samples with an analyte concentration (mg/kg) greater than this value would be acceptable. Samples with concentrations less than this action level should be calculated on an individual basis to determine acceptability. No action is needed for sample analyte concentrations greater than the sample action value; data are acceptable as reported. Samples less than the sample action value must be blank-corrected and assigned either a B or Z qualifier and an E qualifier as an estimate. Blank-correction calculations are the same calculations described for low-level samples. A worksheet and raw data

sheet are shown in Figure 5-3 as examples for reviewing blank determinations and calculating action levels.

5.4.4 Certified ~~Standard~~ Reference Material

A CRM ~~SRM~~ of a matrix similar to the samples is analyzed with each batch of samples to monitor the efficiency of the digestion procedure and to evaluate overall accuracy of the method. Examples of the laboratory report sheets are shown in Figure 5-4 along with Sections VII and VIII of the PSEP/PSDDA worksheet to aid in reviewing QA results.

Requirements—The following QA checks are required:

- The CRM ~~SRM~~ must closely match the sample matrix. It must be a certified standard (e.g., EPA, National Bureau of Standards) with values for the analytes of interest. Currently, CRM ~~SRM~~ for silver are not available. For this analyte, evaluation of method accuracy must be based on matrix spike analysis.
- One CRM ~~SRM~~ must be analyzed for every 20 samples or one per batch, whichever is more frequent.
- The CRM ~~SRM~~ must be treated exactly like the samples, from digestion to instrumental analysis.
- All CRM ~~SRM~~ results must fall within the control limits of 80-120 percent recovery.

Evaluation Procedure—During data review, the reviewer should perform the following:

- Review data reports and verify that the proper number of CRM ~~SRM~~ was analyzed, and determine if any analytes were outside control limits.
- Spot check 10 percent of the raw data calculations to verify the reported recoveries. If any calculation errors are found, all CRM ~~SRM~~ data should be recalculated.

Action—The inability of the laboratory to perform successfully on the CRM ~~SRM~~ is dependent on several factors. CRM ~~SRM~~ values outside the control limits indicate an analytical problem related to the digestion procedures and/or instrument operations. The digestion procedure can influence recovery in two ways: 1) incomplete digestion of the sampled matrix, or 2) enhancement or suppression of the instrument signal due to digestate matrix. The TAD method recommended under PSEP protocols (PSEP 1986) can produce substantial matrix interferences for many analytes (particularly antimony, cadmium, and silver) in the final digestate. Poor recovery has been obtained for antimony, independent of the digestion procedure employed. In reviewing data for acceptance, CRM ~~SRM~~ values should be weighed together with matrix spike recoveries to evaluate data accuracy, and not be the only basis for data rejection.

The following guidelines are recommended under CLP (U.S. EPA 1985) for use in evaluating data acceptability when the CRM ~~SRM~~ recoveries do not fall within control limits. These guidelines are summarized on the PSEP/PSDDA data review worksheet in Appendix B.

- If the CRM ~~SRM~~ recovery for an analyte falls within the range of 30-79 percent or greater than 120 percent, flag the positive hit data as estimated (E). In the review narrative indicate the potential bias of the results and the detection levels.
- If the analyte is not detected in the sample and the CRM ~~SRM~~ recovery is greater than 120, the usability of the analytical sample determination is acceptable.

QA/QC Report

BLANKS

Analyte	Calibration Blank (mg/L) 1	2	3	Preparation Blank (mg/Kg)	M
Antimony	<0.001	<0.001	<0.001	<0.08	F
Cadmium	<0.0001	0.0006	<0.0001	<0.0075	F
Copper	<0.025	<0.025	<0.025	<3.1	A
Lead	<0.1	<0.1	<0.1	<1.5	A
Mercury	<0.0002	<0.0002	<0.0002	<0.1	CV
Nickel	<0.1	<0.1	<0.1	<1.5	A
Silver	<0.0001	<0.0001	<0.0001	<0.1	F
Zinc	<0.1	<0.1	<0.1	<1.5	A

Figure 5-3a. Example of calibration and preparation blank results (Figure 5-3a) and data review worksheet (Figure 5-3b)

IV. Blank Analysis Results

Contaminant	Project LODs	Initial Calibration Blank Value	Cont. Calib. Blank			Preparation Blank			Action
			1	2	3	1	2	3	
Antimony									
Arsenic									
Cadmium mg/L		<0.0001	<0.0001	0.0006	<0.0001	<0.0075			0.375
Copper									
Iron									
Lead									
Manganese									
Mercury									
Nickel									
Silver									
Zinc									

Note: Contamination detected above the IDLs should be evaluated and qualified. A separate table should be used for each batch analyzed.

Low Level Samples Action Levels (GFAA Analyses):

Blank Result	Accept	Estimate (B or Z)
ug/L blank <2x IDL	Sample ≤IDL Sample >PLOD	IDL < Sample < PLOD
ug/L blank >2x IDL but ≤10xIDL	Sample ≤IDL	Sample > IDL
ug/L blank >10x IDL	Sample ≤IDL	Sample <10x IDL (also "E") Sample >10x IDL

High Level Samples Action Levels (FAA and ICP) -

Action levels are determined by multiplying the highest concentration determined in any blank. The action level for samples which have been diluted should be multiplied by the dilution factor. Prior to applying action levels to sediments and tissue, it is necessary to convert the aqueous action value (ug/L) to mg/kg for each sample with the following equations:

Action value (ug/L) = 5x highest blank result (ug/L)

TAD and tissue analyses action value (mg/kg) =

$$\text{Action value (ug/L)} \times \frac{\text{volume diluted to (ml)}}{\text{wet weight digested (gm)}} \times \frac{1\ \text{L}}{1{,}000\ \text{ml}} \times \frac{1{,}000\ \text{gm}}{1\ \text{kg}} \times \frac{1\ \text{mg}}{1{,}000\ \text{ug}}$$

SAD action value (mg/kg = $\frac{100}{\%\ \text{solids}}$ x the above equation

Cadmium action value (ug/L) = 5 x (0.6) = 3.0 ug/L

TAD action value (mg/kg) =

$$3.0\,(\text{ug/L}) \times \frac{25\,(\text{ml})}{0.2000\,\text{g}} \times \frac{1}{1000} \times \frac{1000}{1} \times \frac{1}{1000} = 0.375\ \text{mg/kg}$$

Figure 5-3b. Example of calibration and preparation blank results (Figure 5-3a) and data review worksheet (Figure 5-3b)

Sample Name: EBT01
Lab Code: 508-1S

Element	Spike Level	Control Limit	Sample Result	Spike Result	% Recovery
Antimony	7.5	75-125%	.34	1.60	16.8
Arsenic	24	75.125%	5.2	32.4	113.3
Cadmium	2.25	75-125%	0.394	3.47	136.7
Copper	125	75-125%	60	180	96.0
Lead	60	75-125%	48	106	96.7
Mercury	0.46	75-125%.	0.37	0.80	93.5
Nickel	60	75-125%	48	100	86.7
Silver	3	75-125%	0.62	3.78	105.3
Zinc	105	75-125%	104	203	94.3

Figure 5-4a. Example of matrix spike results (Figure 5-4a), standard reference material results (Figure 5-4b), and data review worksheets (Figures 5-4c and 5-4d)

Total Metals
mg/Kg
Dry Basis

Sample Name: Reference NBS 1646
Lab Code:

	Method	Control Limit	True Value	Found Value	% Recovery
Antimony	204.2	20%	0.4	0.39	97.5
Arsenic	206.2	20%	11.6	9.3	80.2
Cadmium	213.2	20%	0.36	0.40	111.1
Copper	220.1	20%	18	19	105.6
Lead	213.9	20%	28.2	23	81.6
Mercury	245.1	20%	0.063	0.064	101.6
Nickel	249.1	20%	32	29	90.6
Silver	272.2		N/A	0.083	-
Zinc	289.1	20%	138	127	92.0

Figure 5-4b. Example of matrix spike results (Figure 5-4a), standard reference material results (Figure 5-4b), and data review worksheets (Figures 5-4c and 5-4d)

VIII. Matrix Spike Percent Recovery Rates

1 Number of matrix spikes analyzed (min. 1 per 20 samples)

✓OK Spot check of raw data - calculation verification.

Contaminants	Sample #: EBT01					Sample #: _____				
	SSR	SR	S	%R	Action	SSR	SR	S	%R	Action
Antimony	1.60	0.34	7.5	16.8	E(+)/R(4)					
Arsenic										
Cadmium	3.47	0.394	2.25	136.7	E(+) A(U)					
Copper										
Lead										
Mercury										
Nickel										
Silver										
Zinc										

If the sample concentration exceeds the spike concentration by a factor of 4 or more, no action is taken. When the sample result (SR) is less than the Project LOD, SR is equal to zero.

Calculation: $\%R = \frac{SSR-SR}{S} \times 100$

Matrix spike results should be applied to all samples of the same matrix.

Accept	Approximate	Reject
%R (75-125%) for SSR	SR(+) + %R < 30% for SSR[1]	SR(U) + %R < 30% for SSR[2]
SR(U) + %R (30-74%) for SSR[5]	SR(+) + %R (30-74%) for SSR[3]	
SR(U) + %R > 125%	SR(+) + %R > 125% for SSR[4]	

NOTE:
- S - amount of spike
- SSR - spiked sample result
- SR - unspiked sample result
- %R - percent recovery
- U - non-detected element
- + - positive result
- 1 - Discuss in summary that sample results could be biased significantly low and that the reported concentration is the minimum concentration at which the analyte is present.
- 2 - Indicate in QA summary memo of the possibility of false negatives, detection limits are elevated over what is reported, and that severe analytical deficiencies exist.
- 3 - Determine percent bias of results. Report that the detection limit may be biased low.
- 4 - Determine percent bias of sample results: false positive results may potentially exist.
- 5 - When the spiked sample results fall between 30-74% recovery, detection limits should be estimated and the percent bias determined.

Comments: Spike recovery low for Antimony

Figure 5-4c. Example of matrix spike results (Figure 5-4a), standard reference material results (Figure 5-4b), and data review worksheets (Figures 5-4c and 5-4d)

VII. Standard Reference Material Results

✓ Standard Reference Material (SRM) analysis was performed for every twenty samples received and met contractual criteria.

_____ Standard Reference Material (SRM) analysis was performed, but did not meet the criteria for the following elements: _____

Calculation: % R = (Observed/True) x 100

Actions:

	Accept	Estimate (E)	Reject
% Recovery	30-79 for U 80-120 for U >120 for U	30-79 for + >120 for + <30 for +	<30 for U

NOTE:

+ - positive result
U - not detected element

Figure 5-4d. Example of matrix spike results (Figure 5-4a), standard reference material results (Figure 5-4b), and data review worksheets (Figures 5-4c and 5-4d)

- If an analyte is not detected in the sample and the CRM ~~SRM~~ recovery is in the range of 30-74 percent, the detection limit may be biased. In this case, the sample concentration may actually be greater than the Project LOD. In the review narrative, report that the detection limit for the sample set may not be accurate and give an estimate of the bias. Flag the samples as UG (estimate is greater than value shown).
- If the CRM ~~SRM~~ recovery falls below 30 percent, severe laboratory or method deficiencies are evidenced, and the data should be rejected. Low CRM ~~SRM~~ recovery is very common for the analyses of antimony in sediments; therefore, other factors (i.e., matrix spike, blank results) should also be closely evaluated prior to data rejection.

5.4.5 Matrix Spike Analysis

Objective—Matrix spike analysis is designed to provide information about the effect of the sample matrix on the digestion and measurement procedures. Examples of laboratory report sheets and Sections VII and VIII of the PSEP/PSDDA worksheet are shown in Figure 5-4. ~~Examples of laboratory report sheets are shown in Figure 5-4, along with Sections VII and VIII of the PSEP/PSDDA worksheet, to aid in reviewing QA results.~~

The following QA checks are required:

- At least one spike analysis is required for every batch or every 20 samples, whichever is more frequent.
- Samples identified as blanks may not be used for spiking purposes.
- The spike recovery control limits are 75-125 percent
- The following calculation is used to determine spike recovery:

$$\text{Percent recovery} = \frac{(SSR-SR)}{SA} \times 100$$

where:

SSR = Spiked sample result

SR = Sample result

SA = Spike added.

- When the sample result is reported as undetected, SR=0 is to be used for the purpose of calculating recovery.
- If data are received from the laboratory with spike recoveries outside the control limits of 75-125 percent, the samples associated with that spiked sample should be flagged. An exception is granted when the sample concentration is 4 times or more the spike concentration.

Evaluation Procedure—During data review, the reviewer should perform the following:

- Review data and verify that the proper number of matrix spikes was analyzed and that results fall within the specified limits.

- Spot check 10 percent of the raw data calculations to verify the reported recoveries. If any calculation errors are found, all matrix recovery data should be recalculated.
- For spiked sample results outside the control limits, verify the correct usage of laboratory flags ~~laboratory~~.

Action—To properly assess spike sample results, the reviewer should consider the following factors that may affect spike recovery:

- Matrix suppression effects
- Matrix enhancement effects
- Duplicate precision results
- Digestion efficiency
- Contamination
- Relative levels of analyte in the spike and sample.

Matrix effects could produce data biased high or low depending on whether the signal was enhanced or suppressed. These effects can be ~~very~~ subtle, and if the reviewer finds indications of a consistent bias, interpretation by an expert is needed. Poor method precision can influence spike recoveries. Nonhomogeneity of the samples that produce poor precision can also result in poor performance on matrix spike samples, yet not reflect true matrix problems. The relative levels of analyte in the spike and sample can influence the percent recovery. If the sample concentration level is greater than 4 times the spike level, the percent recovery results should not be considered accurate or used to determine the accuracy of the sample results.

The following guidelines are recommended under EPA/CLP (U.S. EPA 1985) for use in evaluating data usability when the spike recoveries do not fall within control limits. These guidelines are summarized in the PSEP/PSDDA data review worksheet in Appendix B.

- If the spike recovery is greater than 125 percent and the reported sample results are less than the IDL, these data are acceptable for use.
- If the spike recovery is greater than 125 percent and the reported sample levels are greater than the IDL, flag the data as estimated (E) and indicate in the review narrative the potential bias in the results.
- If the spike recovery falls within the range of 30-74 percent and reportable quantities of analyte were detected, flag the data as estimated (E). In the review narrative, indicate the percent bias of the results.
- If an analyte is not detected in a sample and the spike recovery falls within the range of 30-74 percent, the detection limit may be biased low. In the review narrative, report that the detection limit for that sample set may be elevated and give an estimate of the bias. Flag the data for these samples as estimated (UG).
- If spike recovery results for sample results reported as less than IDL fall below 30 percent, the data should be reported as unusable (R). This result is indicative of severe analytical deficiencies, and the reviewer should state in the narrative that the possibility of a false negative exists and that the detection limits are elevated over what is reported.
- If the spike recovery results for data above detection limits fall below 30 percent, ~~If the spike recovery results for positive hit data fall below 30 percent,~~ the data should be reported as quantitatively questionable (Q). The reviewer should state in the narrative the results could be biased significantly low and the reported concentration is the minimum concentration at which the analyte is present.

When reviewing data for accuracy, both the CRM ~~SRM~~ results and matrix spike recoveries should be taken into consideration. Digestion efficiency is demonstrated by the CRM ~~SRM~~ recovery and, to a lesser extent, the matrix spike recovery. Consistently low recovery for analytes present in the CRM ~~SRM~~ can indicate incomplete digestion, loss of analyte during digestion, or loss of analyte while storing the digestate. Qualifying data solely on the basis of poor CRM ~~SRM~~ recoveries is not recommended, because the CRM ~~SRM~~ and samples may not be of the exact same chemical and physical nature. However, if low recovery is obtained for the matrix spikes as well as the CRM ~~SRM~~ for a particular element, data qualification or rejection is necessary. Contamination of samples, indicated by the method blank values, can affect CRM ~~SRM~~ and spike recoveries. Therefore, blank contamination should be considered when reviewing CRM ~~SRM~~ and spike recoveries, if the blank data show significant analyte contamination.

5.4.6 Duplicate Analysis

Objective—Duplicate analysis is performed to aid in the assessment of precision of sample results. ~~Duplicate analysis is performed to indicate the precision (percent difference) of sample results among laboratory and/or field duplicates.~~

Requirements—The following QA checks are required:

- One laboratory duplicate sample must be analyzed for each group of samples of a similar matrix (i.e., sediment, tissue) for each set of samples or for every 20 samples received, whichever is more frequent. If two different analytical methods are used to obtain the reported values of the same element for a set of samples (e.g., ICP or GFAA), duplicate samples must be run by each method used.
- Samples identified as field blanks cannot be used for duplicate sample analysis.
- A control limit of ±20 relative percent difference (RPD) should be used for sample values greater than 5 times the Project LOD.
- A control limit of ± the Project LOD should be used for samples less than 5 times the LOD.

The RPD for each component is calculated as follows:

$$RPD = \frac{D_1 - D_2}{(D_1 + D_2)/2} \times 100$$

where:

D_1 = First sample value

D_2 = Second sample value.

Evaluation Procedure—During data review, the reviewer should perform the following:

- Verify that the proper number of duplicates was analyzed and that results fall within the control limits.
- Spot check 10 percent of the calculations from the raw data to verify that results have been reported correctly. If any calculation errors are found, all replicate results should be recalculated.

Action—Actions taken as a result of duplicate analysis must be weighed carefully, because it is difficult to determine if poor precision is a result of nonhomogeneity of the sample, method defects, or laboratory technique. The guidelines and acceptance criteria (i.e., ±20 RPD) were designated under the EPA/CLP for water samples. The EPA/CLP requirement for soil samples, because of increased matrix effects, is ±35 RPD. Samples analyzed using PSEP protocols and having analyte concentrations at or near the LOD can be greatly influenced by sample nonhomogeneity and interferences. Therefore, samples with in-solution concentrations near the IDL and a difference between duplicates of 2-3 ppb can produce RPD values outside the control limits.

The following guidelines are recommended under CLP (U.S. EPA 1985) for use in evaluating data acceptability when the RPD between replicates does not fall within control limits:

- If the proper number of duplicates for each matrix has not been analyzed, reject the data.
- If duplicate analysis exceed the ±20 RPD flag the data as estimated (E) for samples with concentrations greater than 5 times the Project LOD. Best professional judgment should be used to evaluate duplicate analyses where the sample concentrations are at or near the IDL.
- If duplicate analysis for a particular analyte exceeds 100 RPD and the sample concentration in the duplicate is greater than 5 times the PSDDA-routinely-achievable LOD, the results should be considered quantitatively questionable and flagged with a Q.

A laboratory report sheet and Section IX of the PSEP/PSDDA worksheet are shown in Figure 5-5 as an example to aid in the review process.

5.5 OVERVIEW OF SPECIFIC INSTRUMENT REQUIREMENTS

This section describes QA/QC requirements specific to AA and ICP analyses. Laboratories are required at a minimum to perform analyses following these guidelines to ensure potential instrument and matrix problems are taken into consideration. ~~Laboratories are minimally required to perform analyses following these guidelines to ensure potential instrument and matrix problems are taken into consideration.~~ Many laboratories perform additional QC checks. Failure to adequately perform the procedures described below demonstrates a laboratory's inability to provide quality data for inclusion in the Puget Sound database.

5.5.1 Graphite Furnace Atomic Absorption Analysis

Objective—GFAA is susceptible to matrix interferences that can affect both precision and accuracy. Duplicate injections (burns) and analytical (post-digestion) spikes are used to detect the presence of matrix effects. The method of standard additions (MSA) is employed if the analytical spike percent recoveries are outside specified control limit ranges ~~(both enhancement and suppression) that could produce false results if not monitored. Duplicate injections and analytical spikes are required to determine the precision and accuracy of individual instrument readings. Method of standard additions (MSA) is used to analyze matrix-affected samples. Not all samples require MSA, and the duplicate injections and post-spike additions are used to monitor if MSA is required.~~

Requirements—The following QA checks are required:

- All furnace analyses, except those requiring full MSA, require duplicate injections and the average concentration should be reported. The raw data should contain all readings.

QA/QC Report
Duplicate Results
Metals
mg/Kg
Dry Basis

Sample Name: EBT01
Lab Code: 508-1

Relative Difference	Method	Control Limit	A	B	Average	%
Antimony	204.2	20%	0.35	0.32	0.34	8.8
Arsenic	206.2	20%	6.1	4.3	5.2	34.6
Cadmium	213.2	20%	0.453	0.334	0.394	30.2
Copper	220.1	20%	60	60	60	0.0
Lead	213.9	20%	49	48	48	0.0
Mercury	245.1	20%	0.38	0.36	0.37	2.7
Nickel	249.1	20%	48	48	48	0.0
Silver	272.2	20%	0.62	0.63	0.62	1.6
Zinc	289.1	20%	105	104	104	1.0

Figure 5-5a. Example of duplicate results (Figure 5-5a) and data review worksheet (Figure 5-5b)

IX. Laboratory Precision Evaluation

1 Number of duplicates analyzed. Required to analyze 1 per 20 samples.

Sample Matrix: Sediment Sample Number EBT01

Element	PLOD ug/L	PLOD mg/kg	Sample Result	Duplicate Sample Result	Criteria (RPD or ± PLOD)	Action
Antimony						
Arsenic		0.1	6.1	4.3	34.6 RPD	E
Cadmium		0.1	0.453	0.334	30.2 RPD	E
Copper						
Lead						
Mercury						
Nickel						
Silver						
Zinc						

Laboratory Duplicate Actions should be applied to all other samples of the same matrix type.

Actions: If both sample results are less than the PLOD, then laboratory precision is not evaluated. If either sample result is less than 5x the PLOD, then "E" results for elements whose absolute difference is > PLOD. If both sample results are greater than 5x the PLOD, then calculate the RPD. For sediment and tissue samples, "E" results for elements which have an RPD > 20%.

Calculation: $RPD = \frac{D1 - D2}{(D1 + D2)/2} \times 100$

NOTE:
PLOD - Project Limit of Detection
RPD - Relative Percent Difference
D1 - First sample value
D2 - Second sample value

Comments: _____

Figure 5-5b. Example of duplicate results (Figure 5-5a) and data review worksheet (Figure 5-5b)

- For concentrations greater than the Project LOD, duplicate injections must agree within ±20 RPD or a third injection is required.
- All analyses must fall within the calibration range.
- Each sample (including method duplicate, ~~CRM, SRM,~~ and blank) requires a single analytical spike to determine if MSA is necessary for quantitation.
- The post-spike concentration should be no more than twice the EPA/CLP contract-required detection limit (see Table 5-6). For low concentration samples, these spike levels are high; no other criteria are specified under PSEP protocols.
- The percent recovery of the spike determines how the sample must be quantitated. Figure 5-6 is a schematic description of the evaluation process.
- If the spike recovery is less than 40 percent, the sample must be diluted by a factor of 5-10 and rerun with another spike. Under CLP guidelines, which were adopted under PSEP protocols, this step must be performed only once. If, after the dilution, the spike recovery is still less than 40 percent, data are flagged with an "E" to indicate interference problems.
- If the spike recovery is greater than 40 percent and the sample absorbance or concentration is less than 50 percent of the spike, report the sample as less than the Project LOD or less than the Project LOD times any dilution factor.
- If the sample absorbance or concentration is greater than 50 percent of the spike and the spike recovery is between 85 and 115 percent, the sample should be quantitated directly from the calibration curve.
- If the sample absorbance or concentration is greater than 50 percent of the spike and the spike recovery is less than 85 percent or greater than 115 percent, the sample must be quantitated by MSA, as follows:
  - MSA data must be within the linear range established by the initial calibration curve.
  - The sample and three spikes must be analyzed consecutively for MSA quantitation. (The initial sample and spike data cannot be used.) Only single injections are required for MSA quantitation.
  - The spikes should be prepared at approximately 50, 100, and 150 percent of the sample absorbance. If the correlation coefficient is less than 0.995, the MSA analyses must be repeated.
  - The data for MSA quantitation should be recorded in the raw data with the slope, intercept, and correlation coefficient for the line. The results should also be recorded. Reported values obtained by MSA should be flagged by the laboratory. If the MSA has been rerun a second time and the correlation coefficient still is less than 0.995, the results should be flagged by the laboratory.

Evaluation Procedure—During data review, the reviewer should perform the following:

- Review GFAA raw data to verify that all analysis requirements have been met
- Verify results by recalculating at least 10 percent of the data for each parameter. If any calculation errors are found, all GFAA data should be recalculated.

Action—The LOD recommended under PSEP protocols will require laboratories to use GFAA for many elements of concern. The QC control limits were established under EPA/CLP requirements and present situations that will require best professional judgment when reviewing data obtained near the IDL. The following review guidelines should be applied:

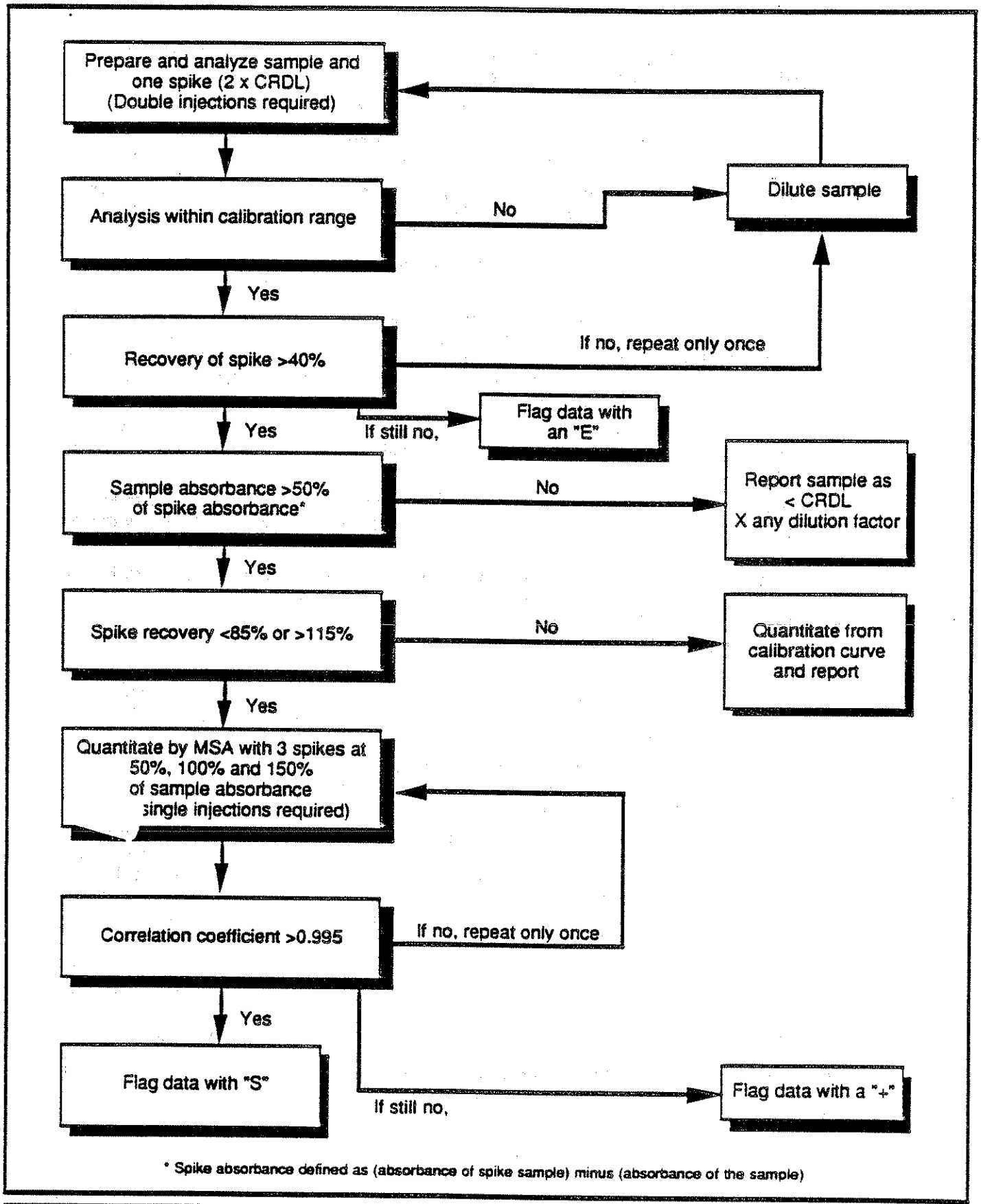

Figure 5-6. Furnace atomic absorption analysis schematic

- If duplicate injections have not been performed, reject the data.
- If duplicate injections are outside the control limits of ±20 percent relative standard deviation (RSD) or CV, and third injection was not analyzed, flag the data as estimated (E).
- If the third injection does not agree with either of the first two injections (±20 percent CV), flag the data as estimated (E).
- Apply best professional judgment to those samples approaching the minimum IDL. Very small differences in absorbance near the IDL can produce samples with duplicate injections outside the control limits, but with sample concentrations differing by only a few parts per billion. Determine the significance of the difference and decide if the data should be qualified. Consult an expert if unsure of the relationship between the duplicate injections and the relative absorbance values.
- If the analytical spike recovery is less than 40 percent and a dilution has not been analyzed, flag the data as estimated (E).
- If the post-digestion spike recovery is less than 10 percent, the data should be rejected (R).
- If MSA is required but has not been performed, flag the data as estimated (E).
- If the MSA correlation coefficient is less than 0.995, flag the data as estimated (E).

These actions are applied on a sample-to-sample basis. Only those samples not meeting these criteria are qualified. For example, one sample in a batch may require MSA, but the others can be quantitated directly. Section VI of the PSEP/PSDDA worksheet ~~in Figure 5-7~~ and two copies of raw data sheets are provided ~~in Figure 5-7~~ as examples of duplicate injection and MSA analysis.

5.5.2 Flame and Cold Vapor Atomic Absorption Analysis

FAA is not routinely used in analyzing samples under PSEP protocols because of the low LOD required. Cold vapor atomic absorption (CVAA) is used in analyzing samples for mercury. For FAA and CVAA, previous calibration requirements apply (i.e., ICV, CCV, ICB, CCA), but no post-digestion spikes or duplicate injections are required. The raw data should include information about the standards and their absorbancies so the reviewer can assess the reliability of the values. FAA readings of less than 0.010 absorbance units greater than the blank should be suspect. Analyses with such low absorbance readings should be performed by a more sensitive method (i.e., GFAA), and it is recommended the FAA sample values be rejected.

5.5.3 Inductively Coupled Plasma Analysis

Two QC checks are required for ICP analyses: interference check sample (ICS) and serial dilution analyses. The laboratory may perform other QC checks, but no additional checks are required under PSEP protocols. Section VI of the PSEP/PSDDA worksheet and laboratory sheets are shown in Figure 5-8 as examples of ICP review.

Objective—The ICP ICS analysis is performed to verify the laboratory's interelement and background correction factors.

SAMPLE NUMBER: 508: 1-5, 7-16	SERVICE REQUEST # : 89508
ANALYSIS FOR: Cd	METHOD: GFAA

DATA

Sample Pos.	Lab Code	Initial Sample (g) (ml)	Initial Dilution (ml)	Aliquot (ml)	Final Volume (ml)	(ug/l) measured	(mg/kg) Sample (actual)
1	ICV (9.94)			1	500	9.59	
2	ICB					<0.1	
3	CCV (10.00)					9.58	
4	PBS	—	30	1	5	<0.1	~~<.005~~
5	PBSA (+10.0)	—				11.27	
6	LCSS	2.00				5.27	~~26.4~~ .40
7	LCSSA					14.72	94.5%
8	508-1					~~4.50~~ 4.49	~~.237~~ MSA
9	508-1A					16.28	118%
10	508-1D					4.48	
11	508-1DA					16.09	116%
12	508-1S			1	20	11.58	.247
13	CCV					10.31	
14	CCB					<0.1	
15	508-2	2.00	30	1	5	4.90	MSA
16	508-2A					16.64	117%
17	508-3					5.28	MSA
18	508-3A					17.72	124%
19	508-4					5.24	.395
20	508-4A					16.96	114%
21	508-5					5.19	.389
22	508-5A					16.63	114%
23	508-6					5.05	See MSA
24	508-6A					16.76	117%
25	CCV					10.81	

COMMENTS: ICV at ~~42~~ 4920 23 5/12/89

SPIKE LEVEL: 150 ug/l

ANALYST	DATE: 5/12/89	TIME:	NB Number:
WITNESS	DATE:		PAGE Number: (1)

Computer File Name is: viv01wk.sht

Figure 5-7a. Example of GFAA laboratory worksheet (Figure 5-7a), MSA laboratory raw data print out (Figure 5-7b), and data review worksheets (Figures 5-7c and 5-7d)

BLANK	0.0		0.005	0.005	
ADDITION 1	150.0	0.0	0.128	0.128	
ADDITION 2	300.0	0.0	0.217	0.217	
ADDITION 3	450.0	0.0	0.288	0.288	
SAMPLE 26	91.9	0.0	0.048	0.048	r=0.9971
BLANK	0.0		0.001	0.001	
ADDITION 1	150.0	0.0	0.080	0.080	
ADDITION 2	300.0	0.0	0.128	0.128	
ADDITION 3	450.0	0.0	0.196	0.196	
SAMPLE 27	55.7	0.0	0.022	0.022	r=0.9979
BLANK	0.0		-0.002	-0.002	
ADDITION 1	150.0	0.0	0.089	0.089	
ADDITION 2	300.0	0.0	0.152	0.152	
ADDITION 3	450.0	0.0	0.210	0.210	
SAMPLE 28	66.2	0.0	0.026	0.026	r=0.9998
BLANK	0.0		-0.002	-0.002	
ADDITION 1	150.0	0.0	0.087	0.087	
ADDITION 2	300.0	0.0	0.151	0.151	
ADDITION 3	450.0	0.0	0.216	0.216	
SAMPLE 29	66.0	0.0	0.029	0.029	r=0.9997
BLANK	0.0		0.001	0.001	
ADDITION 1	150.0	0.0	0.103	0.103	
ADDITION 2	300.0	0.0	0.180	0.180	
ADDITION 3	450.0	0.0	0.249	0.249	

SAMPLE	CONC ppb	%RSD	MEAN ABS	READINGS	
SAMPLE 30	72.1	0.0	0.036	0.036	r=0.9997
BLANK	0.0		0.000	0.000	
ADDITION 1	150.0	0.0	0.080	0.080	
ADDITION 2	300.0	0.0	0.140	0.140	
ADDITION 3	450.0	0.0	0.199	0.199	
SAMPLE 31	59.9	0.0	0.024	0.024	r=0.9999
BLANK	0.0		-0.001	-0.001	
ADDITION 1	150.0	0.0	0.125	0.125	
ADDITION 2	300.0	0.0	0.203	0.203	
ADDITION 3	450.0	0.0	0.276	0.276	
SAMPLE 32	93.3	0.0	0.047	0.047	r=0.9999
BLANK	0.0		0.000	0.000	
ADDITION 1	150.0	0.0	0.081	0.081	
ADDITION 2	300.0	0.0	0.146	0.146	
ADDITION 3	450.0	0.0	0.207	0.207	
SAMPLE 33	58.0	0.0	0.026	0.026	r=0.9995
BLANK	0.0		-0.001	-0.001	
ADDITION 1	150.0	0.0	0.091	0.091	
ADDITION 2	300.0	0.0	0.166	0.166	
ADDITION 3	450.0	0.0	0.222	0.222	
SAMPLE 34	66.5	0.0	0.029	0.029	r=0.9986
BLANK	0.0		0.001	0.001	
ADDITION 1	150.0	0.0	0.082	0.082	
ADDITION 2	300.0	0.0	0.153	0.153	
ADDITION 3	450.0	0.0	0.205	0.205	
SAMPLE 35	58.2	0.0	0.024	0.024	r=0.9984
BLANK	0.0		0.001	0.001	

Figure 5-7b. Example of GFAA laboratory worksheet (Figure 5-7a), MSA laboratory raw data print out (Figure 5-7b), and data review worksheets (Figures 5-7c and 5-7d)

VI. Instrument Specific QA Requirements
CFAA QC Analysis/Method of Standard Additions

A. Duplicate Injections

✓ Duplicate injections were performed for all samples and agreed within ±20% Relative Standard Deviation (RSD). The RSD or Coefficient of Variation (CV) is calculated by dividing the standard deviation by the mean and multiplying by one hundred.

____ Duplicate injections were not performed for the following samples/elements:
Most were okay - Those outside were analyzed by MSA.

Action: Reject (R) data.

____ Duplicate injections were outside the ±20% RSD limit and a third injection was not performed for samples with an absorbance >50% of the spike concentration as required for the following samples/elements:

Action: Estimate (E) data and summarize the lab's deficiency in the QA Summary.

____ Duplicate injections did not agree within ±20% RSD and the third injection did not agree with either of the first two injections (±20% RSD) for the following samples/elements:

Figure 5-7c. Example of GFAA laboratory worksheet (Figure 5-7a), MSA laboratory raw data print out (Figure 5-7b), and data review worksheets (Figures 5-7c and 5-7d)

B. Analytical Spike Percent Recoveries

✓ok One-point analytical spikes were performed for all samples and the spike recoveries met the 85-115% recovery criteria (Accept data).

_____ The analytical spike recoveries were less than 10% for the following samples/elements:

_____

_____

_____

Action: Reject (R) data.

_____ Spike recoveries were 10-40% and the laboratory did not dilute and re-analyze the following samples/elements:

_____

_____

_____

Action: Estimate (E) positive results and reject (R) non-detected results.

_____ Spike recoveries were 10-40% after the following samples/elements were diluted and re-analyzed:

_____

_____

_____

Action: Estimate (E) positive results and reject (R) non-detected results.

_____ Sample Concentrations were less than 50% of the spike value and spike recoveries were greater than 40% (Accept data.)

✓ Sample Concentrations were greater than 50% of the spike value, and spike recoveries did not meet the 85-115% recovery criteria. The following actions should be taken:

_____ Method of Standard Addition (MSA) was not performed as required for sample numbers/elements: _____

_____

_____

_____

Action: Estimate (E) data and summarize the lab's deficiency in the QA Summary.

✓ MSA was used to quantitate analytical results for the following samples/elements when correlation coefficients were greater than 0.995:

508-1, 508-2, 508-3, 508-6

_____

_____

Action: Accept data.

Figure 5-7d. Example of GFAA laboratory worksheet (Figure 5-7a), MSA laboratory raw data print out (Figure 5-7b), and data review worksheets (Figures 5-7c and 5-7d)

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4

ICP INTERFERENCE CHECK SAMPLE

Lab Name: Contract:

Lab Code: Case No.: 11758 SAS No.: SDG No.: MX2185

ICP ID Number: TJA-1 ICS Source: ICSAB-EPA-LV

Concentration Units: ug/L

Analyte	True Sol. A	True Sol. AB	Initial Found Sol. A	Initial Found Sol. AB	Initial Found %R	Final Found Sol. A	Final Found Sol. AB	Final Found %R
Aluminum	511000	508000	526830	511606.8	100.7	530884	521786.5	102.7
Antimony			7	-9.0	0.0	5	-12.8	0.0
Arsenic								
Barium		483	5	474.4	98.2	6	475.4	98.4
Beryllium		474	0	442.2	93.3	1	433.3	91.4
Cadmium		909	7	911.9	100.3	3	946.1	104.1
Calcium	476000	470000	472545	454416.1	96.7	455267	452698.1	96.3
Chromium	48	513	40	484.0	94.3	39	483.0	94.2
Cobalt		478	-5	438.7	91.8	-6	431.9	90.4
Copper		534	3	498.8	93.4	11	494.9	92.7
Iron	219000	211000	205852	200891.1	95.2	199100	198628.0	94.1
Lead		4850	-97	4487.4	92.5	-101	4475.[illegible]	92.3
Magnesium	513000	513000	511411	497418.3	97.0	513045	504917.3	98.4
Manganese		470	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Mercury								
Nickel		916	[illegible]	863.1	94.2	[illegible]	857.1	93.6
Potassium			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Selenium								
Silver		934	-7	944.2	101.1	-5	969.1	103.8
Sodium			218	189.2	[illegible]	226	212.4	0.0
Thallium								
Vanadium		475	100	559.9	117.9	98	552.2	116.3
Zinc		973	-13	852.2	87.6	6	877.2	90.2

11758 00919

Figure 5-8a. Example of ICP interference check sample results (Figure 5-8a), ICP serial dilution results (Figure 5-8b), and data review worksheets (Figures 5-8c and 5-8d)

U.S. EPA - CLP

9

ICP SERIAL DILUTIONS

EPA SAMPLE NO.

MX2185L

Lab Name: Contract:

Lab Code: Case No.: 11758 SAS No.: SDG No.: MX2185

Matrix (soil/water): WATER Level (low/med): LOW

Concentration Units: ug/L

Analyte	Initial Sample Result (I)	C	Serial Dilution Result (S)	C	% Difference	Q	M
Aluminum	1318.79		1336.55		1.3		P
Antimony	130.55		100.00	U	100.0		NR
Arsenic							P
Barium	837.77		849.40	B	1.4		P
Beryllium	14.20		14.85	B	4.6		P
Cadmium	27.80		34.75		25.0		NR
Calcium	9406.67		9630.80	B	2.4		P
Chromium	90.66		95.60		5.4		P
Cobalt	105.04		107.30	B	2.2		P
Copper	137.93		130.70		5.2		P
Iron	1719.60		1621.80		5.7		P
Lead							P
Magnesium	5935.72		6034.00	B	1.7		P
Manganese	291.96		294.55		0.9		P
Mercury							CV
Nickel	207.16		237.40		14.6		P
Potassium	6496.51		11330.20	B	74.4		NR
Selenium							P
Silver	3.00	U	15.00	U			NR
Sodium	13114.40		15337.25	B	1.9		P
Thallium							P
Vanadium	360.05		358.70		0.4		P
Zinc	458.72		471.75		2.8		P

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Figure 5-8b. Example of ICP interference check sample results (Figure 5-8a), ICP serial dilution results (Figure 5-8b), and data review worksheets (Figures 5-8c and 5-8d)

ICP Interference Check Sample Analysis

The ICP interference check sample analysis is performed to verify the contract laboratories' interelement and background correction factors.

✓ Interference QC samples were run at the beginning and end of each sample analysis run (or a minimum of twice per 8 hour working shift, whichever is more frequent) and were within the control limits specified in PSEP.

_____ Interference QC samples were run, but did not meet the control limits.

In general, the sample data can be accepted without qualification if the concentrations of aluminum, calcium, iron, and magnesium are less than 50% of the ICP Interference check sample concentrations.

Note: The 20% contract limit (80-120%) is based on the true value for EPA standards, and on the mean value (run at least five times) for non-EPA standards.

Remarks: Negative values found for lead but samples analyzed by GFAA.

Figure 5-8c. Example of ICP interference check sample results (Figure 5-8a), ICP serial dilution results (Figure 5-8b), and data review worksheets (Figures 5-8c and 5-8d)

QC Analysis Serial Dilution Results

Serial dilution analysis enables the reviewer to evaluate whether significant physical or chemical interferences exist due to sample matrix for samples analyzed by ICP. Sample results for elements analyzed and quantitated by Furnace Atomic Absorption should not be evaluated.

_____ Serial Dilutions were performed for each matrix and results of the diluted sample analysis agreed within ten percent of the original undiluted analysis.

_____ Serial Dilutions were not performed for the following:

_____

_____

✓ Serial Dilutions were performed, but analytical results did not agree within 10% for analyte concentrations greater than 10x the IDL after dilution. The following elements were evaluated for Matrix interferences:

Dilution Factor (DF): 10 Matrix: water

Element	IDL	IDL x 10	Sample #: MX2185	Serial Diluted Sample Result		Action
				without DF	Times DF	
Aluminum						
Cadmium	2.5	25.	27.80	3.475	34.75	E
Calcium						
Chromium						
Iron						
Lead						
Magnesium						
Nickel						
Silver						
Zinc						
Other:						

Actions: All data for samples of the same matrix for that element should be estimated (E) when the serial dilution results do not meet contractual requirements.

Figure 5-8d. Example of ICP interference check sample results (Figure 5-8a), ICP serial dilution results (Figure 5-8b), and data review worksheets (Figures 5-8c and 5-8d)

Requirements—The following QA checks are required:

- The ICP ICS sample must be run at the beginning and end of each sample analysis run (or a minimum of twice per 8-hour working shift), whichever is more frequent. The ICS sample can be obtained from EPA or from a commercial source. ~~The ICS sample can be obtained from EPA if available.~~
- Results for the check sample must fall within the ±20 percent control limit of the true value.
- If no ICP ICS is available from EPA, the laboratory must prepare a sample with analyte and interferant concentrations at the levels described under PSEP protocols.
- If the ICS exceeds the control limits, the laboratory must terminate analysis, correct the problem and reanalyze following all calibration and verification procedures.

Evaluation Procedure—During data review, the reviewer should perform the following:

- Review raw data and verify that the results meet the required criteria
- Spot check raw data (ICP printout) to verify the accuracy of the recoveries reported
- Spot check raw data for negative results
- If the results do not meet the required criteria, verify that all affected samples were reanalyzed.

Action—The ICS is designed to measure the laboratory's ability to analyze samples with high concentrations of analytes that produce spectral interferences in ICP analyses (i.e., aluminum, calcium, iron, and magnesium). There are two solutions analyzed to determine the instrument's interference correction factors. One solution contains aluminum, calcium, iron, and magnesium in very high concentrations (200,000-500,000 mg/L). The second solution contains the same high concentrations of interferents plus smaller concentrations of other elements analyzed by ICP. No elements should be detected in the first solution other than the four interferents. The recovery for the elements analyzed in the second solution should be ±20 percent of the true value. Listed in Table 5-8 are possible problems encountered when reviewing ICP data. For data sets exhibiting problems, an expert should be consulted to interpret data quality and usability.

5.5.4 Serial Dilution Analysis

Objective—Serial dilution analysis is required to ascertain whether significant physical or chemical interferences exist due to sample matrix.

Requirements—The following QA checks are required:

- One sample from each group of samples of similar matrix (i.e., sediment, tissue) or for each group of samples, whichever is more frequent, must undergo at least one serial dilution.

TABLE 5-8. GUIDELINES FOR REVIEWING ICP INTERFERENCE CHECK SAMPLE DATA

Results	Action
Recovery for all elements within ±20% control limits.	Accept sample data. Continue review process.
Recovery for some elements outside control limits. Reported sample results have interferant concentrations considerably lower than interference check sample (ICS) concentrations.	Reject data. Samples should be reanalyzed with instrument properly calibrated.
Recovery for some elements outside control limits. Reported sample results have interferant concentrations comparable or greater than respective levels in the ICS.	Consult an expert. Interelement interferences may be significant.
Positive or negative results for elements not present in the ICS, but the four common interferents present in the samples in concentrations significantly less than their respective concentrations in the ICS (i.e., <50%). All other required control limits met.	Data acceptable without further evaluation. Continue review process.
Positive or negative results for elements not present in the ICS. Samples have comparable or higher concentration of interferents.	Consult an expert. Interelement interferences may be significant.

- Results of the diluted sample analysis and the original must agree within 10 percent. The 10-percent criterion applies only if the analyte concentration is minimally a factor of 10 above the IDL after dilution. If the analyte concentration is greater than 10 times the IDL, and the dilution analysis is not within 10 percent, a chemical or physical interference effect should be suspected.

Evaluation Procedure—During data review, the reviewer should perform the following:

- Review raw data and verify that serial dilutions have been analyzed at the proper frequency for each matrix
- Spot check raw data and verify that serial dilution analysis results compare within 10 percent.

Action—Following data review, the reviewer should perform the following:

- If the 10 percent criterion is not met, and sample analyte concentration is greater than 10 times the IDL after dilution, flag data as estimates (E) for the element
- If serial dilution was not performed, consult an expert as to data usability.

5.6 REFERENCES

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6. QUALITY ASSURANCE FOR SEMIVOLATILES IN SEDIMENTS

6.1 INTRODUCTION

Analysis of semivolatile organic compounds encompasses several classes of compounds including aromatic hydrocarbons, halogenated ethers, organic acids and bases, chlorinated pesticides, and polychlorinated biphenyls (PCB). ~~and PCB.~~ Difficulty in analysis of semivolatiles arises from several factors: sample interferences, analytical losses during extract cleanup and concentration, confirmation of specific compounds in complex extracts (especially with GC/ECD), and obtaining adequate confirmation spectra using mass spectrometry.

A summary of frequencies and control limits of various QA elements of interest when reviewing semivolatile organic compound data is shown in Table 6-1.

6.2 UNIQUE SAMPLING REQUIREMENTS FOR SEMIVOLATILE ANALYSIS

Sample collection for semivolatile analysis must be conducted in a manner that ensures samples are free of contamination. All sampling equipment should be washed with detergent, rinsed with distilled, deionized water (DDW), and rinsed with solvents (three times). Samples should be stored in jars with polytetrafluoroethylene (PTFE)-lined lids. Between samples, the sampling equipment should be rinsed with water, then solvent rinsed to protect against cross contamination. The sample jars should be washed with detergent, rinsed with DDW, and combusted at 400° C for 4 hours. Samples should be frozen at -20° C as soon as possible after collection.

An air pocket in the sample container will allow room for expansion of the sample during freezing and will reduce the possibility of vessel breakage during storage. Samples should be shipped on dry ice. The total time between sample collection and analysis should not exceed 6 months for semivolatile analysis. Extracts should be analyzed within 40 days of the extraction date.

6.3 DATA COMPLETENESS AND FORMAT

All deliverables specified in the SOW should be confirmed upon receipt of the data package. Although complete review of the data probably will not occur immediately upon data receipt, review will be facilitated if all necessary documentation is available. If documentation completeness is not checked until long after data delivery, laboratory staff will be less likely to recall details of the project, and retrieving documents from laboratory files could be time consuming. Often omissions from the data package result from oversights rather than the laboratory's inability to produce missing items.

The data set is considered complete when all items are present (as specified in the SOW) or are accounted for in the cover letter. It is recommended that the following items be included for proper data validation by independent QA review and should always be specified in the original SOW:

- A cover letter referencing or describing the procedure used (noting any procedure modifications) and any analytical problems encountered
- Reconstructed ion chromatograms for analyses for each sample
- Mass spectra of detected target compounds for each sample analyzed by GC/MS and library spectra of all target compounds.

TABLE 6-1. RECOMMENDED FREQUENCIES AND CONTROL LIMITS FOR SEMIVOLATILE QA SAMPLES

Analysis Type	Frequency of Analysis[a]	Control Limit
Method blanks	One per extraction batch or one per 12-hour shift (whichever is most frequent)	Phthalates: 5 μg total or 50% of analyte concentration in samples Other organic compounds: 2.5 μg total or 5% of analyte concentration in samples
Certified reference materials[b]	<50 samples: one per set of samples submitted to laboratory >50 samples: one per 50 samples analyzed	95% confidence interval for certified reference material (±1.96sSD)
Matrix spikes	Not required if complete isotope dilution used <20 samples: one per set of samples submitted to laboratory ≥20 samples: 5% of total number of samples	50% recovery
Analytical replicates	<20 samples: one per set of samples submitted to laboratory ≥20 samples: one triplicate and additional duplicates for a minimum of 5% total replication	±100% coefficient of variation (for >2 replicates) or ±100% ~~RSD~~ RPD (for duplicates)
Surrogate spikes	Every sample	50% recovery (10% if isotope dilution is used)
Initial calibration	Before any samples are analyzed, after each major disruption of equipment, and when ongoing calibration fails to meet criteria	20% coefficient of variation; 30% for highly polar compounds or other analytes at the discretion of the QA reviewer
Ongoing calibration	At the beginning of each work shift, every 10-12 samples or every 12 hours (whichever is more frequent), and at the end of each shift for GC/MS and GC/FID. At the beginning of each work shift, every 6 samples or every 6 hours (whichever is less frequent), and at the end of each shift for GC/ECD.	25% of initial calibration for GC/MS; 15% of initial calibration for GC/ECD

[a] Frequencies listed are minimums; some programs may require higher levels of effort.

[b] As available.

- GC/ECD and/or GC/FID chromatograms for each sample
- Raw data quantification reports for each sample
- A calibration data summary reporting calibration range used (and DFTPP spectra and quantification report for GC/MS analyses)
- Final sample volumes and dilution factors, sample size wet-to-dry ratios, and IDL
- Analyte concentrations with reporting units identified (two significant figures unless otherwise justified)
- Quantification of all analytes in method blanks (μg/sample)
- A list identifying the method blanks associated with each sample
- Tentatively identified compounds (if requested) and methods of quantification (include spectra)
- Recovery assessments and a replicate sample summary (laboratories should report all surrogate spike recovery data for each sample; the range of recoveries should be included in reports using these data)
- Data qualification codes and definitions.

The data should be reported on standard forms so different data sets are of uniform format. This uniformity aids in both internal and external QA review and data validation. Unless otherwise specified, the data package should be complete to avoid misinterpretations based on missing information.

6.4 OVERVIEW OF EXTRACTION, EXTRACT CLEANUP, AND INSTRUMENTAL ANALYSIS

In this section, commonly used analytical techniques and procedures for analysis of semivolatile organic compounds are presented in the approximate analytical sequence. Different methods can be used to attain acceptable results. This list of techniques is not complete, as it concentrates on procedures generally used by laboratories in the Northwest.

During QA review, the reviewer should assess if the analytical scheme is reasonable, considering the target compounds. Most methods used are widely accepted and do not present problems. Modifications of standard procedures merit attention. For example, when less selective GC detectors are substituted for GC/MS, a more thorough cleanup is required to reduce interferences during analysis. GC/MS is preferred for analysis of nonchlorinated analytes; if GC/FID is used, interfering compounds must be removed from the extract. For example, fatty acid methyl esters can present an interference problem for GC/FID analyses of polynuclear aromatic hydrocarbons (PAH) if not removed during extract cleanup.

6.4.1 Extraction

The solvent used during extraction, liquid chromatography, and GC analysis should be chosen so all target compounds are efficiently extracted and carried through the entire analytical scheme. Polarity and volatility are primary factors in determining solvent suitability. Often mixtures of polar and nonpolar solvents are used when trying to extract acid/base/neutral (A/B/N) compounds. According to the organic chemistry maxim "like dissolves like" (i.e., nonpolar compounds are more soluble in nonpolar solvents and polar compounds are more soluble in polar solvents), solvent mixtures containing both nonpolar and polar solvents are more likely to carry all compounds of interest. When solid samples contain water, a polar solvent should be used to permeate the sample. Common extraction solvents are methylene chloride (relatively nonpolar) and acetone or methanol (more polar solvents). The reviewer should verify that the solvents used do not eliminate compounds or classes of compounds during the procedure. Also, boiling points of the solvents should not approach boiling points of low molecular weight semivolatile target compounds.

The extraction of analytes of interest is commonly performed using sonication, Soxhlet extraction, and shakers or rollers. During sonication, a disrupter horn is used to agitate the sample and facilitate partitioning of organic compounds into the solvent phase. Sonication is efficient and fast. Because sonication is typically performed in an open beaker, the possibility exists for sample loss or laboratory contamination. Also, filtration or centrifugation is required to separate the extract from the sediment. Soxhlet extraction is carried out in a closed apparatus in which solvent is cycled through a permeable thimble containing the sample; the solvent cycling is driven by heating and condensation. When Soxhlet extraction is terminated, the sediment and extract are in distinct phases in the apparatus and do not require filtration or centrifugation. Soxhlet extraction is a very efficient method of extraction. Disadvantages of Soxhlet extraction are the time required for efficient extraction (approximately 16 hours) and the possibility of channeling occurring if the sample is not stirred. Shaker and roller table extraction are cold extraction techniques. The shaker or roller table mixes the sample throughout the entire extraction.

Because water from samples is typically contained in extracts (unless samples are dried prior to extraction), the water phase must be removed from the extract by liquid-liquid partitioning (e.g., in a separatory funnel), or by adding sodium sulfate. Residual water can cause considerable difficulties in subsequent steps (e.g., column chromatography, extract concentration). If liquid-liquid partitioning is not performed carefully (e.g., with appropriate pH adjustments), resultant losses of polar analytes can occur.

Anhydrous sodium sulfate ~~Sodium sulfate~~ removes water by forming hydrous sodium sulfate. This method is used to remove water remaining in the organic layer after partitioning. Sodium sulfate is added to the extract until the extract flows freely when swirled or it is passed through a column containing sodium sulfate.

6.4.2 Sulfur Removal

Sediments often contain elemental sulfur, which interferes with GC/ECD analysis. If crystals are present or if the GC/ECD chromatogram shows interference, elemental sulfur may be present in the sample. Sulfur can be removed with metallic mercury, activated copper, or by the tetrabutyl ammonium (TBA)-sulfite method of the EPA/CLP (U.S. EPA 1988). The mercury method is efficient and easy. Problems include the health hazards of working with mercury, the difficulty of recovering mercury when it has been dispersed by high energy agitation, and disposal of the waste mercury. Copper (either in granular form or turnings) is also efficient and easy to work with. A disadvantage of using copper is the potential loss of analytes (e.g., as indicated by reports of loss of mercaptans and possible loss of heptachlor). The TBA-sulfite method involves the addition to the extract of TBA-sulfite in an aqueous phase, requiring a subsequent partitioning step to separate the solvent and aqueous phases. As with any additional procedural step, the potential for analytical losses increases. Depending on the method used, sulfur removal can be performed at any of several stages of analysis (e.g., during extraction or during column chromatography).

6.4.3 Cleanup and Separation

Extracts are fractionated and cleaned up using liquid chromatography. Acceptable methods are gravity column chromatography and high-performance liquid chromatography (HPLC). Column chromatography involves eluting the sample extract through silica gel, alumina, a polymer [gel permeation chromatography (GPC)], or a combination of these. GPC polymers (e.g., Bio-Beads SX-3, Sephadex LH-20) separate macromolecules from the lower molecular weight compounds of interest, thus eliminating biological macromolecules that may cause interferences. Because GPC columns are reusable, it is necessary to calibrate them regularly. When GPC columns degrade, the analytes may elute at a different retention volume than expected (e.g., in the biological macromolecule fraction), and thus may be discarded rather than collected. For this reason, QA review of the documentation for column calibration is strongly recommended. Silica gel and alumina columns are used to separate different classes of compounds (e.g., PAH from polar organic

compounds). Calibration of silica and alumina columns should be checked by the laboratory for each batch of adsorbent and whenever laboratory conditions change significantly, because adsorbent properties vary considerably with moisture content, which in turn may vary with laboratory conditions (especially temperature and humidity). In general, when multiple column elutions are performed, it is likely at the expense of analyte recovery.

HPLC has recently been used to fractionate and clean up sample extracts (Krahn et al. 1988). This method uses two preparatory gel-permeation columns (Phenomenex Phenogel, 100Å) in series. HPLC is capable of performing rapid, sharp separations in small volumes of solvent (total run time, including column cleanup, is 20 minutes). Preparative columns allow samples to be analyzed at low back-pressures and with longer column life.

6.4.4 Extract Concentration

Concentration of fractions can occur at several stages of analytical procedures and presents the risk of analyte loss. Laboratories prefer rapid methods, but should not compromise analyte recoveries. Accepted methods for concentrating extracts to ≥2 mL include rotary evaporation and Kuderna-Danish boiling apparatus; for reducing volumes of several milliliters to final extract volumes (e.g., 0.5 to 1.0 mL), blowing down extracts with purified, inert gas (e.g., nitrogen) is commonly performed. Regardless of the method used for evaporation, samples must never be evaporated to dryness. The recovery of the more volatile analytes drastically decreases when sample extracts are allowed to go to dryness.

6.4.5 Instrumental Analysis

Instrumental analysis of semivolatile organic compounds is performed by GC. The gas chromatograph ideally separates the mixture of contaminants into resolved analyte peaks by passing the sample through a chromatographic column at a specified temperature and carrier gas flow rate. Capillary columns provide superior resolution as compared with packed columns and are strongly recommended. Three kinds of detectors are typically used for analyzing semivolatile compounds. A GC/MS is often used for all A/B/N compounds. A GC/ECD is often used for detection of halogenated analytes (e.g., chlorinated hydrocarbon pesticides and PCB). GC/FID is used for analysis of nonchlorinated compounds.

6.4.6 Commonly Used Analytical Protocols

Although many analytical procedures are used in commercial and government-owned laboratories to analyze semivolatile organic compounds, the QA reviewer is likely to encounter a limited range of procedures. The EPA/CLP method is commonly used for work sponsored by EPA and other regulatory agencies. The National Oceanic and Atmospheric Administration's (NOAA) national monitoring program generates environmental monitoring data by a different standardized procedure. Brief descriptions of the EPA/CLP and NOAA protocols are presented below.

U.S. EPA/CLP Method—EPA has developed methods for the analysis of semivolatile organic compounds as part of the CLP (U.S. EPA 1988). The CLP protocol calls for extraction of a ~~wet~~ sediment/sodium sulfate mixture by sonication using 1:1 methylene chloride:acetone. The extract is filtered, concentrated by Kuderna-Danish apparatus, and run through a GPC column (optional; Bio Beads SX-3). The extract is then split so the PCB/pesticide fraction can be further cleaned up over alumina. If elemental sulfur is present, the TBA-sulfite method is used to remove it. Analysis is performed by GC/ECD for PCB and chlorinated pesticides and by GC/MS for other semivolatile organic priority pollutants (i.e., A/B/N compounds).

NOAA Method—NOAA has developed a method that is used for its national monitoring program, Status & Trends (MacLeod et al. 1985). The method involves extraction of wet sediments on a roller table with methanol, then 1:1 methanol:methylene chloride, then three times with methylene chloride. The combined acidified extract is partitioned into the methylene chloride layer in a separatory funnel, and further dried with sodium sulfate. The extracts are concentrated using a Kuderna-Danish apparatus. Smaller volumes are reduced by blowing down with a purified stream of nitrogen gas. Cleanup and fractionation of sediment extracts is performed with liquid chromatography over silica gel/alumina in the same column. Elemental sulfur is removed by adding granulated copper to the top of the silica/alumina column. If sulfur is still present, copper is added during the concentration step after liquid chromatography. Analysis is performed by GC/ECD for the pesticides and PCB. Ten to twenty percent of these samples are analyzed by GC/MS to verify the peaks used for quantification with GC/ECD. PAH are analyzed by GC/FID (with 10-20 percent verification by GC/MS) or by GC/MS.

NOAA HPLC Method—NOAA has recently modified the MacLeod et al. (1985) method using HPLC for cleanup and separation (Krahn et al. 1988). Extraction and concentration are the same as MacLeod et al. (1985), except for the elimination of the methanol steps. Water is removed by adding sodium sulfate to the extract mixture. A prefiltering of the extract through glass wool is required before HPLC. The samples are eluted isocratically (i.e., using a constant composition of elution solvent) with methylene chloride for 20 minutes. The advantages of the HPLC method are it is automatable, quick, does not require column preparation, and is more precise than gravity columns because chromatographic conditions can be monitored. Initial cost is the primary disadvantage of the HPLC system.

6.4.7 Modifications of Routine Methods

The QA reviewer may encounter procedures that deviate somewhat from routine analytical methods (e.g., the EPA/CLP method). Methods of particular interest are the isotope dilution technique (relevant to the analysis of A/B/N compounds by GC/MS) and procedures for PCB identification and quantification.

The Isotope Dilution Technique—The isotope dilution technique (described for water samples in EPA Method 1625B; U.S. EPA 1984) is a procedure in which the surrogate compounds are stable isotope (deuterium or ^{13}C)-labeled analogs for all (or nearly all) of the target compounds (as available). A detailed discussion of the technique is not possible in this document. However, it is important to understand that the method entails primarily surrogate compound addition and quantification techniques. The isotope dilution technique does not involve specific methods of sediment extraction and extract cleanup. During quantification, concentrations of target compounds are recovery-corrected to account for the observed recovery of the associated surrogate standard. For example, in a sample in which d_8-naphthalene recovery was 60 percent, the detected concentration of unlabeled naphthalene would be multiplied by 1.67 (i.e., 100/60). This recovery correction is appropriate because the surrogate compounds, which are chemically very similar to the target compounds, behave similarly to the target compounds during chemical analysis. Advantages of the isotope dilution technique include the following:

- For a given set of sediment samples, data subjected to compound-specific recovery correction have resulted in better accuracy and precision than data that have not been recovery-corrected. Thus, by accounting for chemical-specific analytical losses on a sample-by-sample basis, the method addresses precision as well as accuracy. A comparison of recovery-corrected and uncorrected data for a sediment reference material is presented in Figure 6-1 (data from PTI 1988). Although performance was relatively good using uncorrected data, improvement in accuracy for more volatile compounds (e.g., naphthalene and 2-methylnaphthalene) is apparent in Figure 6-1. Precision for naphthalene was also better for the recovery-corrected

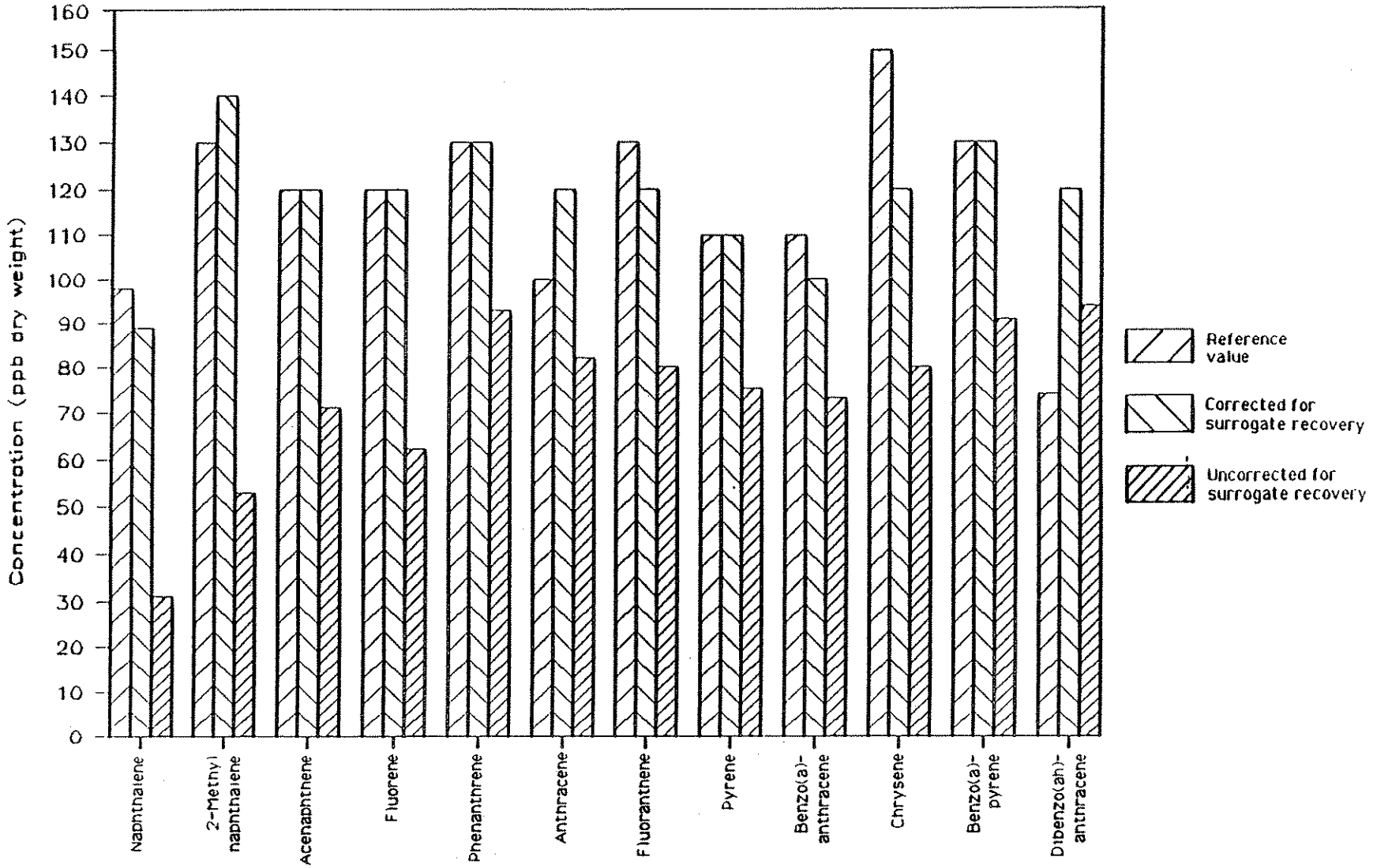


Figure 6-1. Results for a sediment reference material with and without surrogate recovery corrections

data; based on four replicates, uncorrected data had a 36 percent CV for naphthalene, whereas the corrected data had a CV of 15 percent. More dramatic improvements have been observed in other studies.

- Because stable isotopically labeled surrogates are available for the majority of EPA priority pollutants (and for a wide range of other compounds), use of isotope dilution yields considerable QA information not available from more conventional analyses. Specifically, surrogate recoveries are available for most target compounds in all samples when using isotope dilution.
- Isotope dilution precludes the need for matrix spikes.

Disadvantages of the isotope dilution technique include the following:

- Isotope dilution analyses are typically more expensive than more conventional analyses, in part because of the cost of labeled compounds and software required for quantification (recovery correction).
- For relatively uncontaminated samples in which target compounds are not present or occur at concentrations near detection limits, the presence of labeled surrogates can confound GC/MS data interpretation for two reasons: (1) unlabeled impurities, which have been reported for a few compounds by commercial laboratories, could potentially result in false positives, and (2) spectral interferences (in particular, secondary ions of the labeled compounds that are identical to quantification ions of the unlabeled compounds) could present considerable problems in compound identification and quantification when the labeled compound is present at a far higher concentration than the target compound.
- Interferences resulting from the labeled compounds can complicate GC/MS searches for tentatively identified compounds and GC/ECD searches for PCB and chlorinated pesticides (if the same extract is used for GC/MS and GC/ECD analyses). For this reason, separate extracts should be prepared for GC/MS and GC/ECD analyses when isotope dilution is used. Separate extractions for GC/MS and GC/ECD analysis also increases the cost of isotope dilution analysis.

PCB Identification and Quantification—Accurate PCB quantification is difficult to achieve in routine analyses. A common practice is to quantify PCB with packed-column GC/ECD by comparing selected peaks in samples to corresponding peaks in commercial Aroclor formulations closely resembling the sample. The critical difficulties with this procedure relate to two factors:

- Environmental PCB assemblages often differ considerably from commercial Aroclor mixtures because of the variable properties of PCB congeners (e.g., aqueous solubility, volatility, susceptibility to biodegradation)
- GC/ECD has a markedly variable response to the 209 congeners depending on the number and position of chlorine atoms on the biphenyl nucleus (e.g., Mullin et al. 1984).

Although the EPA/CLP has relied upon a packed-column, Aroclor matching technique for PCB quantification, data quality reviewers should be aware that such techniques are not universally accepted. Limitations of such techniques have been discussed in the scientific literature by leading PCB researchers (e.g., Duinker et al. 1980; Gebhart et al. 1985; Brown et al. 1987; Schwartz et al. 1987). For example, the following quotation regarding Aroclor-matching was excerpted from Gebhart et al. (1985), which was co-authored by EPA researchers at the Environmental Monitoring Support Laboratory (Cincinnati):

> This approach to PCB determinations has been widely used, virtually unchanged, for the past 10 years, primarily because it was the only practical approach. It does, however, have a number of disadvan-

> tages. One disadvantage is that gas chromatographic (GC) patterns produced by PCBs in environmental sample extracts frequently are different from Aroclor patterns. Some differences are due to [variations] among different batches of commercial formulations. Other differences are caused by partial degradation, dissolution, and irreversible adsorption of some congeners in the environment. If more than one Aroclor residue is present, the analytical problem is further complicated . . . Another disadvantage to determination of PCBs as Aroclors is that this approach frequently does not provide the information that is most important and most needed. For many environmental samples, determination of a particular Aroclor or mixture of Aroclors is not as important as determination of total PCB contamination and the distribution of congeners among the potentially more toxic and persistent isomer groups.

Significant aspects of the EPA/CLP method from a data quality perspective include the use of packed GC columns, initial calibration requirements, and injection internal standards, as discussed below:

- Capillary columns greatly enhance the resolution between PCB congeners and interferences (including pesticides) and are strongly recommended for PCB/pesticide analyses. A comparison between the resolution of capillary column and packed column analysis is presented in Figure 6-2 (taken from Bush et al. 1982).

- The EPA/CLP procedure does not call for a multipoint initial calibration for PCB; only pesticides require initial and ongoing calibrations to test for instrument linear range. Although it may be appropriate to use pesticides to test instrument performance for PCB, only PCB should be used in calibration procedures (focusing on dominant peaks found in environmental samples).

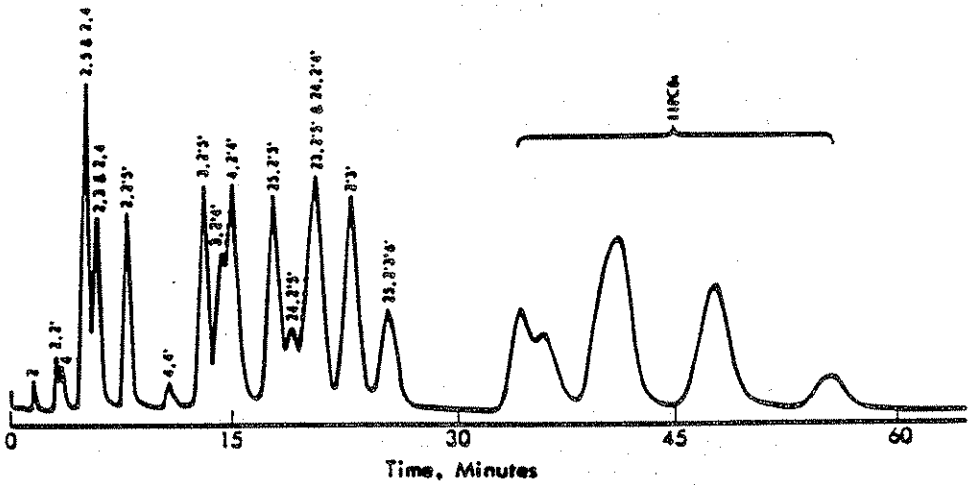
- The EPA/CLP procedure uses an external standard method for quantification rather than an injection internal standard. Use of an internal standard method for quantification is recommended, because it accounts for sample-to-sample variations in ECD response and reduces quantification errors associated with errors in the measurement of injection volumes.

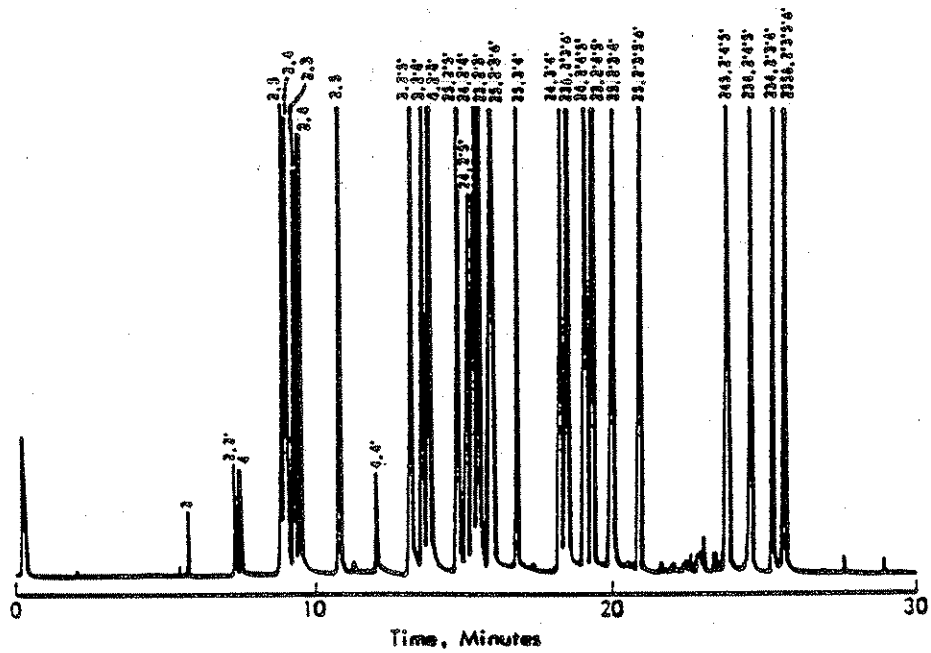
Alternative methods for PCB instrumental analysis exist but tend to require considerably more laboratory expertise, laboratory time, and expense than routine EPA/CLP analyses. Alternative techniques of detection [e.g., Hall electrolytic conductivity detector (HECD) or MS (with selected ion monitoring (SIM)] can provide comparable or superior PCB identification and quantification relative to ECD (e.g., Gebhart et al. 1985; Sonchik et al. 1984). Although ECD is widely available and is more sensitive for PCB than HECD or MS, HECD has a linear response to chlorine content and is more specific to chlorinated compounds, and MS offers more definitive compound identification than ECD.

6.5 DATA VALIDATION AND ASSESSMENT

6.5.1 GC/MS Tuning

Before proceeding with calibration and analysis of samples, the GC/MS must be tuned to established specifications to ensure proper mass resolution, identification, and to some degree, sensitivity.





Taken from Bush et al. (1982)

Figure 6-2. Comparison of packed column (top) and capillary column (bottom) GC/ECD chromatograms of the same mixture of 29 PCB congeners

Objective—The objective for reviewing the GC/MS tuning data is to verify that the instrument was properly adjusted for optimum performance.~~ObjectiveThe objective for reviewing the GC/MS tuning data is to verify the instrument was properly adjusted for optimum performance.~~

Requirements—GC/MS tuning criteria for DFTPP have been specified for the EPA/CLP (U.S. EPA 1988) and are shown in Table 6-2.

Tuning must be performed and verified before each 12-hour shift. Control limits for GC/MS tuning are shown in Table 6-2. An example of selected DFTPP tuning deliverables is shown in Figure 6-3a,b.

Evaluation Procedures—Calculations should be checked for each GC/MS tuning. Figure 6-3a,b shows a calibration summary and computer-generated GC/MS mass list, respectively. The base peak intensity (i.e., m/e or m/z 198 for DFTPP) is assigned a value of 100 percent by definition. The remaining peak intensities are divided by the base peak intensity to give "% relative abundance" (RA in Figure 6-3b). Criteria for several ions are normalized to ions other than the base peak (e.g., m/z 68, 70, 441, and 443 for DFTPP). The values in parentheses in Figure 6-3a are the intensity ratios for the ions that were compared. ~~The values in parentheses are the intensity ratios for the ions that were compared.~~

Evaluation considerations for GC/MS tuning are:

- Compare the data transcribed onto the GC/MS tuning form (Figure 6-3a) with GC/MS mass listings (Figure 6-3b).
- Ensure the laboratory has not made transcription errors or calculation errors. For example, calculate the ratio of the intensity of m/z 443 relative to m/z 442 (as percent).
- The following guidance generated by the EPA/CLP is useful for applying judgment to results outside specifications (U.S. EPA 1988):

> The most critical factors in the DFTPP criteria are the non-instrument specific requirements that are also not unduly affected by the location of the spectrum on the chromatographic profile. The m/z 198/199 and 442/443 ratios are critical. These ratios are based on the natural abundances of Carbon-12 and Carbon-13 and should always be met. Similarly, the m/z 68, 70, 197, and 441 relative abundances indicate the condition of the instrument and the suitability of the resolution adjustment and are very important. Note that all of the foregoing abundances relate to adjacent ions - they are relatively insensitive to differences in instrument design and position of the spectrum on the chromatographic profile. For the ions at m/z 51, 127, and 275, the actual relative abundance is not as critical. For instance, if m/z 275 has 40 percent relative abundance (criteria: 10-30 percent) and other criteria are met, the deficiency is minor. The relative abundance of m/z 365 is an indicator of suitable instrument zero adjustment. If m/z 365 relative abundance is zero, minimum detection limits may be affected. On the other hand, if m/z 365 is present, but less than the 1 percent minimum abundance criteria, the deficiency is not as serious.

TABLE 6-2. GC/MS TUNING CRITERIA FOR DECAFLUOROTRIPHENYLPHOSPHINE

m/z	Ion Abundance Criteria
51	30.0-60.0% of m/z 198
68	<2.0% of m/z 69
70	<2.0% of m/z 69
127	40.0-60.0% of m/z 198
197	<1.0% of m/z 198
198	base peak, 100% relative abundance
199	5.0-9.0% of m/z 198
275	10.0-30.0% of m/z 198
365	>1.0% of m/z 198
441	present, but <m/z 443
442	>40.0% of m/z 198
443	17.0-23.0% of m/z 442

SEMIVOLATILE ORGANIC GC/MS TUNING AND MASS CALIBRATION - DECAFLUOROTRIPHENYLPHOSPHINE (DFTPP)

Lab File ID: F216250327A
Instrument ID: FINN 2
Matrix: Mixed
Level: Low

Client:
DFTPP Injection Date: 03/27/89
DFTPP Injection Time: 11:05

m/e	Ion Abundance Criteria	% Relative Abundance	
51	30.0-60.0% of mass 198	36.3	
68	Less than 2.0% of mass 69	0.4 (1.4)1	
69	Mass 69 relative abundance	31.2	
70	Less than 2.0% of mass 69	0.0 (0.0)1	
127	40.0-60.0% of mass 198	47.9	
197	Less than 1.0% of mass 198	0.0	
198	Base peak, 100% relative abundance	100	* See Figure 6-3b
199	5.0-9.0% of mass 198	6.9	
275	10.0-30.0% of mass 198	27.3	*
365	Greater than 1.00% of mass 198	3.12	
441	Present, but less than mass 443	10.9	
442	Greater than 40.0% of mass 198	90.8	
443	17.0-23.0% of mass 442	17.1 (18.8)2	*

1-Value is % mass 69 2-Value is % mass 442

THIS TUNE APPLIES TO THE FOLLOWING SAMPLES, MS, MSD, BLANKS, AND STANDARDS:

Client Sample ID	ARI Sample ID	Lab File ID	Date Analyzed	Time Analyzed
SSTD50	1650327A	F21650327A	03/27/89	11:05
Meth.Blk(Tissue)	2607MB	F22607MB	03/27/89	12:08
Meth.Blk (Soil)	2744MB	F22744MB	03/27/89	12:59
SQ-1	2744L	F22744L	03/27/89	13:50
O1-A&B	2607SR	F22607SR	03/27/89	16:09

FORM V SV

Figure 6-3a. Example of DFTPP summary report (Figure 6-3a) and associated raw data (mass listing) (Figure 6-3b)

Mass List

03/27/89 11:05:00 + 25:23 Data: F216250327A #1523 Base m/z: 198
Sample: 1525 STD. + ABN CAL 50 NG Call: C0112 # 7 RIC: 215040
Conds.:
#1521 to #1525 summed - #1530 X1.00

39 0.00 0. Minima Min Inten: 53.
444 Maxima # 0

Mass	%RA	Inten.	Mass	%RA	Inten.
39.00	2.39	647.	175.00	2.63	710.
50.00	8.02	2128.	176.00	1.56	420.
51.00	36.27	9808.	177.00	0.83	220.
52.00	1.30	351.	179.00	3.38	913.
56.00	0.40	107.	180.00	2.62	708.
57.00	1.41	381.	181.00	0.80	217.
63.00	1.32	356.	185.00	2.00	542.
68.00	0.44	120.	186.00	12.88	3484.
69.00	31.18	8432.	187.00	3.74	1012.
74.00	3.61	976.	189.00	0.40	107.
75.00	6.66	1800.	192.00	1.75	473.
76.00	3.15	853.	193.00	1.16	314.
77.00	42.49	11488.	196.00	2.55	359.
78.00	3.15	851.	198.00	100.00	27040.
79.00	2.82	763.	199.00	6.87	1858.
80.00	2.13	576.	204.00	3.65	987.
81.00	2.51	678.	205.00	6.64	1796.
82.00	0.39	105.	206.00	23.22	6280.
93.00	4.20	1136.	207.00	3.18	859.
98.00	3.34	903.	211.00	0.88	238.
99.00	3.70	1000.	217.00	7.13	1928.
101.00	1.17	316.	221.00	8.42	2276.
104.00	0.88	238.	223.00	1.26	342.
105.00	0.83	225.	224.00	14.17	3832.
107.00	14.35	3880.	225.00	3.71	1002.
108.00	2.35	635.	227.00	4.80	1298.
110.00	22.57	6104.	229.00	1.00	270.
111.00	3.64	983.	244.00	11.80	3192.
116.00	0.38	103.	245.00	1.61	435.
117.00	10.93	2956.	246.00	1.41	380.
118.00	0.44	120.	255.00	51.83	14016.
122.00	0.37	101.	256.00	7.68	2076.
123.00	1.07	290.	258.00	2.67	723.
127.00	47.87	12944.	265.00	0.97	262.
128.00	4.09	1106.	273.00	2.03	549.
129.00	16.45	4448.	274.00	6.23	1684.
130.00	1.12	302.	275.00	27.25	7368.
135.00	1.23	333.	276.00	3.50	947.
137.00	0.39	106.	277.00	2.16	584.
141.00	2.05	553.	296.00	6.57	1776.
147.00	0.99	267.	297.00	0.38	103.
148.00	3.20	866.	323.00	2.05	554.
153.00	1.02	276.	324.00	1.53	413.
156.00	2.25	608.	365.00	3.12	843.
160.00	0.43	115.	372.00	0.53	143.
161.00	0.94	253.	423.00	6.01	1624.
167.00	5.18	1400.	441.00	10.86	2936.
168.00	4.82	1302.	442.00	90.77	24544.
169.00	0.38	102.	443.00	17.10	4624.
174.00	0.86	232.	444.00	0.43	116.

base peak = m/z 198

$$\frac{m/z\ 275}{m/z\ 198} = \frac{7368}{27040} \times 100 = 27.25\%$$

$$\frac{m/z\ 443}{m/z\ 442} = \frac{4624}{24544} \times 100 = 18.8\%$$

Figure 6-3b. Example of DFTPP summary report (Figure 6-3a) and associated raw data (mass listing) (Figure 6-3b)

Action—Unless otherwise specified, these criteria must be met; samples should not be run when the GC/MS is out of tuning specifications. If they are not met, the associated sample data should be qualified as estimated (E) (if the standards are near the set criteria) or rejected. Note that laboratories commonly report the samples associated with each tuning (see lower portion of Figure 6-3a).

6.5.2 Initial Calibration

Initial calibration is performed to determine the response of the instrument across a range of concentrations of each analyte of interest. The relationship between response and concentration is often called linearity. Response factors (RF) of analytes to standards at various concentrations are established by calibration. The standards may be surrogate compounds (for isotope dilution) or injection internal standards.

Objective—The objective of the reviewer is to verify that the GC was properly calibrated over a wide range of concentrations prior to sample analysis. Quantification of target compounds in samples is suspect if initial calibration criteria were not met.

Requirements—The frequency of initial calibration is dependant upon control limits set and failure to meet these criteria. Initial calibration should be performed at the onset of a project, whenever there is major disruption in instrumentation, and when the criteria for ongoing calibration are not met (see Section 6.5.3).

RF values must be determined for at least three concentration levels (five concentration levels, or a five-point calibration, is preferable). The standard concentrations tested should encompass the range of expected sample concentrations. One standard concentration for each target chemical must be within 150 percent of the stated detection limit (PSEP 1986) (see Section 6.5.5).

The RF of most target compounds should not differ by more than 20 percent CV (also known as RSD) over the range of concentrations tested. Hence, the response of the instrument is assumed to increase in direct proportion to concentration of the analyte when <20 percent deviation in response is observed over the concentration range bracketed by the calibration curve. EPA/CLP recommends a less stringent control limit (±30 percent CV) (U.S. EPA 1988).

Evaluation Procedures—Calculations of RF and CV values should be checked against the raw data provided by the laboratory. A typical initial calibration summary sheet for GC/MS is shown in Figure 6-4a. The pertinent information in this summary includes RF (RF10 through RF150 in Figure 6-4a) for each target compound at each standard concentration (five standard concentrations are used in this example, from 10 to 150 ng/μL), average RF values for each chemical ("RF Avg" in Figure 6-4a), and the RSD for RF values for each target compound. All summary sheets will not have this exact format, but should contain the above information. The following items should be confirmed during evaluation of initial calibration:

- Verify that all RF values are at least 0.05.
- Check several RF calculations (calculation checks should account for several chemicals and standard concentrations). The general formula for calculation of RF is:

$$RF = \frac{\frac{A_x}{C_x}}{\frac{A_{is}}{C_{is}}} = \frac{A_x}{A_{is}} \times \frac{C_{is}}{C_x}$$

Initial Calibration Data
Semivolatile HSL Compounds
Method 1625 (Modified List)

QC Report No:
Contractor:
Project:

Instrument ID: FINN II
Calibration Date: 06/01/88

Laboratory ID	162510	162520	162550	1625100	1625150		
Compound (CCC* SPCC**)	RF10	RF20	RF50	RF100	RF150	RF Avg	%RSD
Phenol *	3.489	3.615	3.524	3.291	3.032	3.390	6.9
1,3-Dichlorobenzene	1.440	1.418	1.363	1.294	1.323	1.367	4.5
1,4-Dichlorobenzene *	1.349	1.355	1.323	1.211	1.229	1.293	5.3
Benzyl Alcohol	0.122	0.136	0.155	0.194	0.226	0.167	25.7
1,2-Dichlorobenzene	1.378	1.446	1.381	1.313	1.266	1.357	5.1
2-Methylphenol	1.164	1.118	1.040	1.054	1.050	1.085	4.9
4-Methylphenol	1.241	1.224	1.110	1.106	1.100	1.156	6.1
Hexachloroethane	2.443	2.038	1.863	1.770	1.672	1.957	15.5
2,4-Dimethylphenol	1.787	1.610	1.394	1.350	1.351	1.498	12.9
Benzoic Acid †		0.050	0.144	0.152	0.156	0.126	40.3
1,2,4-Trichlorobenzene	1.137	1.139	1.067	0.971	0.918	1.046	9.5
Naphthalene	0.981	1.031	0.972	0.823	0.745	0.910	13.3
Hexachlorobutadiene *	1.969	1.860	1.712	1.626	1.589	1.751	9.2
2-Methylnaphthalene	0.800	0.823	0.841	0.812	0.800	0.815	2.1
Dimethyl Phthalate	1.114	0.950	0.728	0.853	0.813	0.891	16.5
Acenaphthylene	2.523	2.679	2.410	2.261	2.036	2.382	10.4
Acenaphthene *	1.006	1.076	0.972	0.940	1.045	1.008	5.4
Dibenzofuran	1.329	1.365	1.323	1.136	1.090	1.249	10.1
Diethylphthalate	1.098	1.224	1.018	1.060	1.050	1.090	7.4
Fluorene	1.052	1.168	1.001	0.989	0.948	1.031	8.2
N-Nitrosodiphenylamine *	1.180	1.009	0.960	0.954	0.794	0.979	14.1
Hexachlorobenzene	1.301	1.393	1.516	1.228	1.177	1.323	10.2
Pentachlorophenol *	1.077	1.177	1.136	1.136	1.133	1.132	3.1
Phenanthrene	0.961	1.020	0.949	0.977	0.901	0.962	4.5
Anthracene	0.987	0.969	0.898	0.902	0.835	0.918	6.6
Di-N-Butylphthalate	1.110	1.112	1.062	0.947	0.884	1.023	10.0
Fluoranthene *	0.971	1.027	0.954	0.907	0.870	0.946	6.4
Pyrene	0.995	1.044	0.936	0.905	0.865	0.949	7.5
Butylbenzylphthalate	1.031	1.111	1.032	0.993	0.974	1.028	5.1
Benzo(a)Anthracene	1.202	1.245	1.174	1.126	1.080	1.165	5.5
bis(2-Ethylhexyl)Phthalate	1.095	1.125	1.042	0.996	0.981	1.048	5.9
Chrysene	1.138	1.179	1.123	1.080	1.159	1.136	3.3
Di-n-Octyl Phthalate *	1.242	1.286	1.183	1.139	0.996	1.169	9.6
Benzo(b)Fluoranthene	1.276	1.227	1.141	1.201	0.996	1.169	9.2
Benzo(k)Fluoranthene	1.149	1.223	0.928	0.948	1.131	1.076	12.1
Benzo(a)Pyrene *	1.132	1.079	1.056	1.100	1.084	1.090	2.6
Indeno(1,2,3-cd)Pyrene	1.175	1.222	1.150	1.177	1.573	1.259	14.1
Dibenz(a,h)Anthracene	1.454	1.366	1.256	1.232	1.276	1.317	7.0
Benzo(g,h,i)Perylene	1.740	1.453	1.352	1.314	1.303	1.433	12.7

FORM VI

Figure 6-4a. Example summary sheet (Figure 6-4a), calculations (Figure 6-4b), and raw data (Figures 6-4c-e) for initial calibration using GC/MS

where:

A_x = Area of target peak in calibration standard

A_{is} = Area of injection internal standard peak

C_x = Concentration of target compound in calibration standard (e.g., in ng/μL)

C_{is} = Concentration of injection internal standard (e.g., in ng/μL).

This formula applies to GC/MS as well as GC/ECD and other detectors when internal standard quantification is used. The RF calculated in reference to an internal standard is often called a relative response factor (RRF) rather than a response factor. For GC/MS, the areas (A_x and A_{is}) are based on specific quantification ions for each compound. For GC/ECD or GC/FID, only the total peak areas are available [equivalent to reconstructed ion chromatogram (RIC) peak areas on GC/MS].

- An example calculation of an RF value for naphthalene (10 ng/μL standard) on GC/MS is shown in Calculation 1 of Figure 6-4b. This example is based on isotope dilution, in which the internal standards for target compounds are the corresponding surrogate compounds. Analogous procedures are used to calculate RF for alternative methods (e.g., GC/MS without isotope dilution, GC/ECD). All values used in Calculation 1 are based on computer-generated GC/MS quantitation reports (excerpted in Figure 6-4c). Formats of quantitation reports may differ among laboratories but are relatively consistent for a given GC/MS manufacturer (Finnigan, in this example).

  Figure 6-4c~~,d~~ gives a numerical index for detected peaks based on retention order: d_8-naphthalene and naphthalene are peaks 48 and 49, respectively~~ (Figure 6-4e)~~. ~~Data Information~~ included in Figure 6-4d (moving from left to right) are the peak number, quantitation ion, scan number and retention time, peaks used as an internal standard ("Ref"), ~~("REF"),~~ relative retention time (RRT), and peak area. Additional information in Figure 6-4e includes the nominal standard concentration of the compounds ["Amt(L)"] and the RF calculated from the current run ("R. Fac"). In Figure 6-4d note the internal standard for the labeled compounds is peak number 1 (2-fluorobiphenyl; e.g., see "Ref" for peak number 48). Thus, the RF for d_8-naphthalene is calculated in reference to peak number 1 as an internal standard. RFs of surrogate compounds are necessary for calculating surrogate recoveries.

- For certain analytical methods (e.g., the EPA/CLP method for pesticides/PCB by GC/ECD), an external standard method of quantification is used rather than an internal standard method. In terms of RFs, ~~For RFs,~~ the difference between internal and external standard methods is that the external standard method does not involve an internal standard in any calculations. For external standard methods, a RF (or calibration factor) for a given target compound in a calibration standard is simply:

$$RF = \frac{A_x}{C_x}$$

where:

A_x = Area of target peak in calibration standard

C_x = Amount of the target compound in calibration standard (e.g., in ng injected).

Calculation 1

$$\text{RF (naphthalene)} = \frac{\dfrac{\text{Area (naph.)}}{\text{Conc. (naph.)}}}{\dfrac{\text{Area } (d_8\text{-naph.})}{\text{Conc. } (d_8\text{-naph.})}}$$

$$\text{RF10 (naphthalene)} = \frac{\dfrac{\text{Area (peak no. 49)}}{\text{'Amnt (L)' for peak no. 49}}}{\dfrac{\text{Area (peak no. 48)}}{\text{'Amnt (L)' for peak no. 48}}}$$

$$= \frac{\dfrac{50{,}451}{10\ (\text{ng}/\mu\text{L})}}{\dfrac{205{,}728}{40\ (\text{ng}/\mu\text{L})}}$$

= 0.981 ('R. Fac' for peak no. 49; Figure 6-4e)

Calculation 2

For naphthalene:

$$\text{RF Avg} = \frac{\text{RF10} + \text{RF20} + \text{RF50} + \text{RF100} + \text{RF150}}{5}$$

$$= \frac{0.981 + 1.031 + 0.972 + 0.823 + 0.745}{5}$$

$$= 0.910$$

Calculation 3

For naphthalene:

$$\%\ \text{RSD} = \frac{\text{std. dev.}}{\text{RF Avg.}} \times 100$$

$$= \frac{0.121}{0.910} \times 100 = 13.3\%$$

where:

$$\text{std. dev.} = \sqrt{\frac{\sum_{i=1}^{n} (RF_i - RF_{AVG})^2}{n-1}}$$

Figure 6-4b. Example summary sheet (Figure 6-4a), calculations (Figure 6-4b), and raw data (Figures 6-4c-e) for initial calibration using GC/MS

```
Quantitation Report     File: F2162510

Data: F2162510.TI
06/01/88 13:07:00
Sample: 1625 INIT CALI, DEUT 80/40, H 10
Conds.: FINN-2
Formula: IS/10                    Instrument: FINN2   Weight: 1450.000
Submitted by:                     Analyst:            Acct. No.:

AMOUNT=AREA * REF AMNT/(REF AREA * RESP FACT)
Resp. fac. from average of whole .RL

 No   Name
  1   2-FLUOROBIPHENYL
  2   D5-PHENOL (G.M.=71)
  3   PHENOL (G.M.=94)
  4   D4-2-CHLOROPHENOL (G.M=132)
  5   2-CHLOROPHENOL (G.M.=128)
  6   D4-2-NITROPHENOL (G.M.=143)
  7   2-NITROPHENOL (G.M.=139)
  8   D3-2,4-DIMETHYLPHENOL (G.M.=125)
  9   2,4-DIMETHYLPHENOL (G.M=122)
 10   2,3,5,6-D4-PCRESOL
 11   2-METHYLPHENOL
 12   2,3,5,6-D4-PCRESOL
 13   4-METHYLPHENOL
 14   D3-2,4-DICHLOROPHENOL (G.M.=167)
 15   2,4-DICHLOROPHENOL (G.M=162)
 16   D2-4-CHLORO-3-METHYLPHENOL (G.M.=109)
 17   4-CHLORO-3-METHYLPHENOL (G.M.=107)
 18   D2-2,4,6-TRICHLOROPHENOL (G.M=200)
 19   2,4,6-TRICHLOROPHENOL (G.M=196)
 20   D2-2,4,5-TRICHLOROPHENOL (G.M=200)
 21   2,4,5-TRICHLOROPHENOL (G.M.=196)
 22   D3-2,4-DINITROPHENOL (G.M.=187)
 23   2,4-DINITROPHENOL (G.M=184)
 24   D4-4-NITROPHENOL (G.M=143)
 25   4-NITROPHENOL (G.M=139)
 26   D2-2-METHYL-4,6-DINITROPHENOL (G.M.=200)
 27   4,6-DINITRO-2-METHYLPHENOL (G.M.=198)
 28   *C6-PENTACHLOROPHENOL (G.M.=272)
 29   PENTACHLOROPHENOL (G.M.=266)
 30   D8-BIS(2-CHLOROETHYL)ETHER (G.M=101)
 31   BIS(2-CHLOROETHYL)ETHER (G.M.=93)
 32   D4-1,3-DICHLOROBENZENE (G.M.=152)
 33   1,3-DICHLOROBENZENE (G.M=146)
 34   D4-1,4,DICHLOROBENZENE (G.M.=152)
 35   1,4-DICHLOROBENZENE (G.M.=146)
 36   D4-1,2-DICHLOROBENZENE (G.M.=152)
 37   1,2-DICHLOROBENZENE (G.M.=146)
 38   D12-BIS(2-CHLOROISOPROPYL)ETHER (G.M.=131)
 39   BIS(2-CHLOROISOPROPYL)ETHER (G.M.=121)
 40   *C13-HEXACHLOROETHANE (G.M.=204)
 41   HEXACHLOROETHANE (G.M.=201)
 42   D5-NITROBENZENE (G.M.=128)
 43   NITROBENZENE (G.M.=123)
 44   D6-ISOPHORONE (G.M=88)
 45   ISOPHORONE (G.M.=82)
 46   D3-1,2,4-TRICHLOROBENZENE (G.M.=183)
 47   1,2,4-TRICHLOROBENZENE (G.M.180)
 48   D8-NAPHTHALENE (G.M.=136)
 49   NAPHTHALENE (G.M.=128)
 50   D3-1,2,3-TRICHLOROBENZENE (G.M.=183)
```

Figure 6-4c. Example summary sheet (Figure 6-4a), calculations (Figure 6-4b), and raw data (Figures 6-4c-e) for initial calibration using GC/MS

No	m/z	Scan	Time	Ref	RRT	Meth	Area(Hght)	Amount		%Tot
1	172	930	15:30	1	1.000	A BB	177053.	50.000	NG	1.36
2	71	459	7:39	1	0.494	A BB	44979.	79.028	NG/UL	2.15
3	94	460	7:40	2	1.002	A BB	19614.	10.290	NG/UL	0.28
4	132	449	7:29	1	0.483	A BB	141313.	81.458	NG/UL	2.21
5	128	451	7:31	4	1.004	A BB	20145.	10.639	NG/UL	0.29
6	143	649	10:49	1	0.698	A BB	75426.	79.005	NG/UL	2.15
7	139	651	10:51	6	1.003	A BB	9359.	9.294	NG/UL	0.25
8	125	687	11:27	1	0.739	A BV	79891.	78.976	NG/UL	2.15
9	122	687	11:27	8	1.000	A BB	17848.	11.928	NG/UL	0.32
10	112	594	9:54	1	0.639	A BB	121687.	80.987	NG/UL	2.20
11	108	565	9:25	10	0.951	A BB	17710.	10.730	NG/UL	0.29
12	112	594	9:54	1	0.639	A BB	121687.	80.987	NG/UL	2.20
13	108	594	9:54	10	1.000	A BB	18879.	10.735	NG/UL	0.29
14	167	701	11:41	1	0.754	A BB	62601.	79.262	NG/UL	2.15
15	162	703	11:43	14	1.003	A BB	12734.	10.119	NG/UL	0.28
16	109	856	14:16	1	0.920	A BB	90841.	79.906	NG/UL	2.17
17	107	856	14:16	16	1.000	A BB	18618.	12.024	NG/UL	0.33
18	200	915	15:15	1	0.984	A BV	67646.	62.258	NG/UL	1.69
19	196	915	15:15	18	1.000	A BV	10243.	12.307	NG/UL	0.33
20	200	922	15:22	1	0.991	A VB	72016.	71.951	NG/UL	1.96
21	196	923	15:23	20	1.001	A VB	11139.	12.580	NG/UL	0.34
22	187	1068	17:48	1	1.148	A BB	29290.	74.597	NG/UL	2.03
23	184	1070	17:50	22	1.002	A BB	4058.	9.246	NG/UL	0.25
24	143	1110	18:30	1	1.194	A BB	50062.	82.653	NG/UL	2.25
25	139	1111	18:31	24	1.001	A BB	7438.	11.609	NG/UL	0.32
26	200	1175	19:35	1	1.263	A BB	45381.	76.551	NG/UL	2.08
27	198	1175	19:35	26	1.000	A BB	5647.	11.110	NG/UL	0.30
28	272	1299	21:39	1	1.397	A BB	35188.	73.952	NG/UL	2.01
29	266	1300	21:40	28	1.001	A BB	4736.	9.514	NG/UL	0.26
30	101	445	7:25	1	0.478	A BB	16563.	38.472	NG/UL	1.05
31	93	452	7:32	30	1.016	A BV	14409.	10.886	NG/UL	0.30
32	152	467	7:47	1	0.502	A BV	50541.	40.950	NG/UL	1.11
33	146	469	7:49	32	1.004	A BB	18190.	10.528	NG/UL	0.29
34	152	478	7:58	1	0.514	A VB	57268.	41.547	NG/UL	1.13
35	146	480	8:00	34	1.004	A BB	19318.	10.432	NG/UL	0.28
36	152	510	8:30	1	0.548	A BB	50844.	41.290	NG/UL	1.12
37	146	513	8:33	36	1.006	A BB	17519.	10.158	NG/UL	0.28
38	131	543	9:03	1	0.584	A BB	21913.	39.836	NG/UL	1.08
39	121	554	9:14	38	1.020	A BB	4955.	10.077	NG/UL	0.27
40	204	567	9:27	1	0.610	A BB	15254.	40.454	NG/UL	1.10
41	201	567	9:27	40	1.000	A BB	9317.	12.483	NG/UL	0.34
42	128	587	9:47	1	0.631	A BB	39898.	39.528	NG/UL	1.07
43	123	590	9:50	42	1.005	A BB	9040.	9.591	NG/UL	0.26
44	88	636	10:36	1	0.684	A BB	123491.	48.572	NG/UL	1.32
45	82	643	10:43	44	1.011	A BB	33447.	11.234	NG/UL	0.31
46	183	707	11:47	1	0.760	A BB	59083.	38.180	NG/UL	1.04
47	190	709	11:49	46	1.003	A BB	16792.	10.865	NG/UL	0.30
48	136	713	11:53	1	0.767	A BV	205728.	40.772	NG/UL	1.11
49	128	715	11:55	48	1.003	A BB	50451.	10.774	NG/UL	0.29
50	183	750	12:30	1	0.806	A BB	57069.	40.236	NG/UL	1.09

Figure 6-4d. Example summary sheet (Figure 6-4a), calculations (Figure 6-4b), and raw data (Figures 6-4c-e) for initial calibration using GC/MS

No	Ret(L)	Ratio	RRT(L)	Ratio	Amnt	Amnt(L)	R.Fac	R.Fac(L)	Ratio
1	15:31	1.00	1.000	1.00	50.00	50.00	1.000	1.000	1.00
2	7:43	0.99	0.497	0.99	79.03	80.00	0.159	0.161	0.99
3	7:43	0.99	1.004	1.00	10.29	10.00	3.489	3.390	1.03
4	7:32	0.99	0.485	0.99	81.46	80.00	0.499	0.490	1.02
5	7:35	0.99	1.007	1.00	10.64	10.00	1.140	1.072	1.06
6	10:52	1.00	0.700	1.00	79.01	80.00	0.266	0.270	0.99
7	10:54	1.00	1.003	1.00	9.29	10.00	0.993	1.068	0.93
8	11:31	0.99	0.742	1.00	78.98	80.00	0.282	0.286	0.99
9	11:31	0.99	1.000	1.00	11.93	10.00	1.787	1.498	1.19
10	9:58	0.99	0.642	0.99	80.99	80.00	0.430	0.424	1.01
11	9:27	1.00	0.948	1.00	10.73	10.00	1.164	1.085	1.07
12	9:58	0.99	0.642	0.99	80.99	80.00	0.430	0.424	1.01
13	9:58	0.99	1.000	1.00	10.73	10.00	1.241	1.156	1.07
14	11:45	0.99	0.757	1.00	79.26	80.00	0.221	0.223	0.99
15	11:46	1.00	1.001	1.00	10.12	10.00	1.627	1.608	1.01
16	14:18	1.00	0.922	1.00	79.91	80.00	0.321	0.321	1.00
17	14:18	1.00	1.000	1.00	12.02	10.00	1.640	1.364	1.20
18	15:17	1.00	0.985	1.00	62.26	80.00	0.239	0.307	0.78
19	15:18	1.00	1.001	1.00	12.31	10.00	1.211	0.984	1.23
20	15:24	1.00	0.992	1.00	71.95	80.00	0.254	0.283	0.90
21	15:25	1.00	1.001	1.00	12.58	10.00	1.240	0.985	1.26
22	17:51	1.00	1.150	1.00	74.60	80.00	0.103	0.111	0.93
23	17:53	1.00	1.002	1.00	9.25	10.00	1.108	1.199	0.92
24	18:32	1.00	1.194	1.00	82.65	80.00	0.177	0.171	1.03
25	18:33	1.00	1.001	1.00	11.61	10.00	1.189	1.024	1.16
26	19:38	1.00	1.265	1.00	76.55	80.00	0.160	0.167	0.96
27	19:39	1.00	1.001	1.00	11.11	10.00	0.995	0.896	1.11
28	21:42	1.00	1.398	1.00	73.95	80.00	0.124	0.134	0.92
29	21:42	1.00	1.000	1.00	9.51	10.00	1.077	1.132	0.95
30	7:28	0.99	0.481	0.99	38.47	40.00	0.117	0.122	0.96
31	7:36	0.99	1.018	1.00	10.89	10.00	3.480	3.197	1.09
32	7:50	0.99	0.505	0.99	40.95	40.00	0.357	0.349	1.02
33	7:53	0.99	1.006	1.00	10.53	10.00	1.440	1.367	1.05
34	8:01	0.99	0.517	0.99	41.55	40.00	0.404	0.389	1.04
35	8:04	0.99	1.006	1.00	10.43	10.00	1.349	1.293	1.04
36	8:33	0.99	0.551	1.00	41.29	40.00	0.359	0.348	1.03
37	8:36	0.99	1.006	1.00	10.16	10.00	1.378	1.357	1.02
38	9:06	0.99	0.586	1.00	39.84	40.00	0.155	0.155	1.00
39	9:17	0.99	1.020	1.00	10.08	10.00	0.904	0.898	1.01
40	9:30	0.99	0.612	1.00	40.45	40.00	0.108	0.106	1.01
41	9:30	0.99	1.000	1.00	12.48	10.00	2.443	1.957	1.25
42	9:50	0.99	0.634	1.00	39.53	40.00	0.282	0.285	0.99
43	9:54	0.99	1.007	1.00	9.59	10.00	0.906	0.945	0.96
44	10:40	0.99	0.687	0.99	48.57	40.00	0.872	0.718	1.21
45	10:48	0.99	1.012	1.00	11.23	10.00	1.083	0.964	1.12
46	11:49	1.00	0.762	1.00	38.18	40.00	0.417	0.437	0.95
47	11:52	1.00	1.004	1.00	10.86	10.00	1.137	1.046	1.09
48	11:55	1.00	0.768	1.00	40.77	40.00	1.452	1.425	1.02
49	11:58	1.00	1.004	1.00	10.77	10.00	0.981	0.910	1.08
50	12:32	1.00	0.808	1.00	40.24	40.00	0.403	0.401	1.01

Figure 6-4e. Example summary sheet (Figure 6-4a), calculations (Figure 6-4b), and raw data (Figures 6-4c-e) for initial calibration using GC/MS

- Average RF calculations should be checked for several chemicals. An example calculation of RF Avg for naphthalene is shown in Calculation 2 of Figure 6-4b.
- RSD calculations should be checked for several chemicals. An example calculation of percent RSD for naphthalene is shown in Calculation 3 of Figure 6-4b. Note the SD calculation includes (n-1), not n, in the denominator.
- Verify that all target compounds have RSD of ≤ 20 percent (≤ 30 percent is allowable for calibration check compounds specified by EPA/CLP and is reasonable for compounds that are very polar or not amenable to GC analysis with typical stationary phases).~~Verify all target compounds have RSD of 20 percent (30 percent is allowable for calibration check compounds specified by EPA/CLP and is reasonable for compounds that are very polar or not amenable to GC analysis with typical stationary phases).~~

Action—If linearity is not established, the laboratory should adjust the instrument and recalibrate before analyzing samples, or the range for reporting data should be reduced to within the observed linear range. If the laboratory failed to take these measures (and was not contractually required to do so), the QA reviewer must determine whether data for compounds out of calibration should be qualified or rejected. Qualification (with E) is appropriate for minor exceedances of control limits (e.g., 23 percent rather than 20 percent RSD), whereas rejection is more appropriate for large exceedances (e.g., 70 percent rather than 20 percent RSD). Data reported out of the calibration range should also be qualified as estimated (E), unless the laboratory can furnish evidence of linearity to the reported level.

6.5.3 Ongoing Calibration

While analyzing sample sets, continuing calibration checks are required to determine that the initial calibration for the instrument is still valid. Ongoing calibration for all GC detectors is verified assuming that the original calibration line or curve is still valid.

Objective—Ongoing calibration should be checked by the reviewer to ensure that the instrument used for analysis was still in calibration when samples were analyzed.

Requirements—Ongoing calibrations are analyzed often as a constant check that the instrument is performing satisfactorily. The standard used to check ongoing calibration should be one of the intermediate standards used for initial calibration.

Frequency: For GC/MS and GC/FID analyses, the ongoing calibration check should be checked at the beginning of each work shift, every 10-12 samples (or every 12 hours, whichever is more frequent), and after the last sample of each work shift. For GC/ECD, calibration should be checked at the beginning of each work shift, every 6 samples (or every 6 hours, whichever is less frequent), and after the last sample of each work shift.

Control Limits: For GC/MS, RF values for all target compounds should be within 25 percent (measured as percent difference) of average RF values from the initial calibration. RF values determined for PCB and pesticides with GC/ECD should agree within 15 percent of the initial calibration.

Evaluation Procedure—A typical ongoing calibration summary sheet for GC/MS analysis is shown in Figure 6-5. The pertinent information in this summary includes the RF Avg from the initial calibration, the RF (RF50) for each target compound at an intermediate concentration (50 ng/μL in this example) analyzed during the initial calibration, and the percent difference between the RF Avg and the RF50. Although all summary sheets may not be in this format, the above information should be included for all ongoing calibration summaries. The following items should be confirmed when evaluating ongoing calibration:

- Verify that all RF values are $\geq$0.05.
- Verify that the average RF values reported for the ongoing calibration are the same as the values determined from the relevant initial calibration. Check for transcription errors or use of the wrong initial calibration (i.e., only the most recent initial calibration should be used).
- RF values from the ongoing calibration should be calculated for several target compounds. An example RF calculation is shown in Calculation 1 of Figure 6-4b ~~Figure 6-5~~.
- Percent difference values should be recalculated for several target compounds. Percent difference is calculated as:

$$\%D = \frac{RF_1 - RF_2}{RF_1} \times 100$$

where:

%D = Percent difference

RF_1 = Average response factor from initial calibration

RF_2 = Response factor from ongoing calibration.

For example, the percent difference value for naphthalene in Figure 6-5 would be calculated as follows:

$$\frac{0.910 - 0.948}{0.910} \times 100 = -4.2$$

- Verify that ongoing calibration has been performed at the appropriate frequency.
- Verify that the percent difference for each target compound is $\leq$25 percent for GC/MS or $\leq$15 percent for GC/ECD.

Action—Failure to attain the control limit for ongoing calibration should have resulted in an additional initial calibration and reanalysis of the samples analyzed between the last valid calibration and the invalid calibration. If such laboratory actions were not taken, data for the samples run between the last valid calibration and the invalid calibration should be qualified as estimates (E); qualification applies only to chemicals with greater than 25 percent difference (GC/MS) or greater than 15 percent difference (GC/ECD) from the initial calibration. ~~qualification applies only to chemicals with greater than 25 (GC/MS) or greater than 15 (GC/ECD) percent difference from the initial calibration.~~ The laboratory should have provided a chronological list of samples and calibrations in order of instrumental analysis (e.g., Figure 6-3a for GC/MS analysis), which can be used to determine the samples associated with each calibration.

Continuing Calibration Check
Semivolatile HSL Compounds
(Page 1)

QC Report No:
Laboratory:
Project No:
Instrument: FINN II

Calibration Date: 03/27/89
Time: 1105 hrs
Laboratory ID:
Initial Calibration Date: 06/01/88

Minimum RF(avg) for SPCC is 0.050 Maximum %D for CCC is 25%

Compound	RF Avg	RF50	% D	CCC	SPCC
Phenol	3.390	2.405	29.1	*	
1,3-Dichlorobenzene	1.367	1.229	10.1		
1,4-Dichlorobenzene	1.293	1.209	6.5	*	
Benzyl Alcohol	0.167	0.260	-55.7		
1,2-Dichlorobenzene	1.357	1.263	6.9		
2-Methylphenol	1.085	1.118	-3.0		
4-Methylphenol	1.156	1.157	-0.1		
Hexachloroethane	1.957	1.774	9.4		
2,4-Dimethylphenol	1.498	0.699	53.3		
Benzoic Acid	0.126	0.090	28.6		
1,2,4-Trichlorobenzene	1.046	1.013	3.2		
Naphthalene	0.910	0.948	-4.2		
Hexachlorobutadiene	1.751	1.676	4.3	*	
2-Methylnaphthalene	0.815	0.956	-17.3		
Dimethyl Phthalate	0.891	0.965	-8.3		
Acenaphthylene	2.382	1.909	19.9		
Acenaphthene	1.008	0.869	13.8	*	
Dibenzofuran	1.249	1.267	-1.4		
Diethylphthalate	1.090	1.078	1.1		
Fluorene	1.031	1.023	0.8		
N-Nitrosodiphenylamine	0.979	0.953	2.7	*	
Hexachlorobenzene	1.323	1.264	4.5		
Pentachlorophenol	1.132	1.102	2.7	*	
Phenanthrene	0.962	0.761	20.9		
Anthracene	0.918	0.764	16.8		
Di-N-Butylphthalate	1.023	0.832	18.7		
Fluoranthene	0.946	0.738	22.0	*	
Pyrene	0.949	0.785	17.3		
Butylbenzylphthalate	1.028	0.890	13.4		
Benzo(a)Anthracene	1.165	0.897	23.0		
bis(2-Ethylhexyl)Phthalate	1.048	0.913	12.9		
Chrysene	1.136	0.902	20.6		
Di-n-Octyl Phthalate	1.169	0.969	17.1	*	
Benzo(b)Fluoranthene	1.169	0.751	35.8		
Benzo(k)Fluoranthene	1.076	1.472	-36.8		
Benzo(a)Pyrene	1.090	0.938	13.9	*	
Indeno(1,2,3-cd)Pyrene	1.259	1.681	-33.5		
Dibenz(a,h)Anthracene	1.317	1.408	-6.9		
Benzo(g,h,i)Perylene	1.433	1.213	15.4		

Figure 6-5. Example summary form for continuing calibration on GC/MS

6.5.4 Compound Confirmation

Objective—The primary objective of compound confirmation is to confirm that compounds reported as detected in samples are present (i.e., to investigate the possibility of false positives) and to verify, to the extent possible, that target compounds reported as undetected are not present (i.e., to investigate the possibility of false negatives). Compound confirmation during QA review focuses on false positives rather than false negatives, because detected compounds are associated with data supporting positive identifications (e.g., mass spectra), whereas undetected compounds are largely associated with an absence of data. To some extent, false negatives are addressed during QA review of factors relating to analytical sensitivity (e.g, detection limits, analytical recovery).

Requirements—The degree of compound confirmation for GC/MS differs from that for less specific detectors (e.g., GC/ECD, GC/FID). Although all GC methods provide retention time data (chromatograms and associated quantitation reports) as a tool for compound identification, GC/MS also provides a more reliable and powerful means of identification (i.e., mass spectra). Thus, in this section, GC/MS will be treated separately from other instrumental methods.

Because the evaluation of GC/MS data requires professional expertise, the specifications for retention time and mass spectra below should be considered as guidelines rather than firm criteria. These guidelines are based on requirements of the EPA/CLP, which is designed to preclude false positives rather than to ensure there are no false negatives.

Mass spectra of the target compound in a sample and a recent laboratory-generated standard should agree according to the following criteria:

- The RRT of the target compound should be within ±0.06 RRT units of the calibration standard
- All ions present in the standard mass spectrum at a relative intensity greater than 10 percent must be present in the sample spectrum
- The relative intensities of ions specified above must agree within ±20 percent between the standard and sample spectra (e.g., for an ion with an abundance of 50 percent in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70 percent)
- Ions greater than 10 percent in the sample spectrum and not present in the standard spectrum should be considered as possible interferences due to co-eluting compounds, or as possible evidence the overall spectrum is not that of the target compound.

For analyses not conducted by GC/MS (primarily pesticide/PCB analyses by GC/ECD), the following guidelines apply:

- Retention times of target compounds must be within appropriate retention time windows (e.g., based on multiple analyses of the calibration standard, three SD around the mean retention time) for two GC columns of dissimilar polarity.
- When concentrations are sufficient, GC/MS should be used to confirm the presence of the reported compounds.

Evaluation Procedures—For GC/MS, compounds reported as detected in each sample should be confirmed by examining RRT and, more importantly, mass spectra:

- Confirm the retention time of the compound is within a reasonable retention time window as compared to the calibration standard (±0.06 RRT units). GC/MS data

systems are programmed to search in a specified window for the target compound; thus, if the window was reasonably specified by the laboratory, little effort need be expended during QA review. For reference, computer-generated GC/MS quantitation reports will typically list RRT along with absolute retention times (e.g., see Figure 6-4d). Software used for the isotope dilution technique typically locates the surrogates first and then searches for the associated target compounds. If the surrogate is not found (i.e., if surrogate recovery is 0 percent), the computer may not search for the target compound, which could result in false negative results for that compound.

- When GC/MS is used, the mass spectrum is far more important than retention time in confirming compound identification. The laboratory can report the mass spectral information in two general formats: (1) as a histogram, in which relative intensity is plotted vs. mass/charge (m/z) ratio (Figure 6-6a,b), and (2) in tabular form, where relative intensities are listed with corresponding ions (i.e., m/z ratios) (~~discussed as "mass-listings" in~~ Figure 6-3b). The output in Figure 6-6a (or a similar format with the sample and library spectra on the same page), available with most GC/MS data systems, is very useful for comparing sample spectra to library spectra. In Figure 6-6a, good agreement exists between the spectrum in the sample and the library spectrum of benz(a)anthracene. Relatively low levels of spectral interference (e.g., <10 percent relative intensity on the y-axis) are apparent in the sample spectrum for m/z below 200. Also, note the presence of ions in the region around m/z 240 that derive from d_{12}-benz(a)anthracene, a surrogate compound that partially co-eluted with the target compound in the sample. Figure 6-6b displays a spectrum of fluoranthene in raw form and after being enhanced by computer software. Use of enhanced or background-subtracted spectral data is common and is generally acceptable. Note that enhancement has significantly reduced interferences in Figure 6-6b. The tabular data format facilitates quantitative comparisons of relative ion intensities, but is not always necessary (i.e., the visual comparisons in Figure 6-6a are often sufficient) or included in data packages.

Compound identification by GC/ECD is based largely on retention time. Because some compounds could potentially co-elute on a given column, dual-column analyses are required by the EPA/CLP (and other programs) to reduce the possibility of false positives. The reason for using dual-column analyses (i.e., two columns with stationary phases of dissimilar polarity) is that it is unlikely two different compounds would co-elute on two different columns even if they co-elute on one column. The use of capillary columns (highly recommended, as discussed previously in reference to PCB) enhances resolution and also decreases the possibility for co-elution. Interferences are a far more prevalent problem in GC/ECD analyses than in GC/MS analyses, because mass spectral interferences occur only when compounds co-elute and have common ions, whereas GC/ECD interferences only require co-elution. Interferences can result in overestimation of target compounds, false positives, and even false negatives (e.g., if a large elemental sulfur peak masked target compounds present at relatively low concentration). The following steps should be taken during compound confirmation with relatively nonselective detectors:

- Confirm that any reported compounds eluted within appropriate retention time windows on both the analytical column and the confirmation column. Chromatograms should be examined carefully for presence of apparent interferences (e.g., elevated baseline, clusters of peaks in area of target compound), and judgment must be used to evaluate whether the target compound was present. In complex extracts, such as those containing relatively high concentrations of PCB, it is unlikely that certain pesticides such as DDT will be resolvable from certain PCB congeners. Comparing PCB and pesticide calibration standards will highlight potential co-elution problems for target compounds.

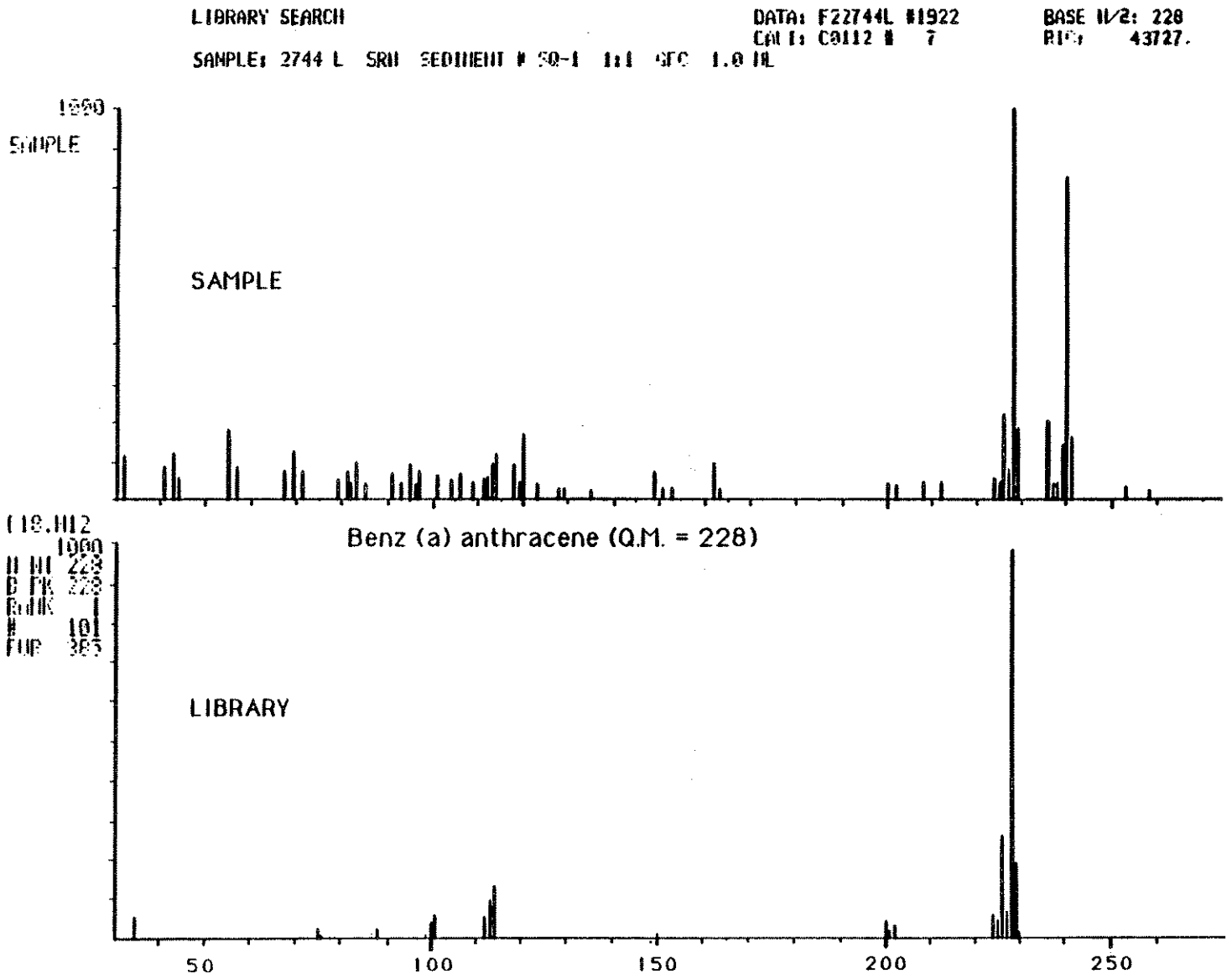


Figure 6-6a. Example mass spectrum for benz(a)anthracene identified in a sample sediment extract (upper) and authentic spectrum stored in computerized GC/MS library (lower)

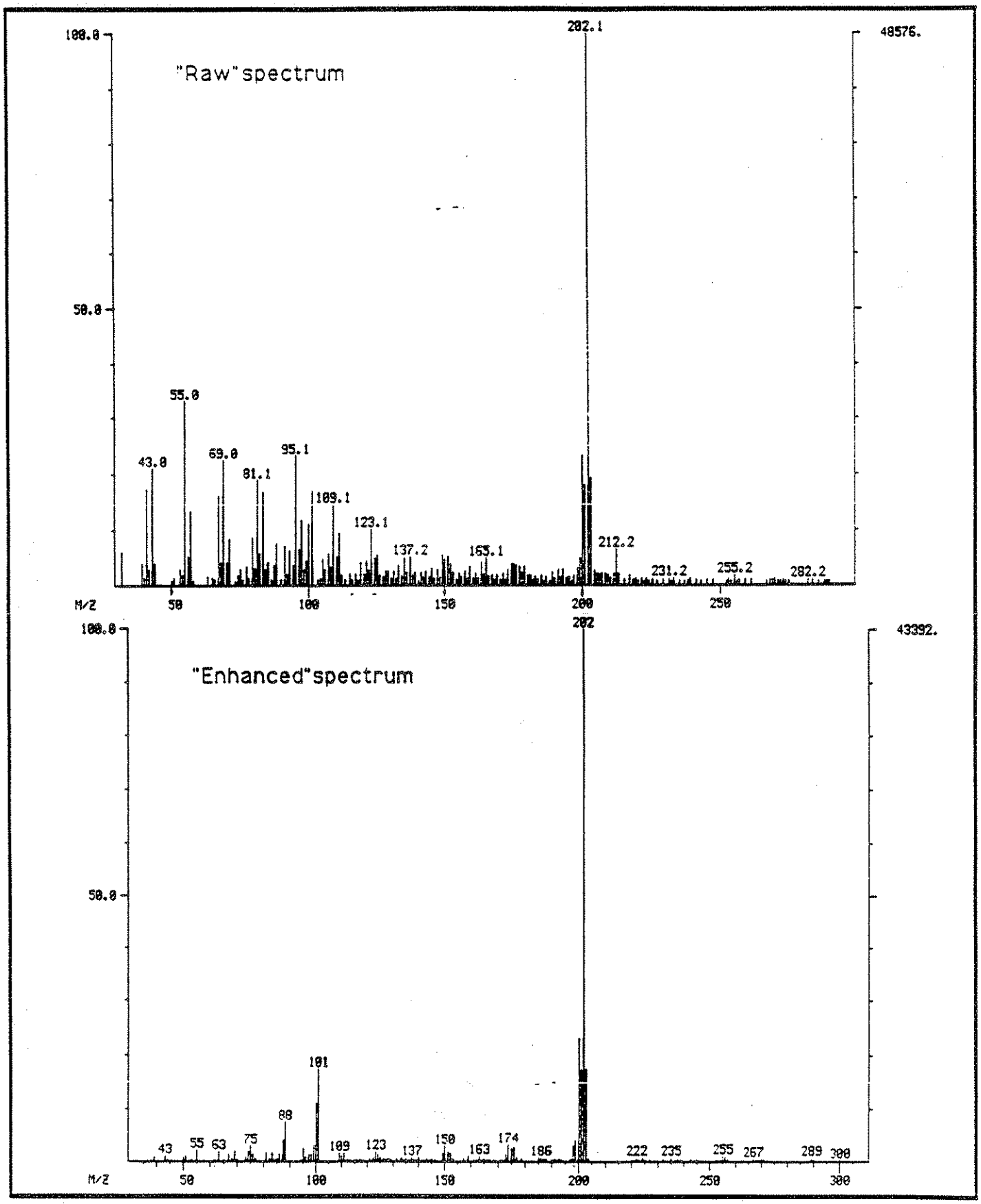


Figure 6-6b. Examples of "raw" and "enhanced" spectra of fluoranthene in a sediment extract

- When complex mixtures (e.g., PCB as Aroclors) are reported, quantitative examination of retention times is of limited use. Chromatograms of the appropriate standards (i.e., the Aroclor mixtures reported by the laboratory) should be overlaid on the sample chromatogram using a light table. In this manner, similarities and differences between the sample and standard will be more apparent. The laboratory should have marked the chromatographic peaks used for identification and quantification, and these peaks should be carefully reviewed. Laboratories do not always specify the peaks they have used for PCB quantification, so this should be specified in their contract. A comparison of GC/ECD chromatograms of a sediment extract and common Aroclor standards is presented in Figure 6-7. Analyses were performed with a wide-bore capillary column. The sample assemblage appears most similar to Aroclor 1254 and was quantified in relation to that standard. Aroclors 1016 (or 1242) and 1260 are often combined in standards because their overall elution ranges tend not to overlap.

Action—If GC/ECD sample interferences appear overwhelming, the laboratory should be requested to perform further cleanup (e.g., if a large, poorly resolved sulfur peak is present in a GC/ECD chromatogram), or, if concentrations permit, to reanalyze by GC/MS. Such contingencies should be specified in the laboratory's contract. The EPA/CLP requires the GC/MS confirmation of pesticides and PCB when concentrations permit.

Professional judgment is a critical aspect of compound confirmation. If chromatographic and/or mass spectral evidence suggest false positive results, the compound should be reported as undetected (U) at an appropriate detection limit. The appropriate detection limit should account for high levels of interferences, if present. If the reviewer is convinced the compound is present but the supporting evidence is only marginally acceptable, the compound should be reported as an estimate (E).

6.5.5 Detection Limits

Detection limits are a critical but often overlooked aspect of data quality. ~~Detection limits are a critical aspect of data quality often overlooked.~~ Environmental analytical chemists have not universally agreed upon a convention for determining and reporting the detection limit associated with a particular chemical analysis. Detection limits are variously based on instrument sensitivity, levels of blank contamination, matrix interferences, and various levels of statistical significance. ~~and statistical significance.~~ CEI defined the following detection limits to standardize the reporting procedures of environmental laboratories (Keith et al. 1983):

- Instrumental detection limit (IDL)—the smallest signal above background noise an instrument can detect reliably. This measure by itself does not account for matrix factors that may constrain the ability to detect the presence of a chemical in a particular sample.
- Limit of detection (LOD)—the lowest concentration that can be determined to be statistically different from the blank. The recommended value for LOD is 3σ, where σ is the SD of the blank in replicate analyses. This concentration is assumed to exceed the measurement uncertainty with 99 percent confidence.
- Method detection limit (MDL)—the minimum concentration of a substance that can be identified, measured, and reported with 99 percent confidence that the analyte concentration is greater than zero. The MDL is determined from seven replicate analyses of a sample of a given matrix containing the analyte (Glaser et al. 1981; Code of Federal Regulations 1987). This procedure is not used routinely by most laboratories to establish detection limits, although in concept the MDL reflects a method's ability to quantify the presence of a target chemical in a sample matrix, regardless of its origin (Taylor 1988).

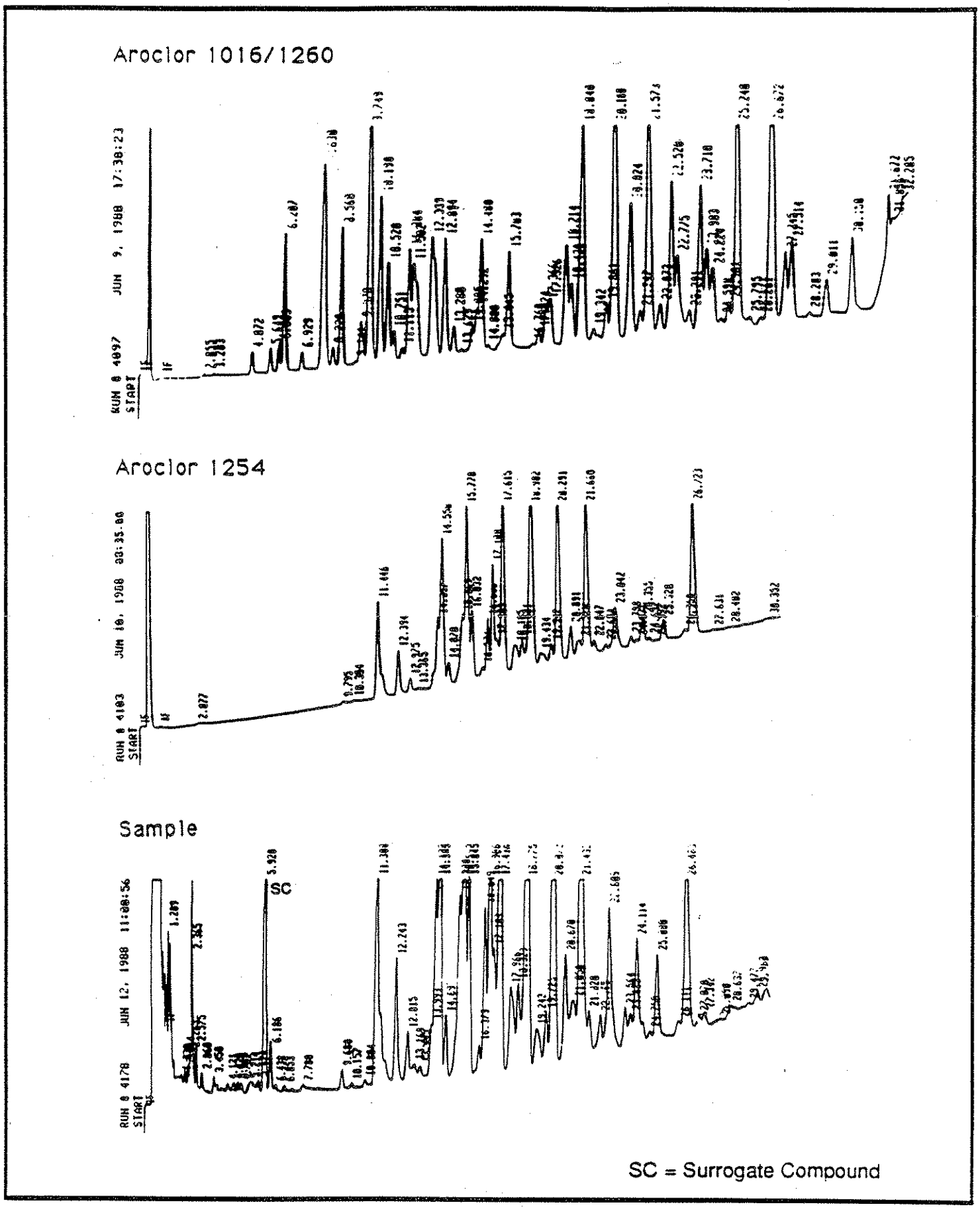


Figure 6-7. PCB chromatograms of Aroclor 1016/1260 combined standard (top), Aroclor 1254 standard (middle), and sediment sample (bottom) analyzed by GC/ECD. The sample assemblage is most similar to Aroclor 1254.

- Limit of quantification (LOQ)—the level above which quantification results may be obtained with a specified degree of confidence. The recommended (but arbitrary) value for LOQ is 10σ, where σ is the SD of blanks in replicate analyses. Assuming normally distributed data, 10σ provides well above 99.99 percent confidence that the result exceeds the LOD. At this concentration, the relative confidence in the measured value is approximately ±30 percent at the 95 percent probability level (Taylor 1988).

The CEI recommended that results below 3σ be reported as "not detected" (ND) and that the detection limit (or LOD) be given in parenthesis. In addition, if the results are near the detection limit ($3-10\sigma$, the "region of less-certain quantitation"), the results should be reported as detections with the LOD given in parentheses.

Objective—The objective of reviewing detection limits is to confirm that the detection limit is consistent with the requirements of the contract and to validate that detection limits have been correctly calculated.

Requirements—Detection limit requirements are project-specific and should be specified in the laboratory's contract. On GC/MS, IDL of approximately 1-2 ng on-column should be attainable for compounds such as PAH, whereas polar compounds such as pentachlorophenol and benzoic acid may have IDL that are 5-10 times higher, or more. Notably, the lowest concentration A/B/N standards specified by the EPA/CLP are 20 ng on-column, which do not reflect a high degree of sensitivity. On GC/ECD, IDL for individual pesticides often fall within the range of 0.005 to 0.05 ng on-column, and Aroclor mixtures are typically discernable at levels of 0.10 ng on-column.

The reported detection limit should be within the control limits set by the SOW at the beginning of the project. Samples with high interferences should have correspondingly higher detection limit. This should be evaluated, but no action should be taken, as interference levels are not within the control of the laboratory. As specified by PSEP (1986), detection limits for semivolatile organic compounds should fall between 1 and 50 μg/kg dry weight for sample sizes of 50-100 grams wet weight of sediment.

Evaluation Procedures—Detection limits typically reported by laboratories do not conform strictly with methods defined by Keith et al. (1983). Often, reported sample detection limits are based on IDL and account for sample weight, injection volume, and total extract volume. For example, the following formula is often used to calculate sample detection limits:

$$\text{Sample detection limit} = \frac{\text{IDL (on-column)} \times (\text{injection volume/extract volume})^{-1}}{\text{sample weight}}$$

IDL are sometimes determined by the EPA/CLP method. The preferable method is by injection of calibration standards at lower and lower concentrations until a concentration corresponding to an appropriate signal/noise ratio (e.g., approximately 3) is determined.

In general, any factor affecting the calculation of detected concentrations in a sample should also affect the calculation of detection limits. For example, if the sample required dilution, detection limits should increase by the dilution factor. If the extract was split for any reason (e.g., 80 percent for GC/MS, 20 percent for GC/ECD), the detection limit should be adjusted accordingly (e.g., if only 80 percent of the extract is used, the detection limit should be multiplied by 100/80 or 1.25). During QA review, the reviewer should determine whether detection limits accounted for such adjustments.

Detection limit validation should address the following considerations:

- Calculations for some samples should be checked to determine the method for calculation, whether this method was reasonable, and whether it was applied consistently.
- The lowest initial calibration standard should be in the range of the IDL; if not, the IDL itself is questionable. Mass spectra (for GC/MS) or chromatograms for the lowest concentration standard are helpful to confirm a reasonable signal/noise ratio is met for that standard.
- CRM results should be consistent with detection limits. For example, if a compound known to be present at 100 ppb in a sediment CRM ~~SRM~~ was reported as undetected at 25 ppb, the detection limits may be poor estimates. However, if the compound was reported as undetected at 200 ppb, no inconsistency is apparent.
- Matrix spike recoveries should be consistent with detection limits. For example, if a matrix spike was added at 1,000 ppb and was not detected, the detection limit for that compound in that particular sample would be ≥1,000 ppb. If stated detection limits for the same compound in most samples were 50 ppb, it would be reasonable to suspect those detection limits as underestimates (unless the matrix spike had been diluted to 0.05 its original concentration).
- Evidence of poor recovery (e.g., low surrogate or matrix spike recovery for a specific compound or class of compounds) should be factored into detection limits, if possible. For example, if 20 percent surrogate recovery was observed in a given sample in which the related target compound was undetected, the detection limit for the target compound should be established at 5 times the level that would be set if surrogate recovery were 100 percent.

Action—If the standards used during calibration do not bracket the detection limits, the detection limits should be recalculated. Often, detection limits are not calculated on a sample-by-sample basis. For example, a detection limit of 10 ppb for an analyte may have been appropriately determined for an "average" sample, but when a very contaminated sample (with higher levels of interferences) is analyzed, the same detection limit may be reported. Detection limits should be adjusted in such cases.

6.5.6 Analysis of Blanks

Method blanks are analyzed to assess possible laboratory contamination of samples associated with all stages of preparation and analysis of sample extracts. Contamination is of concern because it can result in false positives (i.e., erroneous reports of the compound as present in the sample) or overestimates of sample concentrations. Phthalates are common laboratory contaminants, and have less rigorous control limits.

Objective—The objective of the reviewer is to assess the contaminant levels in method blanks. If significant contamination exists, and the QA reviewer deems it necessary, corrections may be applied to the data during QA review to minimize the effects of laboratory contamination on the analyte concentrations. For such corrections, the blank analyses are assumed to be representative of the potential contamination in sample extracts. However, blank correction is not acceptable under the EPA/CLP (U.S. EPA 1988).

Sample contamination can result from sample collection techniques and equipment, sample storage, sample handling, solvents and other reagents, glassware, and analytical equipment. Three common types of blanks are field blanks, method blanks, and reagent blanks.

Field blanks are the most complete blank analysis. A sample container is taken along during the sampling trip where it is opened at a station, then closed and treated like all collected samples. If the container is not opened in the field, it is sometimes called a transport blank. Analysis is performed in the same manner as for samples, except that solvent rinsings of the sample container are added to the blank and no matrix is added to the blank. Field blanks do not assess all possible sources of contamination in the field.

~~Frequency:~~—Method blanks omit the field, transportation, and storage steps of the field blank. A method blank is prepared concurrently with samples and is treated like a sample, although the blank contains no sediment. This blank is carried through the entire analytical scheme concurrently with the samples. The method blank assesses laboratory contamination and is typically the only blank required as a deliverable.

~~Control Limits:~~—Reagent blanks only involve extracting and concentrating the reagents used during each step of the analytical scheme. Reagent blanks should be performed with new lots of solvents and reagents. Reporting these results is not required but should be available from the laboratory.

The remainder of this section pertains only to method blanks.

Requirements—Ideally, blanks should contain no detectable analytes. For each compound, blanks can be expressed in terms of absolute levels (total μg/blank sample) or relative levels (expressed as a percent of the sample concentrations).

~~QA control limits for blanks are typically based on the magnitude of blanks relative to detection limits and sample concentrations. Blanks are relevant to detection limits, because they increase the level of background noise and interfere with the laboratory's ability to discern target chemicals in samples.~~

Frequency: Method blanks should be run with every extraction batch (i.e., every set of samples extracted concurrently) or every 12 hours, whichever is more frequent. It is important to know which blanks correspond with which batch of samples.

Control Limits: For most compounds, blanks should not contain more than 2.5 μg total (absolute control limit) or 5 percent of the amount of analyte present in samples (relative control limit). Phthalates are common laboratory contaminants that warrant special consideration. Blank concentrations of phthalates should not exceed 5 μg total (absolute control limit) or 50 percent of the level of phthalates in samples (relative control limit).

Evaluation Procedures—The reviewer should check chromatograms of all method blanks. A special concern with blank analyses is false negatives. As with all analyses, detected compounds should be confirmed and calculations verified. A typical blank data package should contain a summary sheet with concentrations of detected target compounds (or detection limits, if undetected) and percent recoveries of surrogates, the ~~total~~ reconstructed ion chromatogram and all mass spectra of detected analytes for GC/MS (only chromatograms will be provided for analyses performed by GC/ECD and GC/FID), and the instrumental quantitation reports.

The following items should be verified during blank evaluation:

- Examine the chromatogram of the blank ~~(i.e., overlay a blank and a standard chromatogram),~~ quantitation reports, and mass spectra (if applicable). When isotope dilution is used, comparison to calibration standards is useful for determining whether surrogate solutions contain unlabeled analytes.

- Examine surrogate recoveries. If they are below control limits (see Section 6.5.7), the blank may underestimate contamination.

- Evaluate the absolute and relative concentrations of any detected contaminants. The laboratory should directly report absolute concentrations (μg/blank). Relative blank concentrations must be calculated, which can be a time-consuming process. As an example of such calculations, if a blank associated with Sample A had a reported concentration of 4 μg of di-n-octyl phthalate (per blank) and the sample (90 grams dry weight) had a reported concentration of 75 μg/kg (dry weight), the following calculation would be performed to determine the relative blank contamination (DW = dry weight):

{blank concentration (μg/blank)/[sample concentration (μg/kg DW) × sample wt. (kg DW)]} × 100

or, in this specific case:

$$[4\ \mu g\ /\ (75\ \mu g/kg \times 0.09\ kg)] \times 100 = 59\%$$

In this case, the blank contamination was below the absolute control limit (5 μg for phthalates), but exceeded the relative control limit (50 percent for phthalates) (see Table 6-1).

- Verify that a blank was analyzed with each extraction batch or every 12 hours, whichever is more frequent. The laboratory should provide a list of the blanks that correspond to each sample set.

Action—No action is required when there are no detectable contaminants in the blank. If contaminant concentrations exceed both the absolute and relative control limits, the data for the particular analyte(s) should be rejected. If there are detectable contaminants that are within at least one of the control limits, the data should be blank-corrected and qualified with a B or Z, as described below.

When any concentration is detectable in blanks, blank correction is recommended. Blank correction entails subtracting the total μg of the compound in the blank from the sample and then expressing the difference as a concentration (i.e., dividing by the sample weight). Using the preceding example, blank correction for di-n-octyl phthalate would be calculated as follows (DW = dry weight):

$$\{[75\ \mu g/kg\ (DW) \times 0.09\ kg] - 4\ \mu g/blank\}/0.09\ kg = 31\ \mu g/kg\ (DW)\ \text{di-n-octyl phthalate}$$

Blank-corrected data should be qualified with a B (if the corrected concentration is above the detection limit) or with a Z (if the corrected concentration is below the detection limit). When the isotope dilution technique is used, be certain that recovery-corrected data for the sample and blank are used in the calculation.

The laboratory's contract should include appropriate actions to be taken if absolute control limits are exceeded. If contaminants exceed the control ~~above~~ limits, sources of contamination should be tracked down, eliminated, and discussed in the cover letter of the data report. If problem contaminants (i.e., phthalates) cannot be traced or eliminated, the blanks should be replicated and confidence levels for contaminants should be determined.

6.5.7 Surrogate Spike Compounds

Surrogate spike compounds, or recovery internal standards, are compounds with chemical characteristics similar to those of target compounds that are used to assess analytical recovery on a sample-specific basis.

Objective—Known amounts of surrogate compounds are added to each sample prior to extraction to evaluate recovery for every sample. Surrogate recovery is the only QA check performed for every sample. Surrogate recoveries can be used to correct analyte concentrations if it is known that the actual analyte (or class of analytes represented by the surrogate) and the surrogate compounds behave similarly during sample preparation and analysis.

Requirements—The concentration of individual surrogates should be within the expected range of analyte concentrations as bracketed by the calibration standards. The isotope dilution method is recommended (see Section 6.4.7), but a minimum of five spike compounds should be added for neutral/acid compound analysis (three neutrals and two acids). A volatile and a degradable PAH [e.g., d_{12}-perylene or d_{12}-benzo(a)pyrene] should be included. The surrogate spikes should cover as much of the entire elution range as possible. Isotopically labeled analogs of target compounds are strongly recommended and many are commercially available.

At least one pesticide/PCB surrogate spike is required to assess recovery of chlorinated compounds analyzed by GC/ECD. The surrogates must be resolvable from target compounds and should behave similarly to the target compounds. Possible surrogate compounds include dibutylchlorendate (used by the EPA/CLP), dibromoctafluorobiphenyl [used routinely by NOAA/National Marine Fisheries Service (NMFS)], decachlorobiphenyl (used as a second surrogate spike only), and isodrin (endo-endo isomer of aldrin). However, no single surrogate or small group of surrogates can be representative of all PCB congeners (209 total) and chlorinated hydrocarbon pesticides. If isotope dilution is used for A/B/N analytes, a separate extraction for chlorinated compounds should be performed.

Frequency: Surrogates are required for each sample analysis as the only means of checking the accuracy of individual samples.

Control Limits: Applicable control limits for GC/MS depend on whether or not the isotope dilution technique was used. When using isotope dilution, recoveries should be greater than 10 percent of the amount added. When isotope dilution is not used and for pesticide/PCB analysis, surrogate recoveries should be greater than 50 percent.

Evaluation Procedures—The following items should be verified when assessing surrogate recoveries:

- Verify that the minimum required number of surrogates was used (not applicable when isotope dilution was used). Determine which analytes were represented by each surrogate. If the isotope dilution technique was not used, surrogates should represent compounds based on similar chemical behavior and retention times.
- Determine whether recovery correction was applied during quantification of target compounds. Recovery correction should only be performed if isotope dilution has been used or if the surrogates have been shown to behave in a manner similar to the target analytes (e.g., MacLeod et al. 1984). When few surrogates are used for many types of compounds, recovery correction cannot be justified. More detail on quantification is presented in Section 6.6.
- Check chromatograms to ensure proper identification of surrogates. Particularly for GC/ECD, if interferences are apparent (e.g., the baseline is elevated or the surrogate peak is not well resolved), recovery may be overestimated.
- Surrogate recovery calculations should be checked for several samples using the following equation:

$$\text{Surrogate recovery (\%)} = \frac{\text{amount detected (ng)}}{\text{amount added to sample (ng)}} \times 100$$

When using the internal standard method, the amount of surrogate detected can be calculated as:

$$\frac{A_{surr}}{A_{is}} \times \frac{Amnt_{is} \cancel{\times V_{tot}}}{RF \cancel{\times V_{inj}}}$$

where:

A_{surr} = Area of the surrogate compound

A_{is} = Area of the internal standard

$Amnt_{is}$ = Amount of internal standard added to final extract ~~(as ng injected)~~

RF = Response factor.

~~V_{tot} = Total extract volume (µL)~~

~~V_{inj} = Injection volume of extract (µL).~~

When using external standard quantification, the amount of surrogate detected can be calculated as:

$$\frac{A_{surr}}{RF} \times \frac{V_{tot}}{V_{inj}}$$

where terms are defined as above, and:

V_{tot} = Total extract volume (µL)

V_{inj} = Injection volume of extract (µL).

It is important that the amount of surrogate added and the amount detected be adjusted to account for any dilutions (or splits) of the sample or sample extract that occurred after surrogate addition.

- Verify that surrogates were added to each sample.
- Verify that all surrogates were above the 50-percent control limit (for GC/ECD and GC/MS when isotope dilution was not used) or the 10-percent control limit (when isotope dilution was used).

Action—QA review of surrogate recoveries may be complicated by factors arising from the sample itself. Matrix problems such as interferences and high target compound concentrations may be outside the control of the laboratory. Therefore, professional judgment and consideration of other QA samples (e.g., matrix spikes and CRM ~~SRM~~) should complement the assessment of surrogate recoveries that exceed control limits.

When using isotope dilution, compounds with associated surrogate recoveries of less than 10 percent should be qualified with an X, because of the uncertainty introduced when using large correction factors. If surrogate recoveries are less than 1 percent (using isotope dilution), analytes should be recovery-corrected assuming a 1 percent recovery, and qualified as underestimates. When the isotope dilution technique is not used, qualification of the compounds with an E should be considered when surrogate recoveries below 50 percent are observed. If CRM ~~SRM~~ and matrix spike data also indicate low recovery, a G qualifier (greater than) may be more appropriate. Professional judgment is necessary to determine which surrogates should apply to which target compounds, as surrogates will not be available for all compounds. Consideration should be given to relative elution time and chemical similarity when determining how to associate surrogates with target compounds.

If performance criteria for surrogate recovery have been specified in the laboratory's contract and the isotope dilution technique is not used, reanalysis of samples with low recoveries is appropriate. Reanalysis results with acceptable recovery should be substituted for the original results.

6.5.8 ~~Standard~~ Reference Materials

Reference materials are samples of the matrices of interest with known concentrations of contaminants. CRM is a reference material, one or more of whose properties are certified by a technically valid procedure, accompanie by or traceable to a certificate or other documentation that is used by a certifying body. Reference materials are designated standard reference material (SRM) if they have been certified through the National Bureau of Standards validation testing program. ~~SRM are samples of the matrices of interest with known concentrations of contaminants. If the samples have been analyzed through an agency validation testing program (e.g., National Bureau of Standards), they are certified.~~ If they are not certified, the samples should have been analyzed by multiple laboratories by several analytical methods. CRM ~~SRM~~ provide information on the accuracy (i.e., how near the measurement is to its true value) as opposed to precision (i.e., how near replicate measurements are to each other). When analyzed in replicate, CRM ~~SRM~~ provide information on both accuracy and precision.

CRM ~~SRM~~ are not readily available for marine sediments, especially for fresh-frozen sediments. However, Northwest NOAA/NMFS has prepared a fresh-frozen marine sediment sample (from Sequim Bay) spiked with PCB, PAH, and selected pesticides for use in Puget Sound studies by EPA, NOAA, and other agencies and laboratories. This reference material ~~SRM~~ is available from the EPA Office of Puget Sound Region 10.

Objective—The objective of the reviewer is to verify the results reported for the CRM ~~SRM~~ to check the overall accuracy of the method. If replicate CRM ~~SRM~~ were analyzed, the reviewer should refer to Section 6.5.10 below to verify the precision evaluation of CRM ~~SRM~~ replicates.

Requirements—Replicate CRM ~~SRM~~ are often analyzed before a project begins to validate the analytical method proposed and the overall laboratory performance. The CRM ~~SRM~~ must be treated exactly as a normal sample throughout the entire analytical procedure.

CRM ~~SRM~~ should be analyzed once per each batch of 5-50 samples. It is recommended that one CRM ~~SRM~~ be analyzed for batches of five or fewer samples.

The reported values should be within the 95 percent confidence interval certified by the agency dispensing the CRM ~~SRM~~. If more than two analytes fall outside of the 95 percent confidence interval, corrective action should be taken. If the reference material is not certified, control limits may not be appropriate, but the CRM ~~SRM~~ can still be used to assess overall accuracy (in conjunction with matrix spikes and surrogate compounds).

Evaluation Procedure—The reviewer should check the chromatograms and associated quantitation reports and mass spectra (if applicable) reported for CRM ~~SRM~~. As with all samples analyzed, compound confirmation and quantification should be assessed along with the method blank, calibration, and surrogate data associated with the CRM ~~SRM~~ analysis. The results should be compared to the mean concentrations (along with their SD) provided by the agency dispensing the CRM ~~SRM~~. The following items should be reviewed during evaluation of CRM ~~SRM~~:

- Determine whether the CRM ~~SRM~~ has a similar matrix as the samples of interest.
- For detected compounds, confirm compound identification and recalculate concentrations for several chemicals (details of calculations are presented in Section 6.6).

- Verify that one CRM ~~SRM~~ was analyzed ~~run~~ for every 50 samples.
- Compare the CRM ~~SRM~~ to the reported values (and SD) of the dispensing agency. Verify that the reported values are within the 95 percent confidence interval. If certified values are not available, calculate the percent recovery for detected compounds as follows:

$$\% \text{ Recovery} = \frac{\text{detected concentration}}{\text{agency-reported concentration}} \times 100$$

Action—If concentrations of certain analytes are outside the 95 percent confidence interval, the reviewer should use professional judgment along with the results of surrogate recoveries and matrix spikes to determine whether all data should be qualified as estimates (E) or underestimates (G), or in very extreme cases (such as recovery <5 percent), rejected. Data should not be qualified based on poor SRM results alone, although poor results are cause for concern. Evaluation of performance based on reference materials that are not certified should carry less weight than the performance based on certified SRM.

The reviewer should note whether biases are apparent. If results are consistently low for all chemicals, poor technique may be the problem. If certain classes of analytes have acceptable recoveries while other classes have poor recoveries, the analytical protocol may not be suitable for some target compounds.

At least one SRM analysis should be performed at the beginning of a project to demonstrate acceptable laboratory performance. Analysis of actual samples can be made contingent on QA review and acceptance of SRM results.

6.5.9 Matrix Spikes

Matrix spikes are currently the most common form of recovery data provided by laboratories and are required by the EPA/CLP. Matrix spikes are samples that are spiked with a known amount of analytes of interest (not their isotope-labeled analogs) prior to extraction. Generally, a sample assumed to be uncontaminated is chosen for matrix spike analysis, as the spikes should be added at 1-5 times the concentration of compounds in the sample before spiking. Matrix spike replicates are valuable for assessing accuracy and precision. Use of the isotope dilution technique precludes the need for matrix spikes, as surrogates for most target compounds are available in all samples when using isotope dilution.

Objective—The objective of the reviewer is to verify the results reported for the matrix spike in the context of the overall accuracy of the analytical method. Calculation checks should be performed in the same manner as regular samples (see Section 6.6), except that the sample concentration in an unspiked replicate is subtracted from the total concentration in the matrix spike sample to determine the amount of spike recovered. If matrix spike duplicates are analyzed, refer to Section 6.5.10 ~~6.5.9~~ to assess precision.

Requirements—The spikes added to the sample should include a wide range of representative analytes (i.e., representing the different chemical classes and molecular weights of the target compounds). The spiking level should be 1-5 times the concentrations of the analytes in the samples.

Frequency: Matrix spikes should be run once for every batch of 20 or fewer samples. The total number of matrix spikes analyzed should be at least 5 percent of the total number of samples analyzed. Matrix spike samples are not required if the isotope

dilution technique is used. With the exception of the isotope dilution technique (for A/B/N compounds), matrix spikes should be performed for each kind of analysis (e.g., separate matrix spikes should be performed for pesticides/PCB and A/B/N compounds). The EPA/CLP specifies addition of six pesticides to matrix spikes for pesticide/PCB analysis. If recovery information regarding PCB is more desirable than for pesticides, the laboratory should be instructed to spike PCB (e.g., Aroclor 1254 or 1260) rather than pesticides.

Control Limits: Recovery of spiked compounds should be ≥50 percent.

Evaluation Procedure—The reviewer should evaluate the chromatograms and associated quantification reports and spectra (if applicable) for matrix spikes. As with all samples analyzed, compound confirmation and quantification should be assessed along with the method blank, calibration, and surrogate data associated with the matrix spike samples. The following items should be reviewed during evaluation of matrix spike samples:

- For detected compounds, confirm compound identification and recalculate concentrations for several chemicals (see Section 6.6).
- An example matrix spike summary for six chlorinated hydrocarbon pesticides (analyzed by GC/ECD) is shown in Figure 6-8. Although forms will differ among laboratories, the relevant information that should be summarized on all forms includes sample identification number, compounds spiked, spiking concentrations, unspiked sample results (undetected compounds will be treated as zeros), the results for the matrix spike ("Conc. MS" in Figure 6-8), and the percent recovery. Unspiked sample concentrations should be checked with the original sample. After the matrix spike concentrations have been verified, recalculate the percent recovery as follows for several compounds:

$$\% \text{ Recovery} = \frac{SSR - SR}{SA} \times 100$$

where:

SSR = Spiked sample results

SR = Sample results (unspiked); consider detection limits as zero

SA = Amount of spike added.

All values used in the recovery calculation should be in the same units (e.g., ppb dry weight).

- Verify that one matrix spike was analyzed for every 20 samples.
- Verify that the spike recoveries were above 50 percent after subtracting the amount of analyte present in the unspiked sample.

Action—Low matrix spike recoveries may result from matrix interferences in the sample. Therefore, poor results alone should not be cause for data qualification. In the event of poor matrix spike performance, results of SRM analyses and surrogate recoveries should be considered before any associated sample data are qualified as an estimate (E) or underestimate (G), or in very extreme cases, rejected. Professional judgment must be used to determine which samples are associated with each matrix spike.

SOIL MATRIX SPIKE/MATRIX SPIKE DUPLICATE RECOVERY

FRACTION	COMPOUND	CONC. SPIKE ADDED (ug/Kg)	SAMPLE RESULT	CONC. MS	% REC
PEST PTI SAMPLE NO. EBP 10	Lindane	10.5	0.0	11.3	108 ✓
	Heptachlor	10.5	0.0	10.7	103
	Aldrin	10.5	0.0	11.6	112
	Dieldrin	26.0	0.0	32.0	123
	Endrin	26.0	0.0	29.9	115
	4,4'-DDT	26.0	0.0	24.4	94 ✓

* ASTERISKED VALUES ARE OUTSIDE QC LIMITS.
Percent Recovery: 0 out of 6 outside QC Limits

Comments:

FORM III

Figure 6-8. Example summary form for pesticide matrix spikes analyzed by GC/ECD

6.5.10 Replicate Analyses

Objective—Analytical replicates are multiple analyses of samples, matrix spikes (as in the EPA/CLP), or SRM. Analytical replicates provide information on the precision of the analytical method, assuming that the replicated samples are truly homogenous. Analytical replicates are subsamples of a single homogenized sample. Field replicates are separate samples collected concurrently from the same station. Field replicates provide information on overall variability (analytical plus field variability). To estimate field variability, field and analytical replicates should be analyzed at the same stations. No QA criteria have been established for field replicates, as criteria are not appropriate when measuring field sampling variability and environmental heterogeneity.

Requirements—Replicate analyses should be performed on homogenized aliquots of samples using the same method, and a minimum of 5 percent of the total number of samples analyzed. A minimum of one replicate should always be analyzed (i.e., even for less than 20 samples). With more than 20 samples, one blind triplicate analysis and analytical duplicate analyses should be required for a minimum of 5 percent overall replication. Triplicates yield a better estimate of precision than duplicates.

Precision of analytical replicates should be within ±100 percent CV (for more than two replicates) or within 100 RPD when duplicates are analyzed.

Evaluation Procedure—Replicate analyses are evaluated the same way as samples. If matrix spike duplicates are not used, replicates are actual samples. Chromatograms should be reviewed and compound confirmation and quantification should be assessed along with method blank, calibration, and surrogate data associated with the replicate samples. The replicates are then compared with each other to see if they are within ±100 percent CV or RPD. The following items should be reviewed during evaluation of replicate analyses:

- Compare replicate chromatograms, noting obvious differences (e.g., relative peak heights, levels of interferences).
- If triplicates are analyzed, use the following equation to calculate the CV for each compound:

$$CV = \frac{SD}{mean} \times 100$$

where the SD is calculated as described in Figure 6-4b. If duplicates are analyzed, use the following equation to calculate the RPD for each compound:

$$RPD = \frac{D_1 - D_2}{(D_1 + D_2)/2} \times 100$$

where:

D_1 = Concentration in Duplicate 1

D_2 = Concentration in Duplicate 2.

Detection limits should not be used in CV or RPD calculations (i.e., a CV or RPD should be calculated for a compound only if the compound ~~it~~ is detected in all replicates). However, if a compound is not detected in all replicates and the detection limit and detected value differ considerably (e.g., by a factor of 3 or more), the data should be carefully examined (e.g., for miscalculated detection

limits, false positive results, inconsistent surrogate recovery among replicates, or calibration problems).

- Identify all compounds with CV or RPD values greater than 100 percent.
- Verify that replicates were run for a minimum of 5-percent replication. For sets of less than 20 samples, one duplicate should be analyzed; for 20 or more samples, one triplicate and additional duplicates should be analyzed (if a triplicate was submitted blind to the laboratory).

Action—Quantification (E) of compounds that exceed precision control limits should be based on professional judgment and on consideration of other QA elements (such as matrix spike and surrogate recoveries). If exceedances are relatively minor (e.g., 103 percent CV) and only apply to one or two compounds, qualification may not be necessary. If qualification is considered necessary, professional judgment must be used to determine which samples should be associated with each set of replicates or if the affected chemicals must be qualified ~~quantified~~ for the entire data set.

6.6 CALCULATION CHECKS

Calculation checks specific to QA elements (e.g., RPD, CV, RF values for calibration) have been described previously in this section. However, complete calculations of concentrations of target compounds in samples were not presented previously, and are shown below. Note that the same calculations would be used to determine concentrations of analytes in SRM or matrix spikes, to calculate the concentration of a surrogate compound in a sample, and to calculate concentrations of analytes in blanks (except blank concentrations are expressed as μg/blank rather than μg/kg sediment).

6.6.1 Isotope Dilution

When isotope dilution is used, recovery correction can be applied automatically by assuming the recovery of the surrogate compound (which serves as the internal standard) is 100 percent. (The actual surrogate recovery can be calculated as described in Section 6.5.7.)

$$\text{Conc. (recovery-corrected)} = \frac{C_A\ (\mu g/kg)}{RF} \times \frac{A_T}{A_{is}}$$

where:

C_A = Concentration of labeled surrogate as spiked into the sample (dry weight).

A_T = Area of target compound

A_{is} = Area of labeled surrogate

RF = Response factor of target compound as shown in Figure 6-4b.

6.6.2 Internal Standard Quantification

The internal standard technique, commonly encountered for GC/MS analyses without isotope dilution (and occasionally for GC/FID and GC/ECD), is based on the following general calculation:

$$\frac{A_T}{A_{is}} \times \frac{Amnt_{is}}{RF} \cancel{\times \frac{V_{tot}}{V_{inj}} \times \frac{1}{W_s}} \times y$$

where:

A_T = Area of target compound

A_{is} = Area of internal standard

$Amnt_{is}$ = Amount of internal standard added to final extract ~~(ng injected)~~

RF = Response factor of target compound as discussed in initial calibration

~~V_{tot} = Total extract volume~~

~~V_{inj} = Injection volume of extract~~

W_s = Sample weight (dry)

y = Correction factor (see text).

Note that a dry weight sample weight can be calculated from the sample wet weight (W) and percent total solids (TS) ~~percent moisture (D)~~ as follows:

$$W_s \text{ (dry weight)} = W \times \frac{\overset{TS}{\cancel{100 - D}}}{100}$$

The correction factor (y) in the above equation is specific to a given method, and corrects for dilution or splitting of the extract, or other adjustments. For example, if a sample extract was split before GC/MS analysis and only 75 percent was used for GC/MS, then y would be 100/75 or 1.33.

6.6.3 External Standard Quantification

External standard quantification is used for GC/ECD analysis of pesticides and PCB by the EPA/CLP. The external standard method uses the following general calculation:

$$\frac{A_T}{RF} \times \frac{V_{tot}}{V_{inj}} \times \frac{1}{W_s} \times y$$

where ~~all~~ terms are as defined above, and:

V_{tot} = Total extract volume

V_{inj} = Injection volume of extract

6.7 REFERENCES

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7. QUALITY ASSURANCE FOR VOLATILE ORGANICS IN SEDIMENTS

7.1 INTRODUCTION

In comparison to analyses of semivolatile organic compounds and metals, analyses of VOC typically present infrequent QA problems. The better relative performance for volatile compounds can be attributed to the simplicity of VOC analyses. Purge-and-trap analyses are carried out in a closed system and present little opportunity for analytical losses. Target compound losses likely occur before the addition of surrogate spike compounds. The most commonly encountered problem is laboratory contamination, as many of the analytes are also common laboratory solvents (e.g., methylene chloride, acetone).

A summary of frequencies and control limits of various QA samples of interest when reviewing VOC data is shown in Table 7-1.

7.2 UNIQUE SAMPLING REQUIREMENTS FOR VOLATILE ORGANICS

Solvents in the sampling area, storage unit, or laboratory can contaminate VOC samples; therefore, glassware must be cleaned with detergent, rinsed with deionized, distilled water, and oven-dried at >105° C for 1 hour. Before the sampling event, sampling equipment should be cleaned in the same manner as the glassware. Between samples, equipment should be rinsed well with water to remove all traces of sediment and any organic films. Never rinse sampling gear with solvents, as solvents are a source of contamination for VOC samples. Samples should not be stored in the same refrigerator with solvents or in a refrigerator that has been used for solvent storage.

Samples should be transferred to precleaned jars as soon after recovery as possible. Never homogenize VOC samples, as the more surface area exposed to the atmosphere, the higher the probability of analyte loss. Jars should be filled completely (with no headspace) to ensure that VOC do not diffuse into ~~are not partitioned in~~ the headspace. Care should be taken to ensure that the sample has only come in contact with the scooper and not the sides of the sample device. The sample should be refrigerated or stored on ice at 4° C (not frozen) immediately after the sample is put into the jar. Samples need to be shipped as soon as possible and analyzed within 14 days of collection.

7.3 DATA COMPLETENESS AND FORMAT

All deliverables specified in the SOW should be confirmed upon receipt of the data package. Although complete review of the data will probably not occur immediately upon data receipt, review will be facilitated if all necessary documentation is available. If documentation completeness is not checked immediately upon data receipt, laboratory staff will be less likely to recall details of the project, and retrieving documents from laboratory files could be time consuming. Often, omissions from the data package result from oversights rather than the laboratory's inability to produce missing items.

The data set is considered complete when all items are present (as specified in the SOW) or addressed in the cover letter. The following items should be included for proper data validation by independent QA/QC review and should always be specified in the original SOW:

- A cover letter referencing or describing the procedure used (noting any procedure modifications) and analytical problems encountered
- Reconstructed ion chromatograms for GC/MS analyses for each sample

TABLE 7-1. RECOMMENDED FREQUENCIES AND CONTROL LIMITS FOR VOLATILE QA SAMPLES

Analysis Type	Frequency of Analysis[a]	Control Limit
Method blanks	~~One per extraction batch or one~~ One per 12-hour shift~~; whichever is more frequent~~	~~$MeCl_2$, CH_2Cl_2,~~ acetone: 5 μg total or 50% of the analyte Other organic compounds: 2.5 μg total or 5% of the analyte
Matrix spikes	Not required if complete isotope dilution technique used <20 samples: one per set of samples submitted to laboratory ≥20 samples: 5% of total number of samples	≥50% recovery
Replicates	<20 samples: one per set of samples submitted to laboratory ≥20 samples: one triplicate and additional duplicates for a minimum of 5% total replication	~~None±100% coefficient of variation (for >2 replicates) or ±100% RPD (for duplicates)~~
Surrogate spikes	Every sample	50% recovery (10% if isotope dilution technique is used)
Initial calibration	Before any samples analyzed, after each major disruption of equipment, and when ongoing calibration fail to meet criteria	RF >0.250 for bromoform, RF >0.300 for chloromethane, 1,1,-dichloroethane, ~~1,1,2,2-tetrachloroethane,~~ and chlorobenzene and RSD 20% (≤30% for EPA/CLP) ~~≤30% for 1,1-dichloroethane, chloroform, 1,2-dichloropropane, toluene, ethylbenzene, and vinyl chloride~~
Ongoing calibration	At beginning of each work shift, every 10-12 samples or every 12 hours (whichever is more frequent), and at the end of each shift	>25% initial RF calibration for GC/MS

[a] Frequencies listed are minimums; some programs may require higher levels of effort.

- Mass spectra of detected target compounds for each sample and library spectra of all target compounds
- Raw data quantification reports for each sample
- A calibration data summary reporting calibration range used (and BFB spectra and quantification reports for GC/MS analyses)
- Final sample volumes and dilution factors, sample size, wet-to-dry ratios, and IDL
- Analyte concentrations with reporting units identified (two significant figures unless otherwise justified)
- Quantification of all analytes in method blanks (μg/sample)
- A list identifying the method blanks associated with each sample
- Tentatively identified compounds (if requested) and methods of quantification (including spectra)
- Recovery assessments and a replicate sample summary (laboratories should report all surrogate spike recovery data for each sample; the range of recoveries should be included in reports using these data)
- Data qualification codes and definitions.

Data should be reported on standard forms so different data sets are of uniform format. This uniformity aids in both internal and external QA/QC review and data validation. Unless otherwise specified, the data package should be complete to avoid misinterpretations based on missing information.

7.4 OVERVIEW OF ANALYTICAL METHODS

Two methods have been determined generally acceptable for analysis of VOC. The EPA/CLP method for priority pollutants uses a heated purge-and-trap procedure that is cost-effective and can attain 10-20 μg/kg (dry weight) detection limits. A vacuum extraction/purge-and-trap method (Hiatt 1981; Hiatt and Jones 1984) is under consideration by the EPA for validation as a standard method. The vacuum technique has shown better recoveries of several compounds than the CLP method. While these two methods are used by laboratories in the Northwest, the purge-and-trap procedure is the most common.

7.4.1 Heated Purge-and-Trap Method

An inert gas is bubbled through a mixture of sediment sample (5 grams) and reagent-grade water contained in a purging chamber. In the purge-and-trap device, the VOC are purged from the aqueous phase into a gaseous phase by the inert carrier gas. The VOC are passed into a sorbent column and trapped. After purging is completed, the trap is back-flushed and heated rapidly to desorb the compounds into a GC/MS.

GC/MS analysis is performed according to EPA Method 624 (U.S. EPA 1984). The isotope dilution technique is not required, because the system is closed and no problems with recovery have been observed using Method 624. Method 624 requires spiking samples with three surrogate compounds to assess recovery ~~check recoveries~~ (e.g., p-bromofluorobenzene, ~~4-BFB,~~ d_4-1,2-dichloroethane, and d_8-toluene are used for EPA/CLP).

7.4.2 Vacuum Extraction Technique

Hiatt (1981), and Hiatt and Jones (1984) used a vacuum extraction technique to analyze VOC. Their results were equal to or better than the heated purge-and-trap method. With this technique, vacuum-extracted VOC are transferred directly from a cryogenically cooled trap to a fused-silica capillary column for GC/MS analysis. This capillary column technique allows for optimum resolution and rapid conditioning between samples.

7.5 DATA VALIDATION AND ASSESSMENT

General guidelines are presented here for evaluating data independent of the analytical methods employed. Standard methods are widely accepted and should present few problems for routine applications. However, when modifications are made, the reviewer should assess whether the analytical scheme is reasonable.

7.5.1 GC/MS Tuning

Before preceding with calibration and analysis of samples, the GC/MS must be tuned to established specifications to ensure proper mass identification, mass resolution, and sensitivity. A verification of tuning results should be the first step in QA review of the standards data package.

Objective—The objective for reviewing the GC/MS tuning data is to verify that the instrument was properly adjusted for optimum performance.

Requirements—GC/MS tuning criteria for BFB have been specified for EPA/CLP (U.S. EPA 1988) and are shown in Table 7-2.

Tuning must be performed and verified before each 12-hour shift. Control limits for GC/MS tuning are shown in Table 7-2. An example for DFTPP tuning is shown in Figure 6-3a,b.

Evaluation Procedures—Calculations should be checked for each GC/MS tuning. A sample calculation of the tuning results reported by a laboratory is given in Section 6.5.1.

Evaluation considerations for GC/MS tuning are:

- Compare the data transcribed onto the GC/MS tuning form with the GC/MS mass listings.
- Ensure that the laboratory has not made transcription errors or calculation errors. For example, calculate the ratio intensity of m/z 175 to m/z 174 (as percent).
- The following EPA/CLP guidance is useful for applying judgment to results that are outside specifications (U.S. EPA 1988):

  > The most important factors to consider are the empirical results that are relatively insensitive to location on the chromatographic profile and the type of instrumentation. Therefore, the critical ion abundance criteria for BFB are the m/z 95/96 ratio, the 174/175 ratio, the 176/177 ratio, and the 174/176 ratio. The relative abundances of m/z 50 and 75 are of lower importance.

TABLE 7-2. GC/MS TUNING CRITERIA FOR BROMOFLUOROBENZENE

m/z	Ion Abundance Criteria
50	15.0-40.0% of the base peak
75	30.0-60.0% of the base peak
95	Base peak, 100% relative abundance
96	5.0-9.0% of the base peak
173	<2% of m/z 174
174	>50.0% of the base peak
175	5.0-9.0% of m/z 174
176	>95.0%, but <101% of m/z 174
177	5.0-9.0% of m/z 176

Action—Unless otherwise specified, the criteria in Table 7-2 must be met because the criteria are not sample-specific. If they are not met, the data should be qualified as estimated (E) or rejected. Note that laboratories often report the samples associated with each tuning (see lower portion of Figure 6-3a).

7.5.2 Initial Calibration

Initial calibration is performed to determine the response of the instrument across a range of concentrations of each analyte of interest. The relationship between response and concentration is often called linearity. RF of analytes to standards at various concentrations are established by calibration. The standards may be surrogate compounds (for isotope dilution) or injection internal standards.

Objective—The objective of the reviewer is to verify that the GC used for analysis was properly calibrated over a wide range of concentrations prior to sample analysis. Quantification of target compounds in samples is suspect if initial calibration criteria were not met.

Requirements—The frequency of initial calibration is dependent upon the control limits set and failure to meet these criteria. Initial calibration should be performed at the onset of a project, whenever there is a major disruption in instrumentation, or when the criteria for ongoing calibration are not met (see Section 7.5.3).

RF values must be determined for at least three concentration levels (five concentration levels, or a five-point calibration, is preferable). The standard concentrations tested should encompass the range of expected sample concentrations. One standard concentration for each target chemical must be within 150 percent of the stated detection limit (PSEP 1986). RF values for bromoform must be >0.250. RF values for chloromethane, 1,1-dichloroethane, 1,1,2,2-tetrachloroethane, and chlorobenzene must exceed 0.300.

The RF of most target compounds should not differ by more than 20 percent CV (also known as RSD) over the range of concentrations tested for each analyte. Hence, the response of the instrument is assumed to increase in direct proportion to concentration of the analyte when <20 percent deviation in response is observed over the concentration range bracketed by the calibration curve. EPA/CLP recommends a less stringent control limit (±30 percent CV) (U.S. EPA 1988).

Evaluation Procedures—Calculations for RF and CV should be checked against the chromatograms and quantification lists ~~responses~~ provided by the laboratory. A typical initial calibration summary sheet is shown in Figure 6-4a,b,c,d. Pertinent information included in any summary are RF for each target compound at each standard concentration, average RF values for each chemical, and the percent RSD for RF values for each target compound. The following items should be confirmed during the evaluation of initial calibration:

- Verify that all RF values are at least 0.05.
- Check several RF calculations (calculation checks should account for several chemicals and standard concentrations; see Section 6.5.2).
- Average RF calculations should be checked for several compounds. An example calculation of average RF is shown in Calculation 2 of Figure 6-4b.
- Percent RSD calculations should be checked for several compounds. An example calculation of percent RSD is shown in Calculation 3 of Figure 6-4b~~, which includes (n-1), not n, in the denominator~~.
- Verify that all target compounds have RSD of ≤ 20 percent (≤ 30 percent is allowable for calibration check compounds specified by EPA/CLP and is reasonable for

compounds that are very polar or not amenable to GC analysis with typical stationary phases).

- Verify linearity of the calibration range by calculating the equation of the curve (assuming the ongoing calibration criteria have been met as discussed in Section 7.5.3).

Action—If linearity is not established, the laboratory should adjust the instrument and recalibrate before analyzing samples, or the range for reporting data should be reduced to within the observed linear range. If the laboratory failed to take these measures (and was not contractually required to do so), the QA reviewer must determine whether data for compounds out of calibration should be qualified or rejected. Qualification (with E) is appropriate for minor exceedances of control limits (e.g., 23 percent rather than 20 percent RSD), whereas rejection is more appropriate for large exceedances (e.g., 70 percent rather than 20 percent RSD). Data reported out of the calibration range should also be qualified as estimates (E), unless the laboratory can furnish evidence of linearity to the reported level.

7.5.3 Ongoing Calibration

While analyzing sample sets, continuing calibration checks are required to determine whether the calibration for the instrument is still valid. Ongoing calibrations are always used in association with the most current ~~a specific~~ initial calibration curve.

Objective—Ongoing calibration should be checked by the reviewer to ensure the instrument used for analysis was still in calibration while samples were analyzed.

Requirements—Ongoing calibrations are analyzed often as a ~~constant~~ check that the instrument is performing satisfactorily. The standard used to check ongoing calibration should be one of the intermediate standards used for the initial calibration curve.

Frequency: The ongoing calibration check should be performed at the beginning of each work shift, every 10-12 samples or every 12 hours (whichever is more frequent), and after the last sample of the work shift.

Control Limits: RF values for all target compounds should be within 25 percent (measured as percent difference) of average RF values from the initial calibration. ~~The ongoing calibration criterion is satisfied when one of the calibration standards (usually one of intermediate concentration) is analyzed, and all RF values are within 25 percent average RF of the initial calibration at that concentration (i.e., percent difference is 25 percent).~~

Evaluation Procedure—Pertinent information needed for reviewing ongoing calibrations include average RF for each target compound (obtained from the relevant initial calibration), RF of each target compound (from the ongoing calibration; at an intermediate concentration of the initial calibration), and percent difference between the average RF and the RF of the ongoing calibration. The following items should be confirmed when evaluating ongoing calibrations:

- Verify that all RF values are ≥ 0.05.
- Verify that the average RF values reported for the ongoing calibration are the same as the values determined from the relevant initial calibration. Check for transcription errors or use of the wrong initial calibration (i.e., only the most recent initial calibration should be used).

- RF values from the ongoing calibration should be calculated for several target compounds. An example of an RF calculation is shown in Calculation 1 of Figure 6-4b.
- Percent difference values should be recalculated for several compounds. An example of a percent difference calculation is shown in Section 6.5.3.
- Verify that the ongoing calibration was performed at the appropriate frequency
- Verify that the percent difference for each target compound is ≤25 percent.

Action—Failure to attain the control limit for ongoing calibration should have resulted in an additional initial calibration and reanalysis of the samples analyzed between the last valid calibration and the invalid calibration. If such laboratory actions were not taken, data for the samples run between the last valid calibration and the invalid calibration should be qualified as estimates (E); qualification applies only to chemicals with >25 ~~(GC/MS) or >15 (GC/ECD)~~ percent difference from the initial calibration. The laboratory should have provided a chronological list of samples and calibrations in order of instrumental analysis (e.g., Figure 6-3a for GC/MS analysis), which can be used to determine the samples associated with each calibration.

7.5.4 Compound Confirmation

Objective—The primary objective of compound confirmation is to confirm compounds reported as detected in samples are present (i.e., to investigate the possibility of false positives) and to verify, to the extent possible, target compounds reported as undetected are not present (i.e., to investigate the possibility of false negatives). Compound confirmation during QA review focuses on false positives rather than false negatives, because detected compounds are associated with data supporting positive identifications (e.g., mass spectra), whereas undetected compounds are largely associated with an absence of data. To some extent, false negatives are addressed during QA review of factors relating to analytical sensitivity (e.g., detection limits, analytical recovery).

Requirements—Because evaluation of GC/MS data requires professional expertise and judgment, the specifications for retention time and mass spectra below should be considered as guidelines rather than firm criteria. These guidelines are based on requirements of the EPA/CLP, which are designed to preclude false positives ~~rather than to ensure there are no false negatives~~.

Mass spectra of the target compounds in a sample and a recent laboratory-generated standard should agree according to the following criteria:

- The RRT of the target compound should be within ±0.06 RRT units of the calibration standard
- All ions present in the standard mass spectrum at a relative intensity greater than 10 percent should be present in the sample spectrum
- The relative intensities of ions specified above must agree within ±20 percent between the standard and sample spectra (e.g., for an ion with an abundance of 50 percent in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70 percent)
- Ions greater than 10 percent in the sample spectrum, but not present in the standard spectrum, should be considered as possible interferences due to co-eluting compounds, or as possible evidence the overall spectrum is not that of the target compound.

Evaluation Procedures—Compounds reported as detected in each sample should be confirmed by examining RRT and, more importantly, mass spectra.

- Confirm the retention time of the compound is within a reasonable retention time window, as compared to the calibration standard (±0.06 RRT units). GC/MS data systems are programmed to search in a specified window for the target compound. If the window was reasonably specified by the laboratory, little effort need be expended during QA review. For reference, computer-generated GC/MS quantification reports will typically list RRT along with absolute retention time. Note that software used for the isotope dilution technique typically first locates the surrogates, and then searches for the associated target compounds. If the surrogate is not found (i.e., if surrogate recovery is 0 percent), the computer may not search for the target compound. This process could result in false negative results for that compound.

- When GC/MS is used, the mass spectrum is more important than retention time in confirming compound identification (e.g., retention time shifts can occur because of an unusual sample extract matrix). The laboratory can report the mass spectral information in two general formats: 1) as a histogram, in which relative intensity is plotted vs. m/z (mass/charge ratio; see Figure 6-6a,b), and 2) in tabular form, where relative intensities are listed with corresponding ions (i.e., m/z ratios; see Figure 6-3b), discussed as "mass listings" in Section 7.5.1). The output in Figure 6-6a (or a similar format with the sample and library spectra on the same page) is available with most GC/MS data systems and is useful for comparing sample spectra to library spectra. In Figure 6-6a, good agreement exists between the spectrum in the sample and the library spectrum of benz(a)anthracene. Relatively low levels of spectral interference (e.g., <10 percent intensity or <100 on the y-axis) are apparent in the sample spectrum for m/z below 200. Also note the presence of ions in the region around m/z 240 that derive from d12-benz(a)anthracene, a surrogate compound that partially co-eluted with the target compound. Figure 6-6b displays a spectrum of fluoranthene in raw form and after being enhanced by computer software. Use of enhanced or background-subtracted spectral data is common and generally acceptable. Note that enhancement has significantly reduced interferences in Figure 6-6b. The tabular data format facilitates quantitative comparisons of relative ion intensities, but is neither always necessary (i.e., the visual comparisons in Figure 6-6b are often sufficient) nor always included in data packages.

Action—Professional judgment is a critical aspect of compound confirmation. If chromatographic or mass spectral evidence suggest false positive results, the compound should be reported as undetected (U) at an appropriate detection limit. The appropriate detection limit should account for the presence of high levels of interferences. If the reviewer is convinced the compound is present, but the supporting evidence is only marginally acceptable, the compound should be reported as an estimate (E).

7.5.5 Detection Limits

Detection limits are a critical but often overlooked aspect of data quality often overlooked. Detection limits are variously based on instrument sensitivity, levels of blank contamination, matrix interferences, and various levels of statistical significance.

Objective—The objective of reviewing detection limits is to confirm that the detection limit is consistent with the requirements of the contract and to validate that detection limits have been correctly calculated.

Requirements—Detection limit requirements are project specific and should be specified in the laboratory's contract. On GC/MS, IDL of approximately 1-2 ng on-column should be attainable for VOC. The detection limit specified by PSEP and the EPA/CLP are approximately 10-20 μg/kg (PSEP 1986; U.S. EPA 1988).

The reported detection limit should be within the control limits set by the SOW at the beginning of the project. Samples with high interferences should have correspondingly higher detection limit. This should be evaluated, but no action should be taken, as interference levels are not within the control of the laboratory. As specified by PSEP (1986), detection limits for VOC should fall between 10 and 20 μg/kg dry weight for sample sizes of approximately 5 grams ~~50-100 grams~~ wet weight of sediment.

Evaluation Procedures—Detection limits typically reported by laboratories do not conform strictly with methods defined by Keith et al. (1983). Often, reported sample detection limits are based on IDL and account for sample weight~~, injection volume, and total extract volume~~. For example, the following formula is often used to calculate sample detection limits for VOC:

$$\text{Sample detection limit} = \frac{\text{IDL (on-column)} \cancel{\times (\text{injection volume/extract volume})^{-1}}}{\text{sample weight}}$$

IDL are sometimes determined by the EPA/CLP method. The preferable method is by injection of calibration standards at lower and lower concentrations until a concentration corresponding to an appropriate signal/noise ratio (e.g., approximately 3) is determined.

~~In general, any factor affecting the calculation of detected concentrations in a sample should also affect the calculation of detection limits. For example, if the sample required dilution, detection limits should increase by the dilution factor. If the extract was split for any reason (e.g., 80 percent for GC/MS, 20 percent for GC/ECD), the detection limit should be adjusted accordingly (e.g., if only 80 percent of the extract is used, the detection limit should be multiplied by 100/80 or 1.25). During QA review, the reviewer should determine whether detection limits accounted for such adjustments.~~

Detection limit validation should address the following considerations:

- Calculations for some samples should be checked to determine the method for calculation, whether this method was reasonable, and whether it was applied consistently.
- The lowest initial calibration standard should be in the range of the IDL; if not, the IDL itself is questionable. Mass spectra for the lowest concentration standard are helpful to confirm a reasonable signal/noise ratio is met for that standard.
- Matrix spike recoveries should be consistent with detection limits. For example, if a matrix spike was added at 1,000 ppb and was not detected, the detection limit for that compound in that particular sample would be ≥1,000 ppb. If stated detection limits for the same compound in most samples were 50 ppb, it would be reasonable to suspect those detection limits as underestimates ~~(unless the matrix spike had been diluted to 0.05 its original concentration)~~.
- Evidence of poor recovery (e.g., low surrogate or matrix spike recovery for a specific compound or class of compounds) should be factored into detection limits, if possible. For example, if 20 percent surrogate recovery was observed in a given sample in which the related target compound was undetected, the detection limit for the target compound should be established at 5 times the level that would be set if surrogate recovery were 100 percent.

Action—If the standards used during calibration do not bracket the detection limits, the detection limits should be recalculated. Often, detection limits are not calculated on a sample-by-sample basis. For example, a detection limit of 10 ppb for an analyte may have been appropriately determined for an "average" sample, but when a very contaminated sample (with higher levels of interferences) is analyzed, the same detection limit may be reported. Detection limits should be adjusted in such cases.

7.5.6 Analysis of Blanks

Blanks are analyzed to assess possible contamination of samples associated with sample handling. Contamination is of concern because it can result in false positives. Organic solvents (e.g., acetone, methylene chloride) are common laboratory contaminants because of their volatility and exposure of sample extracts to the laboratory atmosphere.

Objective—The objective of the reviewer is to assess contaminant levels in method blanks. If significant contamination exists, ~~and~~ the reviewer may decide that corrections to the data should be applied to minimize the effects of laboratory contamination on the analyte concentrations. For such corrections, the blank analyses are assumed to be representative of the potential contamination in sample ~~extracts~~.

Contamination of VOC samples during analysis can result from impurities in the purge gas, organic compounds out-gassing from the plumbing upstream of the trap, and solvent vapors in the laboratory. The analytical system must be demonstrated to be free from interferences by analyzing blanks initially and with each sample set. After analysis of a high-level sample, contamination of the next sample may result from carryover of traces from the previous sample.

Requirements—Ideally, blanks should contain no detectable analytes. Blanks can be expressed in absolute levels (total ng/blank sample) or relative levels (expressed as a percent of the sample concentrations for each analyte). Relative blank levels are calculated during QA review (not by the laboratory).

~~QA control limits for blanks are typically based on the magnitude of blanks relative to detection limits and sample concentrations. Blanks affect detection limits, because they increase the level of background noise and interfere with the laboratory's ability to discern target compounds in samples.~~

Frequency: Method blanks should be run at least every 12 hours. ~~Method blanks should be run with every extraction batch or every 12 hours, whichever is more frequent. The method blank must be analyzed after calibration verification.~~ The laboratory should provide information showing which blanks correspond with which set of samples.

Control Limits: For most target compounds, blanks should not contain more than 5 percent of the amount of analyte present in samples (or 2.5 μg total). Contamination by common laboratory solvents (e.g., methylene chloride, acetone) is more difficult to control, but should not exceed 50 percent of the level of these compounds in the samples (or 5 μg total).

Evaluation Procedures—The reviewer should check chromatograms of all blanks run with a data set vs. chromatograms of standards. A special concern with blanks is false negatives: a laboratory contaminant that was present but not reported. Peaks present should be confirmed and calculations verified. Any blank containing analytes above the detection limit should be compared to its associated sample set. A typical blank data package should contain a summary sheet with the concentrations of target compounds present (or the detection limit if undetected) and percent recoveries of the surrogates, the total reconstructed ion chromatogram and mass spectra of all

detected analytes for GC/MS, and the quantification report. An example blank calculation is shown in Section 6.5.6.

When any concentration is detectable in blanks, rejection of the data or blank correction during final QA review is recommended. Blank correction entails subtracting the total μg of the compound in the blank from that in the sample and then expressing the difference as a concentration (i.e., divide by the sample weight~~ of sample extracted~~). An example calculation for blank correction is shown in Section 6.5.6. Whether the data are rejected or qualified is a matter of professional judgment. The nature and consistency of the contamination needs to be considered. For example, if common laboratory solvent is present at consistently high levels, data should be rejected~~, qualified.~~

The following should be verified during evaluation of blanks:

- Examine the chromatogram of the blank~~ (i.e., overlay a blank and a standard chromatogram)~~, quantification reports, and mass spectra for each blank analyzed. The reviewer should determine whether peaks were identified correctly and that analytes reported were indeed present.
- Examine surrogate recoveries to check if they are below control limits (see Section 7.5.7). If they are below control limits, the blank may underestimate contamination.
- Evaluate the absolute and relative concentrations of any detected contaminants. The laboratory should report absolute concentrations. Relative concentrations must be calculated during QA review (see Section 6.5.6 for an example calculation).

Action—No action is required when there are no detectable contaminants in the blank. If contaminant concentrations exceed both the absolute and relative control limits, data for the particular analytes should be rejected. If detectable contaminants are within at least one of the control limits, data should be blank-corrected and qualified with either a Z if the blank-corrected concentration exceeds the detection limit, or a B if the blank-corrected concentration is less than or equal to the detection limit.

When any analyte concentration is detected in blanks, rejection or blank correction of the associated data is recommended. See Section 6.5.6 for an example blank-correction calculation.

The laboratory contract should include appropriate actions taken if absolute control limits are exceeded. If contaminant concentrations exceed the control limits, sources of contamination should be identified and discussed in the cover letter of the data report. If problem contaminants (e.g., acetone, methylene chloride) persist, blanks should be replicated and confidence levels for these contaminants should be determined.

7.5.7 Surrogate Spike Compounds

Surrogate spike compounds, or recovery internal standards, are compounds with chemical characteristics similar to those of target compounds. They are used to assess analytical recovery on a sample-specific basis.

Objective—Known amounts of surrogate compounds are added to each sample prior to extraction to evaluate recovery for every sample. Surrogate recovery is the only QA check performed for every sample. Surrogate recoveries can be used to correct analyte concentrations if the actual analyte (or class of analytes represented by the surrogate) and the surrogate compounds are known to behave similarly during sample preparation and analysis.

Requirements—The concentration of individual surrogates added to each sample should be within the expected range of concentrations as bracketed by the calibration standards. A minimum of three spike compounds should be added for VOC. Isotopically labeled analogs of target compounds are recommended and many are commercially available.

Frequency: Because recovery of surrogates is the only means of checking the accuracy of every sample, surrogates should be added to each sample.

Control Limits: The control limit for surrogate recoveries is 50 percent of the amount of each surrogate added. This control limit should be strictly adhered to, because the analytical system is closed and losses greater than 50 percent would indicate a serious problem with the system.

Evaluation Procedures—Recoveries should be verified by checking the chromatograms and raw data. Calculations should be checked by applying the RF of the surrogate to the response from the chromatogram to determine the surrogate concentration in the sample. The following equation is used to determine percent surrogate recovery:

$$\% \text{ Surrogate recovery} = (\text{amt surrogate in sample/amt surrogate added}) \times 100$$

Recovery correction is usually unnecessary for ~~should not be applied to~~ volatile organic data. If poor surrogate recoveries have been obtained, the system should be checked for leaks and the samples reanalyzed. The following items should be verified when assessing surrogate recoveries:

- Verify that at least three surrogates were used. Determine which analytes were represented by each surrogate. Verify that data were not recovery-corrected. If data were recovery-corrected, this correction factor should be removed.
- Check chromatograms to ensure proper identification of surrogate peaks.
- Check several percent surrogate recovery calculations (from several sample sets) using the above equation (see Section 6.5.7 for examples).
- Verify that surrogates were added to each sample.
- Verify that all surrogates were within the 50-percent control limit.

Action—QA review of surrogate recoveries may be complicated by factors arising from the sample itself. Matrix problems, such as interferences and high target compound concentrations, may be outside the control of the laboratory. Therefore, professional judgment and consideration of other QA samples (e.g., matrix spikes) should complement the assessment of surrogate recoveries that exceed control limits. ~~Recoveries of less than 50 percent usually indicate a problem with the system.~~ Data associated with poor surrogate recoveries should be qualified with an E or G (i.e., "estimate" or "estimate is greater than value shown") or rejected. ~~data estimated or estimate is greater than value shown) or rejected.~~

7.5.8 Matrix Spikes

Matrix spikes are currently the most common form of recovery data provided by laboratories and are required by the EPA/CLP. Matrix spikes are samples spiked with a known amount of several target analytes (not their isotope-labeled analogs) prior to extraction. Generally, a sample assumed to be uncontaminated is chosen for matrix spike analysis, as spikes should be added at 1-5 times the concentration of compounds in the sample before spiking. Matrix spike replicates assess accuracy and precision. Matrix spike analysis for VOC are very similar to surrogate recovery analysis, as both are assessments of recovery of compounds added to the closed system. The variability of chemical properties and ranges of recoveries tend to be much narrower for VOC than for A/B/N compounds. ~~as both are assessments of recovery of a known amount of added spike.~~

Objective—The objective of the reviewer is to verify the results reported for the matrix spike in the context of the overall accuracy of the analytical method. Calculation checks should be performed in the same manner as those for regular samples, except that the sample concentration in an unspiked replicate is subtracted from the total concentration in the matrix spike sample to determine the amount of spike recovered. If matrix spike duplicates are analyzed, they should be assessed like regular replicate samples.

Requirements—The spikes added to the sample should include a wide range of representative analytes. The spiking level should be 1-5 times the concentrations of the analytes in the samples.

Frequency: Matrix spikes should be run once for every batch of 20 or less samples. The total number of matrix spike samples should be at least 5 percent of the number of samples analyzed.

Control Limits: The control limit for matrix spike samples is ≥50 percent recovery of the amount of analytes spiked.

Evaluation Procedure—The reviewer should review the chromatograms and associated quantification reports and spectra for matrix spikes. As with all samples analyzed, compound confirmation and quantification should be assessed along with the method blank, calibration, and surrogate data associated with the matrix spike samples. A matrix spike data summary and relevant calculations is shown in Section 6.5.9. The following items should be reviewed during evaluation of matrix spike samples:

- For detected compounds, confirm compound identification and recalculate concentrations for several chemicals (see Section 6.6)
- Determine the spike recovery in the matrix spike (see Section 6.5.9 for equations)
- Verify that one matrix spike was analyzed for every 20 samples
- Verify that spike recoveries exceeded 50 percent after subtracting the amount of analyte present in the unspiked sample.

Action—Low matrix spike recoveries indicate the instrument may not be performing adequately or the analytical technique is inadequate. Because the VOC system is closed, loss of analytes should be minimal ~~not occur~~. If poor matrix spike recoveries do occur, the reviewer should consider results of surrogate recoveries and GC/MS ongoing calibration. Professional judgment is very important in determining whether data should be accepted, qualified, or rejected.

7.5.9 Replicate Analyses

Analytical replicates are subsample analyses of samples or matrix spikes. Replicate analysis provides information on the precision of the analytical method (assuming the samples are homogeneous). VOC samples cannot be homogenized, however, because analytes will be lost from exposed surfaces during mixing. Therefore, the replicate analyses of VOC are considered field replicates. Because VOC samples cannot be homogenized without loss of analytes, there may be poor agreement among replicates if the sample is naturally heterogeneous. Because of their mobility, VOC may be more homogeneously distributed in the environment than semivolatile compounds, but pockets of high VOC concentrations are possible.

Replicate analysis should be performed on 5 percent of the total number of samples analyzed. No control limits are associated with field replicates.

7.6 CALCULATION CHECKS

Calculation checks required for VOC are the same as those checks required for semivolatile organic compounds except fewer calculations are performed (e.g., there are no extract volumes to verify). Refer to Section 6.6 for guidance on calculation checks for VOC.

7.7 REFERENCES

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8. SPECIAL CONSIDERATIONS ASSOCIATED WITH BIOACCUMULATION ANALYSIS

8.1 INTRODUCTION

Analysis of chemicals in tissue is very similar to analysis of chemicals in sediment. The major difference in terms of metals is the need to efficiently digest the lipid-rich tissue and in terms of semivolatile organic compounds is that large quantities of biological macromolecules are co-extracted with the target compounds. These macromolecules can significantly interfere with the detection and quantification of semivolatile organic target compounds in tissue extracts. ~~The major difference is that tissues contain large quantities of biological macromolecules. These macromolecules can significantly interfere with the detection and quantification of target analytes in tissue extracts.~~ For organic chemicals, cleanup of tissue extracts to remove or reduce these interferences is extremely important before analysis, but the cleanup can also contribute to losses of compounds of interest. ~~but the cleanup can also contribute to losses of target analytes.~~

In this section, special considerations associated with the analysis of chemicals in tissue are described. Requirements for data completeness and format, and the data validation process are the same as described for sediments. Section 5 (Quality Assurance for Metals in Sediments) and Section 6 (Quality Assurance for Semivolatile Organic Compounds in Sediments) contain additional information on these topics.

8.2 SAMPLING UNIQUE TO TISSUES

In this section, tissue processing and storage requirements for bioaccumulation analyses are described.

8.2.1 Tissue Processing

To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before each organic sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, soaked in high-purity solvent (e.g., acetone or methylene chloride), and finally rinsed with DDW. Work surfaces should be cleaned with 95 percent ethanol and allowed to dry completely. Before each metals sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, soaked in an acid solution (1:1 DDW:$HN0_3$ or 1:1 DDW:HCl), and finally rinsed with DDW.

The removal of biological tissues should be performed under clean room conditions (i.e., contamination-, dust-free room) by or under the supervision of an experienced biologist. Tissue should be removed with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene (i.e., plastic) surfaces and utensils ~~implements~~ are a potential source of phthalate contamination and should not be used for organic samples. To control contamination while resecting tissues, technicians should use separate stainless steel utensils for removing outer tissue and for resecting tissue for analysis.

For fish samples, special care must be taken to avoid contaminating targeted tissues (especially muscle) with slime or adhering sediment from the fish exterior (skin) during resection. The incision troughs are subject to such contamination and should not be included in the sample. In the case of muscle, a core of tissue is taken from within the area bordered by the incision troughs, without contacting them. Unless specifically sought as a sample, the dark muscle tissue that may

exist in the vicinity of the lateral line should not be mixed with the light muscle tissue that constitutes the rest of the muscle tissue mass. This dark tissue is not always consumed by humans and because of a higher lipid (i.e., fat) content, may contain concentrations of organic chemicals at levels greater than the remaining muscle tissue.

The tissue sample should be placed in a clean glass or PTFE container that has been washed with detergent, rinsed at least once with tap water, rinsed at least twice with distilled water, rinsed with acetone, and finally, rinsed with high-purity methylene chloride. Firing of the glass jar at 450° C may be substituted for the final solvent rinse only if precautions are taken to avoid contamination as the container is dried and cooled.

8.2.2 Storage

Recommended holding times for frozen tissue samples have not been established by EPA, but a maximum 6-month to 1-year holding time similar to the sediment holding times is recommended for Puget Sound studies. (For extended sample storage, precautions should be taken to prevent desiccation). National Bureau of Standards is testing the effects of long-term storage of tissues at temperatures of liquid nitrogen (-120° to -190° C). At a minimum, the samples should be kept frozen at -20° C until extraction. This process will slow biological decomposition of the sample and decrease loss of moisture. Liquid associated with the sample when thawed must be maintained as part of the sample.

8.3 OVERVIEW OF EXTRACTION, DIGESTION, AND EXTRACT CLEANUP

Commonly used analytical techniques for the extraction, cleanup, and digestion of tissue matrices are presented in this section.

8.3.1 Extraction of Semivolatile Organic Compounds

The extraction of target compounds from tissue matrices is commonly performed by Soxhlet extraction (in same manner as sediments), grinding/maceration (e.g., with a Tekmar Tissuemizer or a Brinkman Polytron), or hydrolytic digestion/saponification. The tissue can either be dried and pulverized or extracted in the presence of sodium sulfate. Precautions should be taken to choose appropriate solvents for the procedure.

Soxhlet extraction is performed in a closed apparatus in which solvent is cycled through a permeable thimble containing the sample. The solvent cycling is driven by heating and condensation. The extract and tissue are in separate phases after the extraction is complete. This separation eliminates filtering and centrifugation steps. Soxhlet extraction should be performed for at least 16 hours.

Grinding extraction of tissues is a cold extraction technique. Wet tissues, solvent, and sodium sulfate (to remove water) are mixed together and macerated with a bladed probe. The blades turn rapidly, keeping the slurry well-mixed throughout the extraction. The extract is then separated from the slurry by centrifugation and filtration. Grinding extraction usually takes only a few minutes per sample.

~~Sample dry weight is determined by oven drying a weighed aliquot of the sample at 105° C for 16 hours. Percent dry weight is calculated according to the following equation:~~

$$\cancel{\%\ \text{dry weight} = \left(\frac{\text{dried sample wt.}}{\text{wet sample wt.}}\right) \times 100}$$

8.3.2 Cleanup and Separation of Semivolatile Organic Compounds

Extracts are purified using liquid chromatography. Acceptable methods are gravity column chromatography and HPLC. Column chromatography involves eluting the sample extract through silica gel, alumina, a polymer (GPC), or a combination of these. GPC polymers (e.g., Bio-Beads SX-3, Sephadex LH-20) separate biological macromolecules that are prevalent in extracts from tissue matrices and a source of interferences. Because GPC columns are reusable, it is necessary to calibrate them regularly. When GPC columns degrade, the target compounds may elute at different retention volumes than expected (e.g., in the biological macromolecule fraction) and thus may be discarded rather than collected. For this reason, QA review of documentation for column calibration is strongly recommended. Silica gel and alumina columns are used to separate different classes of compounds (e.g., PAH from polar organic compounds). Calibration of silica and alumina columns should be checked by the laboratory for each different lot of absorbent and whenever laboratory conditions change significantly, because absorbent properties vary considerably with moisture content, which may vary with laboratory conditions (especially temperature and humidity). In general, when multiple column elution are performed, it is likely at the expense of analyte recovery.

HPLC has recently been used to fractionate and clean up tissue extracts (Krahn et al. 1988). This method uses two preparatory gel-permeation columns (Phenomenex Phenogel, Å) in series. HPLC is capable of performing rapid, sharp separations in small volumes of solvent. Total run time, including column cleanup, is 20 minutes. Preparative columns allow samples to be analyzed at low back-pressures and with longer column life.

Due to large concentrations of biological macromolecules, GPC is always required for extracts analyzed by GC/MS. Extracts to be analyzed by GC/ECD also require preparative chromatography (e.g., HPLC or column chromatography; GPC is recommended).

8.3.3 Digestion of Samples for Metals Analysis

Under the PSEP/PSDDA programs (PSEP 1986), ~~(~~Tissue samples are homogenized and digested with 100-percent concentrated nitric acid at room temperature for 15 hours, then at 100° C for 1 hour, then gradually raised to 250° C for approximately 4 hours (until all the tissue is solubilized), and finally cooled to room temperature. Perchloric acid is then added, and the flask is heated to 200° C for 1 hour. The temperature is then raised to 300° C until all the nitric acid is removed. The extract volume is then adjusted with DDW and is ready for analysis.

8.4 LIPID WEIGHT DETERMINATION (AFTER EXTRACTION)

Lipid weight determination of tissue samples is a measure of the amount of extractable organic material, and is dependent on the solvent(s) used to extract the tissue. It is very important for the QA reviewer to know what solvent was used for the lipid weight determination. For example, when a tissue extraction is performed with methylene chloride and the extract is transferred into hexane (during concentration), some insoluble material precipitates out. If the lipid weight determination was made using methylene chloride, the precipitated material would be included in the lipid fraction. If the lipid weight determination was made using hexane, the precipitated material would not be included in the lipid fraction.

Lipid weight is determined by extracting a known amount of tissue and weighing the residue of a known volume (some fraction of the total volume, which is also known). This process is easily performed by dripping a known volume (e.g., 10 μL) of extract on a tared piece of filter paper or aluminum disk. ~~10 L) of extract on a tarred piece of filter paper or aluminum disk.~~ After the solvent evaporates, the filter paper or aluminum disk is reweighed. ~~reweigh the filter paper or aluminum disk.~~ To obtain consistent weights, it is important that a constant time interval be used prior to weighing (some of the lipid material can codistill or evaporate with the solvent, hence,

achieving readings that do not change over time can be difficult). ~~it is important a constant time interval be used prior to weighing (some of the lipid material can codistill or evaporate with the solvent and achieving readings that do not change over time can be difficult).~~ The residue weight must be adjusted for the total volume of the extract, and divided by the sample wet weight. For example, if 3 grams of tissue were extracted and concentrated to 100 μL, and the residue from 10 μL of the extract weighed 39 mg, ~~if 3 grams of tissue were extracted and concentrated to 100 L and 10 L weighed 39 mg,~~ the percent lipid weight would be calculated as follows:

$$\frac{39 \text{ mg}}{10\ \mu\text{L}} \times 100\ \mu\text{L} = 390 \text{ mg total lipids} = 0.39 \text{ g total lipids}$$

$$\frac{0.39 \text{ g}}{3 \text{ g}} \times 100 = 13\% \text{ lipid (wet weight)}$$

Tissue analyses are typically reported based on sample wet weight or normalized to total extractable lipid material. However, if dry weight concentrations are requested, sample dry weight is determined by oven drying a weighed aliquot of the sample at 105° C for 16 hours. Percent dry weight is calculated according to the following equation:

$$\%\text{ dry weight} = \left(\frac{\text{dried sample wt.}}{\text{wet sample wt.}}\right) \times 100$$

8.5 REFERENCES

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9. QUALITY ASSURANCE FOR BIOASSAYS

9.1 INTRODUCTION

This section addresses QA considerations specific to eight sediment bioassays frequently used in Puget Sound. Sediment bioassays are defined as laboratory exposures of organisms to test sediment (or extracts of sediment) collected from the field. Bioassay responses are compared between test and reference sediments to determine whether test sediments are toxic. Adequate QA procedures are required to ensure that the observed bioassay responses are not confounded by extraneous factors such as improper sample collection and processing, variable organism sensitivity, suboptimal experimental conditions, and erroneous endpoint determinations.

The following eight bioassays are considered in this section:

- Amphipod mortality test
  - Species = *Rhepoxynius abronius*
  - 10-day exposure
  - Endpoint = percent mortality (primary) and nonreburial (secondary)
- Juvenile infauna ~~bivalve~~ mortality test
  - Species = *Panope generosa* ~~(preferred)~~, *Protothaca staminea*, *Crassostrea gigas*, *Neanthes* sp.
  - 10-day exposure
  - Endpoint = percent mortality
- Juvenile *Neanthes* growth ~~polychaete mortality~~ test
  - Species = *Neanthes* sp.
  - 20-day ~~10-day~~ exposure
  - Endpoint = biomass (primary) and mortality (secondary) ~~percent mortality~~
- Bivalve larvae abnormality test (solid phase)
  - Species = *Crassostrea gigas*, *Mytilus edulis*
  - 48-hour exposure
  - Endpoint = percent abnormality and mortality
- Bivalve larvae abnormality test (suspended phase)
  - Species = *Crassostrea gigas*, *Mytilus edulis*
  - 48-hour exposure
  - Endpoint = percent abnormality (primary) and mortality (secondary)
- Echinoderm embryo abnormality test
  - Species = *Dendraster excentricus*, *Strongylocentrotus purpuratus* ~~spp.~~
  - 48- to 96-hour exposure
  - Endpoint = percent abnormality (primary) and mortality (secondary)

- Microtox™ test (saline extract)
  - Species = *Photobacterium phosphoreum*
  - 15-minute exposure
  - Endpoint = percent change in luminescence
- Microtox™ test (organic extract)
  - Species = *Photobacterium phosphoreum*
  - 15-minute exposure
  - Endpoint = percent change in luminescence.

Most of the guidelines presented in this section are either derived directly from the Puget Sound protocols (PSEP 1986), or are consistent with those protocols. At present, Puget Sound protocols are available for four of the seven bioassays considered in this report [i.e., amphipod mortality test, bivalve larvae abnormality test (solid phase), and the Microtox™ test (saline and organic extracts)].

The remainder of this section addresses major QA bioassay elements related to the following:

- Sample collection, transport, and storage
- Data completeness and format
- Data validation and assessment.

9.2 SAMPLE COLLECTION, TRANSPORT, AND STORAGE

When collecting a sediment sample for bioassay analysis, it is essential that the sample be representative of the environment being sampled. In addition, this representation must be maintained as the sample is transported to the laboratory or stored prior to analysis. To ensure a representative sample is collected, the following criteria should be specified for judging sample acceptability:

- The sampling station was located with acceptable accuracy (see Section 2.1)
- The sediment sample was not unduly disturbed or winnowed during collection
- The sediment was sampled to a depth sufficient to allow a sample to be collected from the desired horizon (e.g., top 2 cm for surface samples)
- The sample was properly homogenized before a subsample was removed for laboratory analysis.

If a sample does not meet any one of these criteria, it should be rejected and a new sample should be collected. To assist the QA reviewer in judging whether samples were collected appropriately, it is essential the sample acceptability criteria be addressed explicitly on field log sheets.

When bioassays and chemical analyses are conducted concurrently at the same stations, it is preferable to take subsamples from the same sediment homogenate for both kinds of analyses. This technique strengthens the relationship between the two kinds of results, because it minimizes the influence of small-scale spatial variability of chemical concentrations. For example, if bioassay and chemical subsamples are collected from different sediment samples, the relationship between the two kinds of results may be obscured because chemical concentrations differed between the two samples.

To ensure the integrity of the sample, samples should be analyzed immediately after collection. However, because immediate analysis is often impractical, the sediment must be stored under conditions that will maintain its integrity. The recommended storage conditions for bioassay samples from Puget Sound are presented in Table 9-1. In most cases, sediment samples should be stored unfrozen because the effect of freezing on the toxicity of sediments is unknown. Unfrozen samples should be stored at 4° C to minimize biological activity. In addition, unfrozen sediment can be stored for only a relatively short period of time (i.e., usually less than 14 days), because it is not known whether prolonged storage at 4° C can alter sediment toxicity. PSDDA allows sediment samples to be stored for periods up to 6 weeks, if the samples are maintained in a nitrogen atmosphere to minimize biological activity. At present, only samples for the Microtox™ test using an organic extract should be ~~are recommended to be~~ stored frozen. This recommendation is based on the rationale that because the extraction procedure results in such a severe alteration of the sample, it probably masks any subtle changes in sediment toxicity resulting from freezing the sediment.

Sediment samples must also be stored in proper containers to ensure the samples are not contaminated by the containers. For all bioassays, sediment samples should be stored in glass containers that have been cleaned in a manner appropriate for collecting samples for analysis of chemical contaminants. In addition, container lids should be lined with PTFE.

9.3 DATA COMPLETENESS AND FORMAT

The QA review process is greatly facilitated if the data are presented in a standardized format. The most efficient means of ensuring a standardized format is used is to develop data report forms or checklists. These forms prompt the data generator to report all pertinent information, and help the data reviewer locate different kinds of information and identify data omissions.

The initial step of the QA process is to review the submitted data for completeness. Data omissions can then be identified before the review process begins, and the omitted information can be requested from the data submitter. The following information is required before a QA review of bioassay data can be conducted:

- Bioassay response for each replicate test chamber
- Experimental conditions for each replicate test chamber
- Results of negative controls
- Results of positive controls
- Any conditions that may have influenced data quality.

The most critical information necessary to evaluate the quality of bioassay data is the results from the negative controls. This information addresses whether the test organisms were adequately healthy to be used for toxicity assessments. If they were not adequately healthy, it is possible that responses judged to be significant were partially or completely the result of unusually sensitive organisms, rather than solely the result of sediment toxicity. Although results of negative controls are important in evaluating the quality of bioassay data, other information is also desirable for this purpose (see Section 9.4).

9.4 DATA VALIDATION AND ASSESSMENT

General guidelines are presented in this section for four characteristics of the eight bioassays considered in this report:

- Analytical methods
- Precision

TABLE 9-1. RECOMMENDED HOLDING CONDITIONS FOR BIOASSAY SAMPLES[a]

Bioassay	Container[b,c]	Preservation	Maximum Holding Time
Amphipod mortality	Glass	4° C in the dark	14 days
Juvenile infauna ~~bivalve~~ mortality	Glass	4° C in the dark	14 days
Juvenile *Neanthes* growth ~~polychaete mortality~~	Glass	4° C in the dark	14 days
Bivalve larvae abnormality (solid phase)	Glass	4° C in the dark	14 days
Bivalve larvae abnormality (suspended phase)	Glass	4° C in the dark	14 days
Echinoderm embryo abnormality	Glass	4° C in the dark	14 days
Microtox (saline extract)	Glass	4° C in the dark	14 days
Microtox (organic extract)	Glass	Freeze at -20° C	6 months

[a] Specifications are based on the Puget Sound protocols (Tetra Tech 1986), or are consistent with those protocols.

[b] All glass should be precleaned in a manner appropriate for collecting samples for analysis of chemical contaminants.

[c] Container lids should be lined with polytetrafluoroethylene (PTFE).

- Positive controls
- Negative controls.

To minimize redundancy when discussing these characteristics, the bioassays were grouped into three categories of similar tests:

- Adult/juvenile mortality/growth bioassays
  - Amphipod mortality test
  - Juvenile infauna ~~bivalve~~ test
  - Juvenile *Neanthes* growth ~~polychaete~~ test
- Larval abnormality bioassays
  - Bivalve larvae abnormality test (solid phase)
  - Bivalve larvae abnormality test (suspended phase)
  - Echinoderm embryo abnormality test
- Microtox™ bioassays
  - Saline extract
  - Organic extract.

9.4.1 Analytical Methods

To ensure bioassay testing is conducted in an acceptable manner and results are comparable among different studies, it is essential the tests be conducted according to standardized procedures. Some important elements of the standardized procedures include the following:

- Organism holding, spawning (if required), and acclimation prior to testing
- Preparation of test chambers
- Experimental conditions during testing
- Test duration
- Endpoint determination.

Additional considerations such as replicated measurements, positive controls, and negative controls are considered in Sections 9.4.2–9.4.4. ~~Additional considerations such as replicated measurements, positive and negative controls, and data generation and reporting are considered in Sections 9.3.2–9.3.4.~~ The recommended sources of standardized protocols for the eight bioassays considered in this report are presented in Table 9-2.

Organism Holding, Spawning, and Acclimation—If the test organisms are not handled properly prior to bioassay testing, their sensitivity to toxic chemicals could be altered and the validity of the test results compromised. These organisms may become unusually sensitive to the chemicals used in bioassay testing, which may lead to an overestimate of the toxicity of the sample. ~~which may lead to an overestimated true toxicity of the sample.~~

The main aspects of organism holding for the adult/juvenile mortality/growth tests are that the organisms be acclimated ~~held~~ for a sufficient period of time and under appropriate conditions to ensure they are not stressed by factors other than toxic chemicals when bioassay testing begins. If organisms are not held in the laboratory for a sufficient period after field collection or shipping from laboratory cultures, they could be influenced by the residual stresses related to handling (e.g.,

TABLE 9-2. RECOMMENDED BIOASSAY PROTOCOLS

	Primary	Secondary[a]
Amphipod mortality	Tetra Tech (1986)	Swartz et al. (1985)
Juvenile infauna ~~bivalve~~ mortality	Johns et al. (1989)	U.S. Army COE (1977)~~None~~
Juvenile *Neanthes* growth ~~polychaete mortality~~	Johns (in prep.)	Johns et al. (1989)
Bivalve larvae abnormality (solid phase)	Tetra Tech (1986)	Chapman and Morgan (1983) ASTM (1985)
Bivalve larvae abnormality (suspended phase)	U.S. Army COE (1977)	ASTM (1985)
Echinoderm embryo abnormality	Dinnel and Stober (1985)	None
Microtox (saline extract)	Tetra Tech (1986)	Williams et al. (1986) Beckman Instruments (1982)
Microtox (organic extract)	Tetra Tech (1986)	Schiewe et al. (1985) Beckman Instruments (1982)

[a] Secondary protocols should be used only in conjunction with primary protocols.

capture, sieving, sorting, transport). If organisms are not sufficiently acclimated to the experimental conditions under which bioassay testing will be conducted (e.g., temperature, salinity, DO, pH), they could be stressed by those variables when they are introduced to the test chambers.

The main aspects of organism holding for the larval abnormality tests are related ~~primarily~~ to the conditioning and spawning of the adult organisms that give rise to the actual test organisms, because ~~as~~ the larvae are introduced to the test chambers shortly after fertilization. Because the adults do not spawn throughout the year, they must be conditioned to spawn during periods when they are not ready to spawn naturally. Conditioning usually involves a gradual increase in the temperature of the holding water, which stimulates maturation of gametes. Depending on the physiological and gametogenic status of the organisms at the time of collection, conditioning can extend from several days to several weeks. Because conditioning is an artificial alteration of the natural spawning cycle, the quality of the resulting gametes is sometimes unacceptable. In general, the longer it takes to condition an organism, the higher the probability is that the gametes will be of poor quality. If the gametes are of poor quality, fertilization rate may be unacceptably low or the fertilized embryos may fail to divide normally.

Organism holding is not a major consideration for the Microtox™ bioassays, as the test organisms (i.e., bacteria) can be held for up to a year in a freeze-dried form at -20° C. However, samples are reconstituted in the laboratory prior to testing, and must be used soon after rehydration (i.e., within ~~less than~~ 5 hours). The sensitivity of the bacteria to toxic chemicals can change following prolonged storage in the reconstituted form.

Preparation of Test Chambers—To ensure test organisms in all samples are exposed to the test sediment in the same manner, it is essential the test chambers be prepared using the same standardized techniques. If the preparation procedures vary among samples, they could influence bioassay responses and confound the estimates of sample toxicity.

Preparation procedures are most straightforward for the adult/larval mortality/growth tests, because ~~as~~ the primary exposure route is through bedded test sediment. In those tests, sediment is simply placed in the test chamber and clean seawater is then added until the chamber is nearly full. Every effort is made to avoid sediment disturbance as the seawater is added to each chamber. In addition, the chambers are allowed to equilibrate overnight before the test organisms are added. The test animals are then added to each sample at the sediment surface.

Preparation procedures for the larval abnormality tests are more complex than those for the adult/larval mortality/growth tests, because the exposure route is through both suspended and bedded sediments. In those tests, sediment is added to seawater in the test chamber, which is then shaken vigorously for a fixed period of time (i.e., 10 seconds) to disperse and suspend the sediment in the seawater. The suspended sediment is allowed to settle for an unspecified length of time before the test larvae are introduced to the chamber. Because the act of dispersing and suspending the sediment can release toxic chemicals to the seawater, it is an important component of the exposure route. Therefore, the specified shaking time of 10 seconds must be adhered to. However, the lack of a standardized settling period prior to larval introduction may confound bioassay responses. At a minimum, settling time should be standardized within each study and preferably among all studies.

Preparation procedures for the Microtox™ test are probably the most complex of all the bioassays, because the test organisms are exposed to sediment extracts rather than directly to the test sediment. Extraction procedures must be conducted according to standardized protocols to ensure each sediment sample is extracted in the same manner. If the extraction procedures vary among samples, observed differences in sediment toxicity could be partly the result of differences in extraction effectiveness rather than the inherent toxicity of the samples. The organic extract technique is much more complex than the saline extract method, as the sample must be transferred to a minimally toxic-carrier compound (e.g., ethanol) after sediment extraction is accomplished using a highly toxic compound (e.g., dichloromethane). For the saline extract technique, the

sediment is extracted using nontoxic Microtox™ diluent (i.e., 2.0 percent NaCl in double-distilled organic-free water).

Experimental Conditions—To ensure bioassay results are comparable among different samples, it is essential that testing be conducted under the same standardized experimental conditions (Table 9-3). If all conditions except the test sediment are standardized, any differences in bioassay responses between samples can be attributed with a reasonable level of confidence to the characteristics of the different test sediment. If the experimental conditions differ among samples, it will be uncertain as to what proportion of any observed differences in bioassay responses are the result of the different experimental conditions rather than the test sediment.

Important experimental conditions for the adult/larval mortality/growth tests and the larval abnormality tests are temperature and salinity of the seawater in the test chamber. These values are specified within relatively narrow ranges that should always be adhered to. Although DO and pH of the seawater are also critical experimental conditions, they are allowed to vary in the test chambers. However, if oxygen concentrations fall below 4-5 mg/L or pH falls outside the range of 7-9 in a test chamber, the bioassay response observed in that chamber should be interpreted with caution, especially if it indicates the sample is more toxic than would be expected.

The main experimental condition for the Microtox™ tests is temperature, as the bioluminescent response of the test organisms is very sensitive to that variable. The standard test temperature for both Microtox™ tests (i.e., saline and organic extracts) is 15°±1° C.

Test Duration—Each bioassay has a specified period of time in which the test organisms are exposed to the test sediment or sediment extract. These exposure periods must be adhered to for all samples. The use of different exposure periods among samples could contribute to differences in toxicity among the samples. In general, if the exposure period exceeds the recommended period, the observed bioassay response would be expected to increase in magnitude.

The consistency of the exposure period is probably most critical for the Microtox™ tests, as the recommended exposure period is very short (i.e., 15 minutes), and changes in luminescence can occur rapidly along a continuous scale. The consistency of the exposure period for the larval abnormality tests is also relatively critical, because the exposure period is relatively short (i.e., 48 hours), and the onset of abnormalities can occur relatively quickly along a continuous scale. The adult/larval mortality/growth tests are probably the least sensitive to variations in exposure period, as the period is relatively long and the mortality endpoint is discrete (i.e., present or absent).

Endpoint Determination—Accurate determination of the test endpoint (or response) of each bioassay is essential for estimating the true toxicity of samples. To facilitate accurate determinations, the endpoints should be well defined and relatively easy to measure.

The abnormality endpoint of the larval abnormality tests is probably the most difficult response to determine for the bioassays considered in this report. Because the degree of abnormality usually occurs along a continuous scale, it is not always clear when a larva can be considered abnormal. Given the relatively large degree of subjectivity in determining this endpoint, it is imperative that the definition of abnormality be well defined and standardized. If neither of these two criteria are met, abnormality determinations made by different investigators could differ on the basis of endpoint definition rather than on the basis of sample toxicity. It is also desirable that abnormality determinations be made by experienced personnel, to ensure the definition schemes are implemented correctly. The mortality endpoint for the larval abnormality bioassays is relatively easy to determine because it is based upon the numbers of larvae missing relative to the numbers of larvae in the negative controls.

TABLE 9-3. STANDARD EXPERIMENTAL CONDITIONS FOR BIOASSAYS

Bioassay	Experimental Conditions
Amphipod mortality	Temperature = 15±1° C Salinity = 28±1 ppt pH = 8±1 DO[a] = >5 mg/L
Juvenile infauna ~~bivalve~~ mortality	(not established)
Juvenile *Neanthes* growth ~~polychaete mortality~~	(not established)[b]
Bivalve larvae abnormality (solid phase)	Temperature = 20±1° C Salinity = 28±1 ppt pH = 8±1 DO = >4 mg/L
Bivalve larvae abnormality (suspended phase)	Temperature = 20±1° C Salinity = 28±1 ppt pH = 8±1 DO = >4 mg/L
Echinoderm embryo abnormality	Temperature = 9±2° C Salinity = 30±3 ppt pH = 8±1 DO = >4 mg/L
Microtox (saline extract)	Temperature = 15±1° C
Microtox (organic extract)	Temperature = 15±1° C

[a] DO = Dissolved oxygen.

[b] Interim conditions are available in Johns et al. (1989).

The mortality endpoint of the adult/juvenile mortality/growth tests is probably the easiest to determine. It is a relatively discrete endpoint in which a test organism is considered dead if it is missing at the end of the exposure period or if it shows no sign of movement after gentle prodding. This endpoint does not require extensive experience to determine. The nonreburial endpoint for the amphipod mortality test is also relatively easy to determine, as it relies on the simple enumeration of the number of amphipods that do not rebury. The biomass endpoint of the juvenile *Neanthes* growth test can readily be determined quantitatively using an analytical balance.

The determination of the luminescence endpoint is also relatively easy to determine, as it is simply read directly off the display of the automated Microtox™ analyzer. However, because this endpoint can be very sensitive to how the samples are introduced to the analyzer (e.g., timing, quantity), it is essential that the instrument be operated by experienced personnel.

9.4.2 Test Precision

For bioassays, replicate analyses are required for all samples. The Microtox™ test requires two replicate analyses per extract dilution, whereas the remaining tests require five replicate analyses per sample. At present, the only guideline available for bioassays is for the SD of percent mortality in the amphipod mortality test (i.e., less than 15) (Barrick et al. 1988).

Unusually high variability among replicates acts to reduce the statistical power of comparisons with reference conditions. A reduction in power increases the chance that a "true" adverse effect will not be detected ~~discriminated~~, and is therefore not environmentally protective. It is always advisable to review the variability (e.g., SD of the mean) of the bioassay responses at all stations to check for outliers.

If a station is found with unusually high variability, the raw data for the individual replicate analyses should be inspected. If the high variability is the result of a single anomalous replicate, one might suspect that something unusual happened in the test chamber and that the replicate is not representative of the entire station. The replicate could be deleted from the station set and a new mean response could be calculated on the basis of the remaining replicates. If the high variability is due to variable responses in all replicates, one might suspect that test sediment was not homogenized sufficiently or that the bioassay was not run correctly.

It is helpful to consider the magnitude of the mean response when variability is high among all replicates. In general, high variability would not be expected when mean responses are either very high (i.e., because the sediment is very toxic) or very low (i.e., because the sediment is relatively uncontaminated). By contrast, high variability is sometimes found naturally when mean responses are moderate, and may be the result of variable sensitivities among the individual test organisms.

The larval abnormality tests are different from most of the other bioassays because the sample size is not controlled at the beginning of testing. Instead, sample sizes for abnormality determinations depend on how many organisms survive the test. In some cases, mortality can be very high, leaving few larvae for abnormality determinations. If mortality is 100 percent, the sample size of survivors drops to zero and the abnormality endpoint cannot be assessed. Typically, a value of 100 percent mean abnormality is assigned when 100 percent mortality is observed.

One method of addressing this problem is to specify that a minimum number of larvae (e.g., 40) be evaluated for each replicate test. In cases where mortality is high, this specification could require that additional laboratory time be spent reading slides until the minimum sample size is achieved. If mortality is so high that the minimum number of larvae cannot be achieved with reasonable effort, the value determined for abnormality could be qualified as being based on a suboptimal number of replicates. An alternative approach would be to combine the mortality and abnormality endpoints under the assumption that all organisms that died must have exhibited abnormal development prior to death.

9.4.3 Positive Controls

In bioassay analyses, positive controls involve the exposure of representative test organisms to a reference toxicant. The exposure is usually conducted using a dilution series and extends for a period identical to that used for the definitive testing. The goal of the positive controls is to demonstrate that the test organisms are sensitive to the effects of a known toxic chemical, and that they respond to the chemical in a dose-responsive manner. That is, the magnitude of the bioassay response increases as the concentration of the reference toxicant increases. If the two criteria of sensitivity and dose-responsiveness are satisfied, it can be expected that observed differences in bioassay responses among samples during the definitive testing are the result of differing concentrations of toxic chemicals among the samples. However, if the organisms are not found to be sensitive and dose-responsive, the meaning of the bioassay responses observed during definitive testing will be somewhat uncertain.

The sensitivity of the test organisms is usually estimated as an LC_{50} or EC_{50} derived from the dose-response relationship between chemical concentrations and bioassay responses. The magnitude of sensitivity can be evaluated by comparing the observed values with those found for the same kind of test organisms exposed to the same reference toxicant in other studies. If the observed sensitivity appears unusual relative to other studies, the discrepancy should be resolved prior to definitive testing. If the definitive testing is conducted using test organisms with unusual sensitivity, the interpretation of the results of the testing will be uncertain.

Dose-responsiveness can be evaluated quantitatively by determining statistically the correlation between chemical concentrations and bioassay responses. This characteristic can also be evaluated qualitatively by examining the data for a relatively monotonic relationship between chemical concentrations and bioassay responses. Qualitative evaluations are usually appropriate when the number of observations are small within the responsive range of the test organisms (i.e., between 0 and 100 percent response). The number of observations may be small either because a small number of concentrations were evaluated or because only a small subset of the observations evaluated were within the responsive range of the test organisms.

The following reference toxicants are commonly used for the bioassays considered in the present report:

- Cadmium chloride
  - Adult/larval mortality tests
  - Larval abnormality tests
- Sodium pentachlorophenate
  - Adult/larval mortality tests
  - Larval abnormality tests
- Sodium arsenate
  - Microtox™ saline extract test
- Sodium lauryl sulfate
  - Microtox™ organic extract test.

9.4.4 Negative Controls

In bioassay analyses, negative controls involve the exposure of representative test organisms to clean seawater or sediment. The goal of these controls is to demonstrate that test organisms are adequately healthy to be used for toxicity assessment. This determination is made by exposing the

organisms to optimal, uncontaminated test media and measuring their response over the same exposure period used for the definitive testing. The magnitude of the observed response should not exceed a predetermined maximum level. If the maximum allowable response is exceeded, the test organisms are not considered sufficiently healthy for definitive testing. Inadequate health of the test organisms could result from natural stresses such as reproduction, low food supply, suboptimal water quality conditions, or from various experimental activities such as collection, holding, and acclimation. Results from definitive testing that are associated with failed negative controls cannot be considered valid, because ~~as~~ the observed bioassay responses may have been confounded by the unhealthy condition of the test organisms. If the negative controls fail, definitive testing should be conducted using an alternate supply of test organisms.

The maximum allowable negative control responses for the tests considered in this report are:

- Adult/larval mortality tests
  - 10 percent mortality
- Larval abnormality tests
  - 30 percent mortality
  - 10 percent abnormality
- Microtox™ tests
  - Difference between blank ratios = 0.02.

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10. QUALITY ASSURANCE FOR BENTHIC MACROINVERTEBRATES

10.1 INTRODUCTION

This section addresses QA considerations specific to the collection and analysis of information on benthic macroinvertebrate assemblages in Puget Sound. Benthic macroinvertebrates are defined as the small invertebrates commonly sampled using a bottom grab or box corer, and retained on a 1.0-mm mesh screen after sediment samples are sieved. The characteristics of benthic macroinvertebrate assemblages are compared between test and reference sites to determine whether chemical contaminants in sediments at the test sites result in altered assemblages. Adequate QA procedures are required to ensure that any observed differences in assemblage characteristics between test and reference sites are likely the result of chemical toxicity, and are not confounded by extraneous factors such as improper sample collection and processing, inefficient sorting, and inaccurate taxonomic identifications.

Most of the QA guidelines presented in this section are either derived directly from the Puget Sound protocols (PSEP 1986) or are consistent with those protocols. The remainder of this section addresses the following QA elements:

- Sample collection, transport, and storage
- Data completeness and format
- Data validation and assessment.

10.2 SAMPLE COLLECTION, TRANSPORT, AND STORAGE

To ensure the information collected on benthic macroinvertebrate assemblages is interpretable, the study must adhere to two major design specifications: the device used to collect samples from the environment, and the mesh size used to sieve the samples. Although several devices can be used to sample benthic macroinvertebrate assemblages, each device samples the assemblages in a unique manner, and can thereby influence the characteristics measured for the assemblages. In addition, most samplers are available in various sizes to sample different areas of the sea floor. Because different benthic species can exhibit different scales of patchiness in their horizontal distribution, the characteristics measured for benthic assemblages can differ depending on the size of the device with which they were sampled. Because both the kind and size of the device used to sample benthic assemblages can influence the characteristics measured for the assemblages, it is essential that the study design specification for the sampling device be followed without variation.

A second study design specification critical to interpreting information on benthic macroinvertebrate assemblages is the mesh size used for sieving. Because benthic macroinvertebrates exhibit a wide range of sizes both among species and among different age groups within a species, the mesh size used to sieve these organisms can influence the characteristics measured for the assemblages. For example, larger mesh sizes generally retain fewer individuals and species than smaller mesh sizes. Therefore, the study design specification for sieve mesh size must be followed.

When sampling benthic macroinvertebrate assemblages, samples should be representative of the environment being evaluated. In addition, this representativeness must not be altered as the sample is transported to the laboratory or stored prior to analysis. To ensure a representative sample is collected, the following criteria should be used for judging sample acceptability:

- Sediment should not be extruded from the upper face of the sampler such that organisms may have been lost (a sample would be acceptable if the sediment touched the upper face of the sampler but was not extruded)

- Overlying water should be present in the sample to indicate minimal leakage occurred during retrieval
- The sediment surface should be relatively flat to indicate minimal disturbance and winnowing occurred during sample collection and retrieval
- The entire surface of the sample should be included in the sampler to ensure a full sample was collected
- The following penetration depths (i.e., the maximum depth of sediment sampled) should be achieved at a minimum to ensure most organisms are sampled efficiently:
  - 4-5 cm for medium-coarse sand
  - 6-7 cm for fine sand
  - ≥10 cm for muddy sediment.

If a sample does not meet any one of these criteria, it should be rejected and a new sample should be collected. To assist the QA reviewer in judging whether samples were collected appropriately, it is essential that the sample acceptability criteria be addressed explicitly on field log sheets.

If sample acceptability criteria are not adhered to, the results of subsequent analyses could be strongly biased. For example, amphipods are a group of crustaceans that are generally very sensitive to chemical contamination. However, because they have a relatively low density and are often found near the sediment surface, they are highly susceptible to being lost from a sample if excessive leakage, disturbance, or winnowing occurs during sample collection and retrieval. If amphipods are undersampled at a test site, the toxicity of the sediments at that site could be overestimated. Alternatively, if amphipods are undersampled at a reference site, sediment toxicity at test sites could be underestimated.

Once a representative sample has been collected in the field, it is essential the integrity of the sample be maintained as it is transported to the laboratory and stored prior to analysis. Ideally, samples should be analyzed immediately after collection. However, because immediate analysis is often impractical, the sample must be stored under conditions that will maintain its integrity. Following are recommended storage procedures for samples of benthic macroinvertebrate assemblages in Puget Sound:

- Placement in well-labeled containers that seal adequately
- Immediate fixation in a 10-15 percent solution of buffered formalin in seawater
- Transfer to a 70-percent solution of ethanol or isopropanol within 7-10 days after fixation.

Immediate and adequate fixation is essential to ensure the integrity of organisms is maintained during storage to facilitate accurate taxonomic identifications and accurate measurements of biomass (if required). Adequate fixation is ensured by using the appropriate concentration of formalin solution and by ensuring the volume of fixative in each sample is at least twice the volume occupied by the sample. That is, the sample should only occupy half of the sample container at a maximum, and the fixative should fill the container. The contents of each container should be adequately mixed to ensure the sample is saturated with fixative.

Samples should be transferred to alcohol within 7-10 days after fixation to ensure the fixative has had an appropriate length of time to saturate each organism. If the fixation period is too short, fixation may be inadequate and organisms may decompose to some extent when stored in alcohol. Alternatively, if the fixation period is too long, mollusks and echinoderms could be decalcified. Both decomposition and decalcification can reduce the accuracy with which taxonomic identifications are made.

Samples are usually sieved a second time when they are transferred from formalin to alcohol to ensure that most excess formalin is rinsed from the sample. When rescreening samples, it is essential that the sieve mesh size be at most one-half the size of the sieve used in the field (i.e., 0.5 mm for rescreening if a sample was sieved in the field using a 1.0-mm sieve). Use of a smaller mesh size for rescreening ensures that organisms collected in the field are not lost at this stage.

10.3 DATA COMPLETENESS AND FORMAT

The QA review process is greatly facilitated if the data are presented in a standardized format. The most efficient means of ensuring that a standardized format is used is to develop data report forms or checklists. These forms prompt the data generator to report all pertinent information, and help the data reviewer locate different kinds of information and identify data omissions.

The initial step of the QA process is to review the submitted data for completeness. Data omissions can be identified before the review process begins, and the omitted information can be requested from the data submitter. The following information is needed to review QA data on benthic macroinvertebrate assemblages:

- Collection methods
  - Sampler
  - Sieve mesh size
  - Sample acceptability criteria
  - Fixation and storage procedures
- Laboratory techniques
  - Sorting efficiency
  - Verification of taxonomic identifications
  - Taxon identities and abundances.

10.4 DATA VALIDATION AND ASSESSMENT

In this section, QA ~~QC/QC~~ considerations are discussed for three major elements of laboratory analysis related to benthic macroinvertebrate samples:

- Sample sorting
- Taxonomic identifications
- Intrastation variability.

10.4.1 Sample Sorting

Sample sorting is the removal of benthic organisms from the sieved debris collected in the field for each sample. The debris typically consists of coarse sediment particles, animal tubes, shell fragments, and large pieces of organic material (e.g. macrophytes, wood chips, wood fibers). Efficient sorting can be particularly difficult if the amount of debris in a sample is excessive or if particular organisms resemble the kind of debris present. One method of recognizing organisms in the debris is to stain them with a vital stain (e.g., rose bengal) prior to sorting. However, some taxa (e.g., ostracods, gastropods) do not always stain adequately. In addition, staining may interfere with the taxonomic identification of several taxa.

The most reliable method of achieving a specified level of efficiency in sorting is to re-sort a limited number of whole samples or fractions of whole samples. To avoid bias in the re-sorting analyses, each sample should be selected at random and re-sorting should be conducted by an experienced person other than the person who originally sorted the sample. The recommended sorting method for Puget Sound samples requires at least 20 percent of each sample be selected at random from the total sample and re-sorted. The recommended sorting efficiency is 95 percent of the total number of individuals in the whole sample. That is, the number of organisms found in each 20-percent subsample should be multiplied by five and compared with the total number of individuals in the whole sample. If the 95-percent efficiency criterion is not achieved, the sample should be re-sorted. Suboptimal sorting efficiency should be avoided, because it can lead to underestimates of organism abundances and affect comparisons between test and reference sites. Organisms removed during QA/QC evaluations should subsequently be added to the collection of organisms originally removed from each sample.

10.4.2 Taxonomic Identifications

It is essential that taxonomic identifications are accurate to avoid making erroneous conclusions regarding the effects of chemical toxicity on benthic macroinvertebrate assemblages. Two common methods of evaluating the effects of chemical toxicity are to compare taxon abundances between test and reference sites and to evaluate temporal changes in the taxonomic composition of assemblages at test sites. The results of either method can be confounded by inaccurate taxonomic identifications. For example, certain species are usually important components of the benthic assemblage at each station. If inaccurate taxonomic identifications lead to the erroneous conclusion that an important species is less abundant at the test site relative to the reference site or relative to an earlier survey at the test site, one might mistakenly conclude chemical toxicity is influencing the assemblage at the test site. The problem of inaccurate identifications is often encountered when multiple laboratories analyze samples using different identification criteria.

Four primary methods are available for ensuring that taxonomic identifications are made accurately:

- Use of a reference collection
- Confirmation of identifications by experts
- Confirmation of identifications within a laboratory
- Comparisons with historical species lists.

A reference collection is a collection of archived benthic macroinvertebrates that are representative of the species likely to be found in a particular area. Reference collections should be established for all survey areas. Historical reference collections can be used if they are appropriately accurate and comprehensive. The identity of each species within a reference collection should be verified by a taxonomic expert. Taxonomic identifications made during a particular project can then be checked against those of the reference collection to ensure their accuracy. The reference collection can also be used to teach new taxonomists and to resolve disputed identifications among taxonomists. Reference collections also facilitate the consistency of taxonomic identifications among different studies and investigators.

Despite the existence of reference collections, some taxonomic identifications may be uncertain for a particular project. All uncertain identifications in any project should be verified by an appropriate taxonomic expert. The consistency of taxonomic identifications within a laboratory can be enhanced by having a fraction (e.g., 5 percent) of the samples identified by one taxonomist be re-identified by a second taxonomist. If only one taxonomist in a laboratory is qualified to identify a particular taxon, a taxonomist outside the laboratory should be used to verify the identifications. This intralaboratory checking will ensure any taxonomic discrepancies are found at an early stage.

The species abundances determined during a particular project can be compared with those determined in past studies of the same area or a closely related area (if those historical studies are available). Comparisons among studies can be made with respect to the total number of species found, the total abundances of organisms, and the identities of the numerically dominant species. If major discrepancies are found among the studies, they may indicate one or more of the studies were not conducted properly.

10.4.3 Intrastation Variability

The characteristics of benthic assemblages vary naturally and as a result of sampling techniques. However, variability generally should not be excessive among the replicate samples taken at each station. Unusually high variability among replicates may indicate problems were encountered for one or more of the replicate samples with respect to field sampling, sorting, or taxonomic identifications. To evaluate intrastation variability, the relative abundances of the dominant species should be compared among replicates to ensure the abundances are relatively consistent. In addition, the total abundances of macroinvertebrates should be compared among replicates. Within-station variability of total abundances can be compared among stations by calculating coefficients of variation for each station. If this variable is unusually large for any station (i.e., relative to most other stations), the reasons for the anomaly should be investigated.

10.5 REFERENCES

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APPENDIX A

List of Acronyms and Glossary

LIST OF ACRONYMS

AA	atomic absorption
A/B/N	acid/base/neutral
AET	apparent effects threshold
BFB	bromofluorobenzene
CCB	continuing calibration blank
CCV	continuing calibration verification standard
CEI	Committee on Environmental Improvement
CLP	Contract Laboratory Program
CRM	certified reference material
CV	coefficient of variation
DDW	distilled, deionized water
DFTPP	decafluorotriphenylphosphine
DO	dissolved oxygen
Ecology	Washington Department of Ecology
EPA	U.S. Environmental Protection Agency
FAA	flame atomic absorption
GC/FID	gas chromatography/flame ionization detection
GC/MS	gas chromatography/mass spectrometry
GC/ECD	gas chromatography/electron capture detection
GFAA	graphite furnace atomic absorption
GPC	gel permeation chromatography
HECD	Hall electrolytic conductivity detector
HPLC	high-performance liquid chromatography
ICB	initial calibration blank
ICP	inductively-coupled plasma
ICS	interference check sample
ICV	initial calibration verification standard
IDL	instrument detection limit
LOD	limit of detection
LOQ	limit of quantification
MDL	method detection limit
MSA	method of standard additions
ND	not detected
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
PAH	polynuclear aromatic hydrocarbon
PCB	polychlorinated biphenyl
PSDDA	Puget Sound Dredged Disposal Analysis
PSEP	Puget Sound Estuary Program
PSWQA	Puget Sound Water Quality Authority
PTFE	polytetrafluoroethylene
QA	quality assurance
QA/QC	quality assurance/quality control
RF	response factor
RIC	reconstructed ion chromatogram
RPD	relative percent difference
RRF	relative response factor
RRT	relative retention time
RSD	relative standard deviation
SAD	strong acid digestion
SD	standard deviation
SIM	selected ion monitoring

SOW	statement of work
SRM	standard reference material
TAD	total acid digestion
TBA	tetrabutyl ammonium
TOC	total organic carbon
TS	total solids
TVS	total volatile solids
~~TS~~	~~total solid~~
~~TVS~~	~~total volatile solid~~
U.S. COE	U.S. Army Corps of Engineers
VOC	volatile organic compound

GLOSSARY

Accuracy—The closeness of a measured or computed value to its true or expected value.

Amphipods—Small shrimp-like crustaceans (e.g., sand fleas). Many live on the bottom, feed on algae and detritus, and serve as food for marine species. Amphipods are commonly used in laboratory bioassays to test the toxicity of sediments because they are relatively sensitive to chemical toxicity.

Analyte—The specific component measured in a chemical analysis.

Analytical (Post-Digestion) Spike—An analysis conducted by adding a known amount of analyte to the digestate prior to analysis by GFAA. An analytical spike is required for each sample and QA samples as a measure of matrix interferences. The percent recovery determines whether the sample can be quantitated directly from the calibration curve or method of standard additions is required.

Apparent Effects Threshold (AET)—The sediment concentration of a contaminant above which statistically significant biological effects would always be expected.

Area Ranking—The designation of a dredging area relative to its potential for having sediment chemicals of concern. Rankings range from "low" potential to "high" potential, and are used to determine the intensity of dredged material evaluation and testing that might be required.

Batch—Usually refers to the number of samples that can be prepared or analyzed at one time. A typical commercial batch size is 20 samples for extraction of organic compounds.

Bioaccumulation—The accumulation of chemicals in the tissues of an organism (e.g., certain chemicals in food eaten by a fish tend to accumulate in its liver or other tissues).

Bioassay—A laboratory test used to evaluate the toxicity of a material (commonly sediments or wastewater) by exposing organisms to the material under controlled conditions and measuring their behavioral, physiological, or lethal responses.

Biota—The animals and plants that live in a particular area.

Blank-Corrected—The concentration of a chemical in a sample adjusted for the concentration of that chemical in the method blank carried through the procedure concurrently with the sample.

Bottomfish—Fish (e.g., English sole) that live on or near the bottom of a body of water in close contact with the sediment.

Bulk Chemical Analyses—Chemical analyses performed on an entire sediment sample, without separating water from the solid material in a sample.

Calibration—The systematic standardization of either the response of instruments used for measurements or the chemical separation achieved by a laboratory cleanup procedure.

Certified Reference Material—A reference material, one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation that is issued by a certifying body. A standard reference material (SRM) is a certified reference material issued by the National Bureau of Standards. ~~A reference material accompanied by, or traceable to, a certificate stating the concentration of chemicals contained in the material. The certificate is issued by a public or private organization that routinely certifies such material (e.g., National Bureau of Standards, American Society for Testing and Materials).~~

Coefficient of Variation—The standard deviation expressed as a percentage of the mean.

Confined Disposal—A disposal method that isolates the dredged material from the environment. Confined disposal may be in aquatic, nearshore, or upland environments.

Contaminant—A chemical or biological substance in a form or in a quantity that can harm aquatic organisms, consumers of aquatic organisms, or users of the aquatic environment.

Contaminated Sediment

Technical Definition: A sediment that contains measurable levels of contaminants.

Management or Common Definition: A sediment that contains sufficient concentration(s) of chemicals to produce unacceptable adverse environmental effects and thus require restriction(s) for dredging and/or disposal of dredged material (e.g., is unacceptable for unconfined, open-water disposal or conventional land/shore disposal, requiring confinement).

Control Limit—Defines the minimum quality of data as measured by some indicator (e.g., recovery) required to assume that the system or method is performing as expected. Exceedance of a control limit triggers action by the laboratory to correct the problem before data are reported.

Conventional Variables—Sediment parameters and characteristics other than chemical contaminants that have been routinely measured in assessing sediment quality. These include sulfides, organic carbon, etc.

Corrective Action—Measures taken to remove, adjust, remedy, or counteract a malfunction or error so that a standard or required condition is met.

Detection Limit—The smallest concentration or amount of some component of interest that can be measured by a single measurement with a stated level of confidence. In practice, detection limits can be determined by different methods in different laboratories and are not always assigned a statistical level of confidence.

Disposal Site—The bottom area that receives discharged dredged material; encompassing, and larger than, the target area and the disposal area.

Dredged Material—Sediments excavated from the bottom of a waterway or water body.

Dredged Material Management Unit—The maximum volume of dredged material for which a decision on suitability for unconfined open-water disposal can be made. Management units are typically represented by a single set of chemical and biological test information obtained from a composite sample. Management units are smaller in areas of higher chemical contamination concern (see **Area Ranking**).

Dredger—Private developer or public entity (e.g., federal or state agency, port, or local government) responsible for funding and undertaking dredging projects. This is not necessarily the dredging contractor who physically removes and disposes of dredged material (see below).

Dredging—Any physical digging into the bottom of a water body. Dredging can be done with mechanical or hydraulic machines and is performed in many parts of Puget Sound for the maintenance of navigation channels that would otherwise fill with sediment and block ship passage.

Dredging Contractor—Private or public (e.g., U.S. Army Corps of Engineers) contractor or operator who physically removes and disposes of dredged material for the dredger (see above).

Duplicate Analysis—A second analysis made on the same (or identical) sample of material to assist in the evaluation of measurement variance.

Evaluation Procedures Work Group (EPWG)—The PSDDA work group that is developing chemical and biological testing and test evaluation procedures for dredged material assessment.

Gas Chromatography (GC)—An instrumental technique used to separate a complex mixture into its component compounds by partitioning the compounds between a mobile gaseous phase (under pressure) and a stationary solid or liquid phase.

Gas Chromatography/Electron Capture Detection (GC/ECD)—An instrumental technique useful for the determination of organic compounds containing halogens (e.g., chlorine).

Gas Chromatography/Flame Ionization Detection (GC/FID)—An instrumental technique useful for the detection of organic compounds that can be converted to ions during exposure to a flame.

Gas Chromatography/Mass Spectroscopy (GC/MS)—An instrumental technique useful for breaking organic compounds into characteristic fragments that can be used to determine the original structure of the compound.

Gel Permeation Chromatography (GPC)—A cleanup procedure used to remove interfering biological macromolecules from sample extracts.

Gravid—Having eggs, such as female crabs carrying eggs.

Habitat—The specific area or environment in which a particular type of plant or animal lives. An organism's habitat provides all of the basic requirements for life. Typical Puget Sound habitats include beaches, marshes, rocky shores, bottom sediments, mudflats, and the water itself.

High Pressure (or High Performance) Liquid Chromatography (HPLC)—An instrumental technique used to separate a complex mixture into its component compounds by partitioning the compounds between a mobile liquid phase (under high pressure) and a stationary solid phase.

Hydrocarbon—An organic compound composed of carbon and hydrogen. Petroleum and its derived compounds are primarily hydrocarbons.

Injection Internal Standards—A standard added to a sample extract just prior to instrumental analysis. This standard is used to determine the actual percent recovery of the surrogate spike compounds. When the isotope dilution technique is not used, the injection internal standard is also used to quantify compounds of interest in the sample relative to standards.

Isotope Dilution Technique—A technique for quantification of organic compounds that uses a large number of stable isotopically labeled compounds (i.e., compounds for which some hydrogen atoms have been replaced with deuterium, or some carbon-12 atoms have been replaced with carbon-13) spiked in the sample before sample extraction to correct for compound losses during sample workup. The labeled compounds are analogs of the compounds of interest and behave similarly.

Matrix—The sample material in which the chemicals of interest are found (e.g., water, sediment, tissue).

Matrix Spike—An analysis conducted by adding a known amount of chemicals of interest to an actual sample (i.e., matrix), usually prior to extraction or digestion, and then carrying the spiked sample through the analytical procedure. The final matrix spike results are reduced by the amount of each chemical found in a replicate analysis of the sample conducted without spikes. A comparison of these results with the known concentration of spike added to the sample enables an evaluation of the effect of the particular sample matrix on the recovery of compounds of interest.

Metals—Metals are naturally occurring elements. Certain metals, such as mercury, lead, nickel, zinc, and cadmium, can be of environmental concern when they are released to the environment in unnatural amounts by man's activities.

Method Blank—A measure of the contribution of analytes from all laboratory sources external to the sample. The method blank value is determined by proceeding through all phases of extraction and analysis with no addition of sample.

Method Spike—A method blank to which a known amount of surrogate standards and analytes (compounds of interest) has been added.

Microtox—A laboratory bioassay using luminescent bacteria and measuring reductions in light production as the test endpoint, often used to assess toxicity of saline or organic sediment extracts.

Noise—The electronic signal intensity attributed to instrument "background" or electronic current from chemical interferents (i.e., any part of an electrical signal that cannot be related in a known way to the electronic current from a target compound).

Overdepth Material—Dredged material removed from below the dredging depth needed for safe navigation. Though overdepth is incidentally removed due to dredging equipment precision, its excavation is usually planned as part of the dredging project to ensure proper final water depths. Common overdepth is 2 feet below the necessary dredging line.

Oxygen Demanding Materials—Materials such as food waste and dead plant or animal tissue that use up dissolved oxygen in the water when they are degraded through chemical or biological processes. Chemical and biological oxygen demand (COD and BOD) are different measures of how much oxygen a particular substance demands.

Parameter—A quantifiable or measurable characteristic of something (e.g., height, weight, sex, and hair color are all parameters that can be determined for humans). Water quality parameters include temperature, pH, salinity, dissolved oxygen concentration, and many others.

Permit—A written warrant or license, granted by an authority, allowing a particular activity to take place. Permits required for dredging and disposal of dredged material include the U.S. Army Corps of Engineers Section 404 permit, the Washington State Department of Fisheries Hydraulics Permit, the city or county Shoreline Development Permit, and the Washington Department of Natural Resources Site Use Disposal Permit.

Pesticide—A general term used to describe any substance, usually chemical, used to destroy or control organisms (pests). Pesticides include herbicides, insecticides, algicides, and fungicides. Many of these substances are manufactured and are not naturally found in the environment. Others are natural toxins that are extracted from plants and animals.

pH—The degree of acidity or basicity of a solution, which is a function of hydronium ion concentration. A pH of less than 7.0 indicates an acidic solution, and a pH greater than 7.0 indicates a basic solution. The pH of water influences many of the types of chemical reactions that occur in it.

Phase I—The PSDDA study is divided into two 3-year overlapping phases. Phase I covers the central area of Puget Sound including Seattle, Everett, and Tacoma. Phase I began in April 1985.

Phase II—The PSDDA study is divided into two 3-year overlapping phases. Phase II covers north and south Puget Sound (including Olympia, Bellingham, and Port Angeles)--the areas not covered by Phase I. Hood Canal is not being considered for location of a disposal site. Phase II began in April 1986.

Polychlorinated Biphenyls (PCB)—A group of manufactured organic chemicals, comprising 209 different but closely related compounds (congeners) made up of carbon, hydrogen, and chlorine. If released to the environment, they persist for long periods of time and can concentrate in food chains. The manufacture and use of PCB are regulated by EPA under the Toxic Substances Control Act.

Polycyclic (Polynuclear) Aromatic Hydrocarbon (PAH)—A class of organic compounds, some of which are persistent and carcinogenic. These compounds are formed from the combustion of organic material and are ubiquitous in the environment. PAH are commonly formed by forest fires and by the combustion of fossil fuels. PAH often reach the environment through atmospheric fall-out, highway runoff, and oil discharge.

Polytetrafluoroethylene (PTFE)—The generic chemical name for materials such as Teflon, a registered trademark of the duPont Corporation.

Precision—Agreement among two or more results that have been found in an identical fashion. ~~The degree of mutual agreement characteristic of independent measurements as the result of repeated application of a method under specified conditions. It is concerned with the closeness of results.~~

Priority Pollutant—Toxic pollutants defined by EPA in 1976 that are the primary subject of regulation of the Clean Water Act. A list of these substances can be found in the Code of Federal Regulations Volume 40, Section 401.15.

Puget Sound Water Quality Authority (PSWQA)—An agency created by the Washington state legislature in 1985 and tasked with developing a comprehensive plan to protect and enhance the water quality of Puget Sound. The PSWQA adopted its first plan in January 1987.

Quality Assurance (QA)—The total integrated program for assuring the reliability of monitoring and measurement data. A system for integrating the quality planning, quality assessment, and quality improvement efforts to meet user requirements.

Quality Control (QC)—The routine application of procedures for obtaining prescribed standards of performance in the monitoring and measurement process.

Quantification—The determination or expression of the number or amount of a variable.

Reconstructed Ion Chromatogram—A graphical display of the total ionization current resulting from all mass fragments detected over time during a mass spectral analysis. The chromatogram can be used to indicate the relative composition of components in the sample mixture analyzed by GC/MS.

Recovery—The amount of a chemical detected in a sample extract at the end of a procedure relative to the total amount present in a sample before the procedure was begun. Also, the amount of a chemical detected in a sample relative to the amount added (i.e., spike) or known to be present (i.e., in a naturally derived standard reference material). Recovery is usually expressed as a percentage.

Regional Administrative Decisions—A term used in PSDDA to describe decisions that are a mixture of scientific knowledge and administrative judgment. These regionwide policies are collectively made by all regulatory agencies with authority over dredged material disposal to obtain sound-wide consistency.

Relative Percent Difference (RPD)—Difference between two measurements expressed as the percentage of their mean. ~~Difference of two measurements, x1 and x2, divided by the mean of the measurements, multiplied by 100.~~

Replicate—One of several identical experiments, procedures, or samples. Duplicate is a special case of replicates consisting of two samples or measurements.

Reproducibility—The ability to produce the same results for a measurement. Often measured by calculation of relative percent difference or coefficient of variation.

Resection—The surgical removal of tissue from an organism during sampling (dissection is the sectioning of tissues within the organism, but does not entail removal of the tissues).

Response Factor—Generally, the ratio of the amount (mass) of a substance to a measurement of its response over time measured by the detector of an analytical instrument. The ratio of response factors for a chemical and a surrogate spike in a sample, or a chemical in a sample and a standard calibration is used to quantify the concentration of chemicals in a sample.

Sediment—Mineral and organic material suspended in or settling to the bottom of a liquid, such as the sand and mud that make up much of the shorelines and bottom of Puget Sound. Sediment input to Puget Sound comes from natural sources, such as erosion of soils and weathering of rock, or anthropogenic sources, such as forest or agricultural practices or construction activities. Certain contaminants tend to collect on and adhere to sediment particles. The sediments of some areas around Puget Sound contain elevated levels of contaminants.

Semivolatile Organic Compounds—Organic compounds with moderate vapor pressures that can be extracted from samples using organic solvents and analyzed by gas chromatography. In this document, semivolatile organic compounds include the EPA acid/base/neutral compounds, pesticides and PCB, as well as numerous other neutral and organic acid compounds of regional interest (e.g., carbazole, retene, coprostanol, 4-methylphenol).

Sensitivity—Capability of a method or instrument to discriminate between samples having differing concentrations of a chemical. The degree to which an instrument responds to low concentrations of a chemical.

Significant Difference—A quantitative determination of the probability that two measurements of the same parameter are different, given the variability of the measurements.

Significant Figure—A digit in a number that is known with certainty. Zeros used to place a decimal point are not significant. ~~A figure that remains to a number or decimal after the zeros to the right or left are cancelled.~~

Spike—The addition of a known amount of a substance to a sample.

Standard—A substance or material, the properties of which are believed to be known with sufficient accuracy to permit its use to evaluate the same property of a sample. In chemical measurements, a standard often describes a solution of chemicals, commonly prepared by the analyst, to establish a calibration curve or the analytical response function of an instrument.

Standard Reference Material (SRM)—A material or substance for which one or more properties are sufficiently well established to be used for the assessment of a method or the calibration of an instrument.

Surrogate Spike Compound—A known amount of a compound that has characteristics similar to that of a compound of interest, added to a sample prior to extraction. The surrogate compound can be used to estimate the recovery of chemicals in the sample. These compounds are also called "recovery internal standards".

Target Compounds—The chemicals of interest in a sample that can be quantified relative to response factors of reliable standards (in contrast to tentatively identified compounds).

Tentatively Identified Compounds—Chemicals identified in a sample on the basis of mass spectral characteristics held in common with a reference mass spectra of a known chemical. These compounds cannot be more confidently identified unless a reliable standard of the compound is obtained and is confirmed to co-elute with the tentatively identified compound and generate similar mass spectra using the same GC/MS.

Unconfined, Open-Water Disposal—Discharge of dredged material into an aquatic environment, usually by discharge at the surface, without restrictions or confinement of the material once it is released.

Volatile Organic Compounds—Organic compounds with high vapor pressures that tend to evaporate readily from a sample. In this document, volatile organic compounds are the 29 EPA priority pollutants considered as volatiles (e.g., benzene).

Volatile Solids—The material in a sediment sample that evaporates at a given high temperature.

Warning Limit—In Puget Sound programs, a value either above or below which data returned by a laboratory are subjected to qualification before inclusion in a regional database. The principle is identical to that of a control limit, but is less stringent and serves as a warning that the system or method may become out of control.

Water Quality Certification—Approval given by Washington State Department of Ecology acknowledging the compliance of a discharge with Section 401 of the Clean Water Act.

APPENDIX B

PSEP/PSDDA Worksheets

Project Name _____
Contract No. _____

TRACE METAL DATA REVIEW WORKSHEET FOR PSEP/PSDDA DATA PACKAGES

The hardcopied data package from _____ (laboratory name) received at _____ has been reviewed and the quality assurance and performance data summarized. The data reviewed included:

Laboratory No. _____ Sampling Date: _____
No. of samples _____ Shipping Date: _____
Matrix _____ Date Received by Lab: _____
Blank No.: _____
Duplicate/Replicate Nos.: _____

The general criteria used to determine the performance were based on an examination of:

- Data Completeness and Format
- Holding Times
- Instrument Calibration Verification
- Lab Blank Analysis
- Detection Limits
- Specific Instrument QA Requirements
- Standard Reference Material Results
- Matrix Spike Percent Recovery Results
- Laboratory Precision Evaluation
- Calculations

Overall Comments:

_____
_____
_____
_____
_____
_____
_____

Definition of Qualifiers:

A - Acceptable data.
B - Blank-corrected down to detection limit
C - Combined with unresolved substances
E - Estimate
G - Estimate is greater than value shown
K - Detected at less than detection limit shown
L - Value is less than the maximum shown
M - Value is a mean
Q - Questionable value
T - Detected below quantification limit shown
U - Undetected at the detection limit shown
X - Recovery less than 10 percent
Z - Blank-corrected, still above detection limit
+ - Positive element identification

Reviewer: _____ Date: _____

I. Data Completeness and Format

A. Data Package Deliverables

_____ The data report contains the required deliverables listed in Table DCF1.

_____ The data package is missing the following sections: _____

_____

_____

_____

Action: The laboratory or contracting agency must be notified and the missing information requested. Depending on what information is missing, continuation of the review may not be possible. The reviewer must assess the severity of the omissions and determine if further review is possible at this time. In some cases, review sections can be completed while waiting for missing sections of the data package.

B. Cover Letter

_____ Cover letter received and the following problems were noted: _____

_____

_____

_____

_____

_____ Cover letter not received. Action: Notify laboratory and request an overview of analyses, noting any special problems (i.e., matrix interferences, deviations from method).

C. Data Report Sheet

_____ Concentrations in proper units and significant figures.

_____ Concentrations not in proper units and/or significant figures for the following samples/elements: _____

_____

_____

Action: Review raw data and correct.

D. Laboratory Qualifiers

_____ Laboratory qualifiers defined.

_____ Laboratory qualifiers not defined. Action: Notify laboratory and request explanation.

II. Holding Times

Date samples received: _____
Date prepared (Hg): _____
Date prepared (all others) by: _____

Action:

If samples are prepared for analysis of mercury (28 days) or any other element (6 months) in excess of the holding times, approximate results for that element. If mercury is held for over one month in excess of the contract required holding time, also reject non-detected results.

Remarks: _____

_____

_____

_____

_____

III. Initial and Continuing Calibration Verification

_____ Calibrations were performed at the beginning of sample analysis and at a minimum frequency of ten percent or every two hours during the analysis, and met PSEP criteria.

_____ Calibrations were not performed as specified and/or did not meet PSEP-specified windows. Action: The sample set for all analytes affected should be rejected and all associated data assigned an R qualifier. Failure to meet calibration criteria is an indication of serious problems in the analytical system.

Remarks: _____

_____

_____

_____

_____

IV. Blank Analysis Results

Contaminant	Project LODs	Initial Calibration Blank Value	Cont. Calib. Blank			Preparation Blank			Action
			1	2	3	1	2	3	
Antimony									
Arsenic									
Cadmium									
Copper									
Iron									
Lead									
Manganese									
Mercury									
Nickel									
Silver									
Zinc									

Note: Contamination detected above the IDLs should be evaluated and qualified. A separate table should be used for each batch analyzed.

Low Level Samples Action Levels (GFAA Analyses):

Blank Result	Accept	Estimate (B or Z)
ug/L blank <2x IDL	Sample ≤IDL Sample >PLOD	IDL < Sample < PLOD
ug/L blank >2x IDL but ≤10xIDL	Sample ≤IDL	Sample > IDL
ug/L blank >10x IDL	Sample ≤IDL	Sample <10x IDL (also "E") Sample >10x IDL

High Level Samples Action Levels (FAA and ICP) -

Action levels are determined by multiplying the highest concentration determined in any blank. The action level for samples which have been diluted should be multiplied by the dilution factor. Prior to applying action levels to sediments and tissue, it is necessary to convert the aqueous action value (ug/L) to mg/kg for each sample with the following equations:

Action value (ug/L) = 5x highest blank result (ug/L)

TAD and tissue analyses action value (mg/kg) =

$$\text{Action value (ug/L)} \times \frac{\text{volume diluted to (ml)}}{\text{wet weight digested (gm)}} \times \frac{1\ \text{L}}{1{,}000\ \text{ml}} \times \frac{1{,}000\ \text{gm}}{1\ \text{kg}} \times \frac{1\ \text{mg}}{1{,}000\ \text{ug}}$$

$$\text{SAD action value (mg/kg} = \frac{100}{\%\ \text{solids}} \times \text{the above equation}$$

V. Detection Limits

_____ Instrument detection limit results were present and found to be less than the Project Required LODs.

_____ Detection limit results were not included in the data package.

_____ Detection limits were present, but the criteria were not met for the following elements:

_____

_____

_____

Action: Adjust sample detection limits for elements not meeting contractual criteria listed above. Elements detected below the adjusted detection limit should be rejected (R'd).

Calculating detection limits for soil samples:

SAD Sample detection limit (mg/kg) =

$$100 \times \frac{\text{IDL (ug/l)}}{\text{\% solids}} \times \frac{\text{Volume diluted to (ml)}}{\text{wet weight digested (g)}} \times \frac{1\text{L}}{1{,}000\text{ ml}} \times \frac{1{,}000\text{ gm}}{1\text{ kg}} \times \frac{1\text{ mg}}{1{,}000\text{ ug}}$$

TAD Sample detection limit (mg/kg) =

$$\text{IDL (ug/l)} \times \frac{\text{Volume diluted to (ml)}}{\text{dry weight digested (g)}} \times \frac{1\text{L}}{1{,}000\text{ ml}} \times \frac{1{,}000\text{ gm}}{1\text{ kg}} \times \frac{1\text{ mg}}{1{,}000\text{ ug}}$$

VI. Instrument Specific QA Requirements
GFAA QC Analysis/Method of Standard Additions

A. Duplicate Injections

_____ Duplicate injections were performed for all samples and agreed within ±20% Relative Standard Deviation (RSD). The RSD or Coefficient of Variation (CV) is calculated by dividing the standard deviation by the mean and multiplying by one hundred.

_____ Duplicate injections were not performed for the following samples/elements:

_____

_____

_____

Action: Reject (R) data.

_____ Duplicate injections were outside the ±20% RSD limit and a third injection was not performed for samples with an absorbance >50% of the spike concentration as required for the following samples/elements:

_____

_____

_____

Action: Estimate (E) data and summarize the lab's deficiency in the QA Summary.

_____ Duplicate injections did not agree within ±20% RSD and the third injection did not agree with either of the first two injections (±20% RSD) for the following samples/elements:

_____

_____

_____

B. Analytical Spike Percent Recoveries

_____ One-point analytical spikes were performed for all samples and the spike recoveries met the 85-115% recovery criteria (Accept data).

_____ The analytical spike recoveries were less than 10% for the following samples/elements:

_____

_____

_____

Action: Reject (R) data.

_____ Spike recoveries were 10-40% and the laboratory did not dilute and re-analyze the following samples/elements:

_____

_____

_____

Action: Estimate (E) positive results and reject (R) non-detected results.

_____ Spike recoveries were 10-40% after the following samples/elements were diluted and re-analyzed:

_____

_____

_____

Action: Estimate (E) positive results and reject (R) non-detected results.

_____ Sample Concentrations were less than 50% of the spike value and spike recoveries were greater than 40% (Accept data.)

_____ Sample Concentrations were greater than 50% of the spike value, and spike recoveries did not meet the 85-115% recovery criteria. The following actions should be taken:

_____ Method of Standard Addition (MSA) was not performed as required for sample numbers/elements: _____

_____

_____

_____

Action: Estimate (E) data and summarize the lab's deficiency in the QA Summary.

_____ MSA was used to quantitate analytical results for the following samples/elements when correlation coefficients were greater than 0.995:

_____

_____

_____

Action: Accept data.

_____ MSA was performed for the following samples/elements and correlation coefficients were less than 0.995:

_____

_____

_____

Comments: _____

_____

_____

_____

_____

_____

_____

_____

ICP Interference Check Sample Analysis

The ICP interference check sample analysis is performed to verify the contract laboratories' interelement and background correction factors.

_____ Interference QC samples were run at the beginning and end of each sample analysis run (or a minimum of twice per 8 hour working shift, whichever is more frequent) and were within the control limits specified in PSEP.

_____ Interference QC samples were run, but did not meet the control limits.

In general, the sample data can be accepted without qualification if the concentrations of aluminum, calcium, iron, and magnesium are less than 50% of the ICP Interference check sample concentrations.

Note: The 20% contract limit (80-120%) is based on the true value for EPA standards, and on the mean value (run at least five times) for non-EPA standards.

Remarks: _____

_____

_____

_____

_____

_____

QC Analysis Serial Dilution Results

Serial dilution analysis enables the reviewer to evaluate whether significant physical or chemical interferences exist due to sample matrix for samples analyzed by ICP. Sample results for elements analyzed and quantitated by Furnace Atomic Absorption should not be evaluated.

_____ Serial Dilutions were performed for each matrix and results of the diluted sample analysis agreed within ten percent of the original undiluted analysis.

_____ Serial Dilutions were not performed for the following:

_____

_____

_____ Serial Dilutions were performed, but analytical results did not agree within 10% for analyte concentrations greater than 10x the IDL after dilution. The following elements were evaluated for Matrix interferences:

Dilution Factor (DF): _____ Matrix: _____

Element	IDL	IDL x 10	Sample #: _____	Serial Diluted Sample Result		Action
				without DF	Times DF	
Aluminum						
Cadmium						
Calcium						
Chromium						
Iron						
Lead						
Magnesium						
Nickel						
Silver						
Zinc						
Other:						

Actions: All data for samples of the same matrix for that element should be estimated (E) when the serial dilution results do not meet contractual requirements.

ICP QC Analysis Serial Dilution Results

_____ Serial Dilutions were performed, but analytical results did not agree within 10% for analyte concentrations greater than 50x the IDL in the original sample. The following elements were evaluated for Matrix interferences:

Element	IDL	IDL x 10	Sample #: _____	Serial Diluted Sample Result	Action
Aluminum					
Cadmium					
Calcium					
Chromium					
Iron					
Lead					
Magnesium					
Nickel					
Silver					
Zinc					

Actions: All data for samples of the same matrix for that element should be estimated (E) when the serial dilution results do not meet contractual requirements.

VII. Standard Reference Material Results

_____ Standard Reference Material (SRM) analysis was performed for every twenty samples received and met contractual criteria.

_____ Standard Reference Material (SRM) analysis was performed, but did not meet the criteria for the following elements: _____

_____

_____

_____

Calculation: % R = (Observed/True) x 100

Actions:

	Accept	Estimate (E)	Reject
% Recovery	30-79 for U 80-120 for U >120 for U	30-79 for + >120 for + <30 for +	<30 for U

NOTE:

+ - positive result
U - not detected element

VIII. Matrix Spike Percent Recovery Rates

_____ Number of matrix spikes analyzed (min. 1 per 20 samples)

_____ Spot check of raw data - calculation verification.

Contaminants	Sample #: _____					Sample #: _____				
	SSR	SR	S	%R	Action	SSR	SR	S	%R	Action
Antimony										
Arsenic										
Cadmium										
Copper										
Lead										
Mercury										
Nickel										
Silver										
Zinc										

If the sample concentration exceeds the spike concentration by a factor of 4 or more, no action is taken. When the sample result (SR) is less than the Project LOD, SR is equal to zero.

Calculation: $\%R = \frac{SSR-SR}{S} \times 100$

Matrix spike results should be applied to all samples of the same matrix.

Accept	Approximate	Reject
%R (75-125%) for SSR	SR(+) + %R < 30% for SSR[1]	SR(U) + %R < 30% for SSR[2]
SR(U) + %R (30-74%) for SSR[5]	SR(+) + %R (30-74%) for SSR[3]	
SR(U) + %R > 125%	SR(+) + %R > 125% for SSR[4]	

NOTE:

S - amount of spike
SSR - spiked sample result
SR - unspiked sample result
%R - percent recovery
U - non-detected element
\+ - positive result
1 - Discuss in summary that sample results could be biased significantly low and that the reported concentration is the minimum concentration at which the analyte is present.
2 - Indicate in QA summary memo of the possibility of false negatives, detection limits are elevated over what is reported, and that severe analytical deficiencies exist.
3 - Determine percent bias of results. Report that the detection limit may be biased low.
4 - Determine percent bias of sample results: false positive results may potentially exist.
5 - When the spiked sample results fall between 30-74% recovery, detection limits should be estimated and the percent bias determined.

Comments: _____

IX. Laboratory Precision Evaluation

_____ Number of duplicates analyzed. Required to analyze 1 per 20 samples.

Sample Matrix: _____ Sample Number _____

Element	PLOD		Sample Result	Duplicate Sample Result	Criteria (RPD or ± PLOD)	Action
	ug/L	mg/kg				
Antimony						
Arsenic						
Cadmium						
Copper						
Lead						
Mercury						
Nickel						
Silver						
Zinc						

Laboratory Duplicate Actions should be applied to all other samples of the same matrix type.

Actions: If both sample results are less than the PLOD, then laboratory precision is not evaluated. If either sample result is less than 5x the PLOD, then "E" results for elements whose absolute difference is >PLOD. If both sample results are greater than 5x the PLOD, then calculate the RPD. For sediment and tissue samples, "E" results for elements which have an RPD >20%.

Calculation: $RPD = \frac{D1 - D2}{D1 + D2/2} \times 100$

NOTE:
PLOD - Project Limit of Detection
RPD - Relative Percent Difference
D1 - First sample value
D2 - Second sample value

Comments: _____

X. Calculations

A. Sample Results

For sediment and tissue samples, the following equation may be necessary to convert raw data values (usually reported in ug/L) to actual sample concentrations (mg/kg):

For TAD, SAD and tissue analyses, the following equation is used to determine mg/kg:

$$\text{Digest Result (ug/L)} \times \frac{\text{Volume Diluted to (mL)}}{\text{Weight Digested (gm)}} \times \frac{1\text{L}}{1{,}000\text{ mL}} \times \frac{1{,}000\text{ gm}}{1\text{ kg}} \times \frac{1\text{ mg}}{1{,}000\text{ ug}}$$

In addition, SAD results must be converted to dry weight using the percent solids calculation. TAD are done on dried sample, and tissue results are reported on a weight basis, so no correction is necessary.

$$\frac{\text{wet weight sample result (mg/kg)}}{\text{\% solids}} \times 100 = \text{Final Concentration, mg/kg (Dry Weight)}$$

B. Percent Solids

It is recommended that percent solids determination be validated 100% due to the impact an error could have on the results for an entire sample.

$$\text{\% solids} = \frac{\text{Sample Dry Weight}}{\text{Sample Wet Weight}} \times 100$$

Comments: _____

_____

_____

_____

_____

_____

APPENDIX C

Treatment of Chemistry Data: Calculations and Qualifier Codes

TREATMENT OF CHEMISTRY DATA: CALCULATIONS AND QUALIFIER CODES

Most of SEDQUAL's procedures involve the manipulation of sediment chemistry data, and proper interpretation of the results depends upon an understanding of how these manipulations are carried out. The way chemical measurements are qualified and the effect of these qualifiers are most important.

Each sediment chemistry value stored in SEDQUAL is assigned up to five qualifier codes (in practice, most have only one). These qualifier codes and their meanings are displayed in Table 5-3. Many SEDQUAL procedures (in particular, sediment quality value calculations and comparisons) use only detected data. These values are ignored when calculating apparent effects thresholds or comparing sediment quality values to the data.

Qualifiers also affect the way sediment chemistry values are combined. Data are combined when laboratory replicates must be averaged to provide a single value for a sample, and when samples are averaged (across field replicates or dates) to summarize data by station. The following rules are used to average laboratory replicates:

1. If all values are undetected, the lowest detection limit is used.

2. If any values have been detected, all detected and undetected data are averaged, excluding undetected values greater than the highest detected value.

Treatment of significant digits used varies with the procedure. In particular, the sediment quality value calculations differ from other retrieval and analytical procedures. Ordinarily, as many significant digits as were entered are used. Averages of laboratory replicates are computed with no more significant figures than the inidividual measurements. For sediment quality value calculations and comparisons, however, values are always rounded to two significant figures. This is done because published criteria are expected to have no more than two digits of precision, and comparison to data reported to greater precision can lead to spurious predictions of biological effects.

When values are averaged, their qualifier codes are also combined. Only the first of the five qualifiers associated with each measurement is used to generate a combined qualifier. During data entry, it is important that the qualifiers for each measurement be listed in priority. Qualifiers are combined in a pairwise fashion, using the matrix shown in Table C-1. If more than two values are to be averaged, the first two are used to develop a resultant qualifier, which is then combined with the third qualifier. This process continues until all values have been averaged and a final combined qualifier is produced. SEDQUAL adds an M qualifier to all averaged data.

Sediment chemistry data can be stored in units of either parts per million (ppm), parts per billion (ppb), or percent, and by either wet- or dry-weight basis. When averaging data, SEDQUAL automatically converts units and does not mix data measured by dry- and wet-weight basis. In some cases, the units and measurement basis are predetermined by the procedure being carried out; in others (such as some retrievals) they are not. In the latter case, SEDQUAL reports the concentration in terms of the first units and measurement basis encountered when searching the database. Note the units and measurement basis may differ from chemical to chemical.

TABLE C-1. QUALIFIER CODES MATRIX

	U	E	G	L	K	T	B	Z		Q	X	C	M
U	U	L	E	L	L	L	B	L	L	Q	L	L	L
E	L	E	E	E	E	E	L	E	E	E	E	E	E
G	E	E	G	E	E	E	E	G	G	E	G	G	G
L	L	E	E	L	L	L	L	L	L	E	L	L	L
K	L	E	E	L	K	L	L	L	L	E	L	L	L
T	L	E	E	L	L	T	L	L	L	E	L	L	L
B	B	L	E	L	L	L	B	L	L	E	L	L	L
Z	L	E	G	L	L	L	L	Z	E	E	E	E	E
	L	E	G	L	L	L	L	E		E			
Q	Q	E	E	E	E	E	E	E	E	Q	E	E	E
X	L	E	G	E	L	L	L	E		E	X	C	
C	L	E	G	L	L	L	L	E		E	C	C	
M	L	E	G	L	L	L	L	E		E			