STANDARD OPERATING PROCEDURES

for

Laboratory Analysis: Benthic Macroinvertebrate Indicator

Prepared by



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March 2017

A1. TITLE AND APPROVAL SHEET

Document Title:

Quality Assurance Project Plan for Laboratory Analysis: Benthic Macroinvertebrate Indicator

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Day/Month/Year

07/March/2017

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Gary T. Lesten 🗸 🕖 March 2017

EcoAnalysts, Inc. Quality Assurance Manager:

Robert Bobier / 07 March 2017



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Table 1. Acronyms and Abbreviations

BMI Benthic Macroinvertebrate
CEO Chief Executive Officer

EPA United States Environmental Protection Agency

DQO Data Quality Objective

EcoAnalysts EcoAnalysts, Inc.

LIMS Laboratory Information Management System

MQO Measurement Quality Objective

QA Quality Assurance

QAM Quality Assurance Manager
QAPP Quality Assurance Project Plan
QA/QC Quality Assurance/Quality Control

QC Quality Control

SOP Standard Operating Procedure

US EPA United States Environmental Protection Agency



DOCUMENT CONTROL

This document has been prepared according to the United States Environmental Protection Agency publication, *EPA Requirements for Quality Assurance Project Plans* (EPA QA/R5, March 2001). This QAPP will be reviewed annually and updated as needed. Updated versions of this QAPP will bear a new (x + 1) revision number.

GROUP A: PROJECT MANAGEMENT

A3. DISTRIBUTION LIST

Each person listed on the Approval Signature Page and each person listed in Table 2 or his/her successor will receive a copy of the final approved version of this Quality Assurance Project Plan. A copy will also be made available to other persons taking part in the project and to other interested parties.

Table 2. QAPP for Laboratory Analysis: BMI Distribution List

Name	Title/Affiliation	Address	Phone/email
Gary Lester	CEO, Project	1420 South Blaine Street,	208-882-2588 ext 21
	Manager	Suite 14	glester@ecoanalysts.com
	EcoAnalysts, Inc.	Moscow, ID 83843	
Robert Bobier	QA Manager	1420 South Blaine Street,	208-882-2588 ext 34
	EcoAnalysts, Inc.	Suite 14	rbobier@ecoanalysts.com
		Moscow, ID 83843	
William LaVoie	Taxonomy	1420 South Blaine Street,	208-882-2588 ext 80
	Coordinator	Suite 14	blavoie@ecoanalysts.com
	EcoAnalysts, Inc.	Moscow, ID 83843	
Megan Payne	Sorting Lab Manager	1420 South Blaine Street,	208-882-2588 ext 59
	EcoAnalysts, Inc.	Suite 14	mpayne@ecoanalysts.com
		Moscow, ID 83843	

A4. PROJECT/TASK ORGANIZATION

The primary responsibilities of the principals are as follows:

EcoAnalysts Project Manager – Gary Lester, CEO

 Provides overall coordination of the project and makes decisions regarding the proper functioning of all aspects of the project; and



• Makes assignments and delegates authority as needed, to other parts of the project organization.

EcoAnalysts QA Manager – Robert Bobier

- Oversees transfer of samples and related records for the benthic macroinvertebrate indicator;
- Ensures the validity of data for the benthic macroinvertebrate indicator;
- Interacts with EcoAnalysts Project Manager on issues related to sample processing and schedules for conduct of activities;
- Collects copies of all official forms, evaluation checklists and reports;
- Oversees and maintains records of laboratory operations, but is not part of laboratory operations; and
- Directs laboratory audits.

<u>EcoAnalysts Laboratory Managers – William LaVoie and Megan Payne</u>

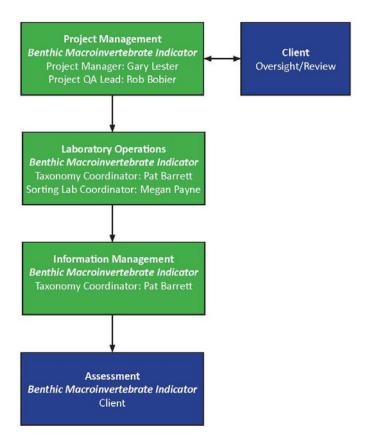
- Oversee analysis of benthic macroinvertebrate samples; and
- Ensure the validity of data for the benthic macroinvertebrate indicator.

Table 3. Principal Contact List

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Gary Lester	Robert Bobier			
CEO, Project Manager	QA Manager			
EcoAnalysts, Inc.	EcoAnalysts, Inc.			
1420 South Blaine Street	1420 South Blaine Street			
Suite 14	Suite 14			
Moscow, ID 83843	Moscow, ID 83843			
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William LaVoie	Megan Payne			
Taxonomy Coordinator	Sorting Lab Manager			
EcoAnalysts, Inc.	EcoAnalysts, Inc.			
1420 South Blaine Street	1420 South Blaine Street			
Suite 14	Suite 14			
Moscow, ID 83843	Moscow, ID 83843			
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Fax: 208-883-4288	Fax: 208-883-4288			
blavoie@ecoanalysts.com	mpayne@ecoanalysts.com			



Figure 1. Project Organization



The QA Manager is independent from project staff that generates data. The QA Manager, Robert Bobier, is responsible for managing this QAPP and is available to address project QA/QC problems and concerns.

A5. PROBLEM DEFINITION/BACKGROUND

This QAPP addresses the laboratory operations and analyses for benthic macroinvertebrate indicator samples. This plan describes elements of project management, data quality objectives, measurement and data acquisition, and information management for processing benthic macroinvertebrate samples.

This QAPP covers in scope the processing of benthic macroinvertebrate samples collected from all water body types including, but not limited to coasts and estuaries, wetlands, lakes, rivers, and streams.



A6. PROJECT/TASK DESCRIPTION

EcoAnalysts is well equipped and staffed to conduct highly specialized analyses related to the benthic macroinvertebrate indicator. EcoAnalysts complies with all methods, procedures, and QA/QC requirements as described in required laboratory methods manuals. Prior to initiation of task orders, EcoAnalysts' laboratory operations may be evaluated by EcoAnalysts' QAM.

Benthic macroinvertebrate samples will be sorted and identified at EcoAnalysts' laboratory to the lowest practicable level or level required. The sample will first be sorted into major taxonomic groups, which then will be identified to the required taxonomic level and counted. The sorting laboratory manager and taxonomy coordinator will oversee, and periodically review, the work performed by sorting technicians.

A7. QUALITY OBJECTIVES AND CRITERIA

Performance objectives as associated primarily with measurement error, are established (following USEPA Guidance for Quality Assurance Plans EPA240/R-02/009) for analyzing benthic macroinvertebrate indicator samples. The following sections describe approaches for evaluating benthic macroinvertebrate indicator sample analyses.

A7.1 Sorting Efficacy – Aliquot Method

To ensure every sample meets a standard minimum level of sorting efficacy, EcoAnalysts, Inc. re-sorts at least 20% of the sorted material of every sample that is processed in the lab.

The resort is performed by a specially trained and designated sorting quality control technician (this will never be the technician who originally sorted the sample).

The QC technician re-sorts at least 20% of the sorted fraction of the sample to check if at least 90% (or percent established by the client) of the organisms have been removed. An estimated percent efficacy is calculated by dividing the number of organisms found in the original sort by the total number of organisms estimated to be in the sorted material, based on those found in the 20% quality control re-sort, using the following equation:

Equation 1. Sorting Efficacy

$$Sorting Efficacy = \frac{Original Count}{Original Count + \left(\frac{QCCount * QCSquares}{QTSquares}\right)} * 100$$

Where:

OriginalCount = the number of organisms picked by the first sorter QCCount = the number of organisms found in the Quality Control sort QCSquares = the number of grids sorted during the QC process QTSquares = the total number of grids in the QC Caton



Sorting efficacy is measured as the estimated percent of the total organisms found during the original sorting process. If the estimated percent sorting efficacy is 90% or greater, the sample passes the quality control check. If the estimate is less than 90%, the sample is re-sorted. When this happens, the sample undergoes the quality control process again until it passes the 90% efficacy requirement. In addition to calculating sorting efficacy, a specially trained and designated sorting quality control technician, who is never the technician who originally sorted the sample, also verifies label accuracy, information capture on the benchsheet, and the presence/absence of non-target organisms in the taxa vials.

A7.2 Taxonomic Precision and Accuracy

Taxonomic precision is quantified by comparing whole-sample identifications completed by independent taxonomists or laboratories. Accuracy of taxonomy is qualitatively evaluated through specification of target hierarchical levels (e.g., family, genus, or species) and the specification of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). To calculate taxonomic precision for benthic macroinvertebrate samples, 10 percent of the samples are randomly selected for reidentification by an independent taxonomist or laboratory. Comparison of the results of whole sample re-identifications provides a Percent Taxonomic Disagreement (PTD) calculated as:

Equation 2. Percent Taxonomic Disagreement (PTD)

$$PTD = \left[1 - \left(\frac{comp_{pos}}{N}\right)\right] \times 100$$

where

 $comp_{pos}$ = the number of agreements

N = the total number of individuals in the larger of the two counts.

The lower the PTD, the more similar taxonomic results are and the overall taxonomic precision is better. A Measurement Quality Objective (MQO) of ≤15% is recommended for taxonomic differences. Individual samples exceeding 15% are examined for taxonomic areas of substantial disagreement, the reasons for disagreement investigated, and corrective measures taken where needed.

Where re-identification by an independent, outside taxonomist or laboratory is not practical, percent similarity will be calculated between each identifying taxonomist. Percent similarity is a measure of similarity between two communities or two samples (Washington 1984). Values range from 0% for samples with no species in common, to 100% for samples that are identical. It is calculated as follows:



Equation 3. Percent Similarity

$$PSC = 1 - 0.5 \sum_{i=1}^{K} |a - b|$$

where:

a and b = for a given species, the relative proportions of the total samples A and B, respectively, which that species represents.

A MQO of ≥85% is recommended for percent similarity of taxonomic identification. If the MQO is not met, the reasons for the discrepancies between analysts should be discussed. If a major discrepancy is found in how the two analysts have been identifying organisms, the last batch of samples counted by the analyst under review may have to be re-identified.

Additionally, percent similarity should be calculated for re-processed subsamples. This provides a quantifiable measure of the precision of subsampling procedures. A MQO of ≥70% is recommended for percent similarity of subsamples. If a sample does not meet this threshold, additional subsamples should be processed from that sample until the MQO is achieved.

Sample enumeration is another component of taxonomic precision. Final specimen counts for samples are dependent on the taxonomist, not the rough counts obtained during the sorting activity. Comparison of counts is quantified by calculation of percent difference in enumeration (PDE), calculated as:

Equation 4. Percent Difference in Enumeration

$$PDE = \left(\frac{|Lab1 - Lab2|}{Lab1 + Lab2}\right) \times 100$$

An MQO of ≤5% is recommended. Individual samples exceeding 5% are examined to determine reasons for the exceedance.

A7.3 MQO Evaluation

For samples exceeding these MQOs, corrective actions can include defining the taxa for which re-identification may be necessary (potentially even by a third party), for which samples (even outside of the 10% lot of QC samples) it is necessary, and where there may be issues of nomenclatural or enumeration problems.

Taxonomic accuracy is evaluated by having individual specimens representative of selected taxa identified by recognized experts. Samples will be identified using the most appropriate technical literature that is accepted by the taxonomic discipline and reflects the accepted nomenclature. Where necessary, the Integrated Taxonomic Information System (ITIS, http://www.itis.usda.gov/) will be used to verify nomenclatural validity and spelling. A reference collection will be compiled as the samples are identified.



A8. SPECIAL TRAINING/CERTIFICATION

Training of EcoAnalysts' project staff, when needed, is done internally through assistance from project operations staff. When appropriate, identifications are verified by taxonomists certified in the applicable area. As verification of EcoAnalysts' taxonomic expertise, Society for Freshwater Science (SFS, formerly NABS) certification information is also available on the Society's Taxonomic Certification Programme website: www.nabstcp.com.

Table 4. Taxonomist's SFS Certifications and Education

Name	Education	SFS Certifications
Chip Barrett	PhD – Zoology	Oligochaeta, 2016
	MS – Zoology	
	BA – Zoology	
Matt Hill	MS – Fishery Science	EPT East, 2013
	BS - Entomology	EPT West, 2013
		General Arthropods East, 2013
		General Arthropods West, 2013
		Oligochaeta, 2013
Wade Hoiland	MS – Zoology	EPT East, 2013
	BS – Secondary Education in Biology	EPT West, 2013
		General Arthropods East, 2016
		General Arthropods West, 2016
		Chironomidae East, 2015
		Chironomidae West, 2015
William Lavoie	MS - Zoology and Physiology	EPT East, 2013
	BS - Fish & Wildlife Resources	EPT West, 2013
		General Arthropods East, 2013
		General Arthropods West, 2013
		Chironomidae East, 2013
		Chironomidae West, 2013
Laura Mills	MS – Aquatic Ecology	EPT East, 2016
	BS - Wildlife and Fisheries Science	EPT West, 2013
Anndrea Navesky	MS - Entomology	EPT East, 2013
•	BS - Biology	EPT West, 2013
		General Arthropods East, 2013
		General Arthropods West, 2013
John Pfeiffer	MS - Entomology	EPT East, 2012
	BS – Fisheries Resource Management	EPT West, 2012
	AAS	General Arthropods East, 2012
		General Arthropods West, 2012
Ben Sloniker	MS – Environmental Science	EPT East, 2013
	BS - Biology	EPT West, 2013
Gregory Wallace	BS - Wildlife Conservation &	EPT East, 2016
	Management	Chironomidae East, 2009
		Chironomidae West, 2009
		Oligochaeta, 2013



A9. DOCUMENTATION AND RECORDS

All versions of the QAPP are retained by EcoAnalysts, Inc. EcoAnalysts retains sorting bench sheets indefinitely. Taxonomic data are entered into EcoAnalysts' custom Laboratory Information Management System (LIMS) by taxonomists during the identification process. Sample data are retained by EcoAnalysts indefinitely following completion of the project.

GROUP B: DATA GENERATION AND ACQUISITION

B1. SAMPLING DESIGN

The protocols for establishing sample and study design associated with different indicators are described in the benthic macroinvertebrate indicator-specific sections of the field QAPP or client field manual.

B2. SAMPLING METHODS

The protocols for the collection of samples associated with different indicators are described in the benthic macroinvertebrate indicator-specific sections of the field QAPP or client field manual.

B3. SAMPLE HANDLING AND CUSTODY

Immediately upon receipt of benthic macroinvertebrate samples, all containers are inspected for damage or leakage. Sample labels are checked against chain of custody forms and/or packing slips and any discrepancies are noted. Receipt records are reported to the client within one business day of sample receipt. Chain of custody logs are reported, throughout the project, according to timelines and methods requested by the client.

Samples are logged into the EcoAnalysts, Inc. custom LIMS database and assigned a unique sample tracking number.

B4. ANALYTICAL METHODS

B4.1 Sorting Benthic Macroinvertebrate Samples

A sample is checked out by a trained sorting technician via the LIMS. A sorting bench sheet is printed that contains all of the sample information and sorting protocols assigned to it. The sorter records the primary matrix type and estimates the volume of detritus in the entire sample prior to rinsing. The standard descriptors for the types of sample matrix are: Inorganic, Coarse Organic, Fine Organic, Vegetation, and Filamentous Algae.

The sample is prepped for subsampling procedures by emptying the matrix into a sieve of a specified mesh size to remove preservative and fine sediment. If the sample matrix is made up



of a significant percentage of inorganic material, the organic material will be elutriated from the inorganic material prior to sorting.

For elutriation, the whole sample is washed into a shallow pan of water where any large pieces of organic material are rinsed and inspected thoroughly by another technician for attached invertebrates. The sample is agitated with water to separate any organic matter from inorganic sediments. After agitating the sample in water, the lighter organic material is poured back into the sieve. The inorganic portion of the sample remaining in the pan is repeatedly washed and decanted into the sieve until no more organic matter remains in the pan with the inorganic material.

The remaining inorganic sediments are inspected under a magnifying lamp (3X) to look for any invertebrates too heavy to have been elutriated (e.g. mollusks, snails, stone-cased Trichoptera, etc.). If there are significant numbers of heavy invertebrates in the inorganic material – too many to easily remove under the magnifying lamp – the inorganic and organic matrix is recombined into the sieve and entire sample matrix will be prepared for subsample. If there are not significant numbers of heavy invertebrates in the inorganic material, they are removed under the magnifying lamp and placed with the organic matrix. A second technician inspects the inorganic material for organisms until it is determined there are no more invertebrates in the inorganic fraction of the sample. Unless otherwise requested, the inorganic elutriate is discarded.

The organic material and other contents of the sieve are then evenly distributed into the bottom of a Caton-style tray. These are trays of various sizes consisting of uniform grids, each grid being 2 inches per side and the bottom is constructed of 250-micron mesh. A grid (or a standardized portion of a grid) is randomly selected and its contents transferred to a Petri dish. The material in the Petri dish is sorted under a dissecting microscope (minimum magnification = 10X). The benthic macroinvertebrates are counted as they are placed into vials containing 70% ethanol.

Sorters are trained to pick and count only benthic macroinvertebrates, with heads, that were alive during sampling and contain the attributes required for taxonomic identification. Organisms picked may include sub-aquatic organisms or other specified organisms according to the specific study design. Specimens rejected according to EcoAnalysts' standard include: Sub-aquatic Adults, Terrestrials, Vertebrates, Collembola, Copepoda, Zooplankton, Exuviae, and any organism without a head. When the target count of organisms has been reached or the target percentage of the sample has been sorted but not fully sorted, a special large and rare protocol may be followed on any remaining unsorted material. Organisms deemed relatively large or rare to the sample (in comparison with the target taxa enumerated in the final count) are found by a naked eye scan in the unsorted sample remnants and are not counted but picked and placed in a separate vial.



Laser-printed labels containing the appropriate sample tracking information are placed in the vial(s). The total number of organisms removed (not including large and rare organisms), the number of grids sorted out of the total, the time spent sorting, and the final volume of the remaining sample volume are all recorded on the sorting bench sheet, as well as comments significant to the preparation, sorting, and/or condition of the sample.

To ensure every sample meets a standard minimum level of sorting efficacy, EcoAnalysts, Inc. standard sorting quality assurance is maintained by re-sorting a portion of the sorted material of every sample that is processed in the lab, and ensuring a minimum efficacy is reached (as required by the project). See Section A7.1 for sorting quality objectives.

B4.1 Taxonomic Identification of Benthic Macroinvertebrates

A taxonomist selects a sample for identification via the LIMS and empties it into a Petri dish. Under a dissecting and/or compound microscope, the invertebrates are identified to the level specified by the study design. Taxonomic references used for the taxonomic analysis of samples may be provided upon request. The taxonomist enters each taxon directly into the project database using a unique taxonomic code (this is done while at the microscope). The number of individuals of each taxon is counted and entered into the database.

As the sample is being identified, the taxonomist enters data directly into the computer using a custom built LIMS database and user interface. The data entry program has several features built into it, including steps for entering taxon names, life stage information, taxonomic notes, etc. There is a visual cue at each step which prompts for a user confirmation. A running tally of invertebrates as well as the number and type of taxa in the sample are displayed on the screen. Therefore, a taxonomist can quickly look for low or high counts as a flag for major discrepancies. Note: With this process, we have successfully eliminated the need for handwritten bench sheets, thereby doing away with a secondary step of data entry and the errors associated with it.

A synoptic reference collection can be prepared, if requested, where at least one specimen (preferably 3-5 specimens) of each taxon encountered is placed into a 1-dram vial containing 70% ethanol and is properly labeled with identity and sample number. Chironomidae reference specimens are permanently slide mounted and labeled with the EcoAnalysts, Inc. sample number and taxonomic determination.

Depending on the requirements of the project, one or several reference collections can be made. Also, organisms can be vouchered by a specified taxonomic level, i.e. vouchered by each taxon per sample. If any synoptic reference collection is made, a second taxonomist examines the reference collection specimens to verify the accuracy of all taxa identified in the project.

If requested, a specified number of the samples are randomly selected for re-identification by a QC taxonomist. All specimens in those samples that were not set aside for the reference



collection are re-identified. See Section A7.2 for taxonomic precision and accuracy measurement quality objectives. The final data is adjusted according to the recommendations of both taxonomists. If requested, reconciliation reports are written and delivered to the client as part of the overall Quality Assurance Report.

B5. QUALITY CONTROL

Each benthic macroinvertebrate sample is checked for quality control. See Sections A7.1 and A7.2 of this QAPP for quality objectives.

B6. INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE

All microscopes and laboratory equipment are inspected regularly according to manufacturer recommendations.

B7. INSTRUMENT/EQUIPMENT CALIBRATION AND FREQUENCY

All microscopes and laboratory equipment, including digital imaging equipment, are calibrated regularly according to manufacturer recommendations. Calibration will be checked throughout the project and equipment will be recalibrated if necessary.

B8. INSPECTION/ACCEPTANCE OF SUPPLIES AND CONSUMABLES

Supplies and consumables include alcohol and sample jars. Supplies and consumables are purchased only from reputable and reliable suppliers and are inspected for usability upon receipt.

B9. NON-DIRECT MEASUREMENTS

EcoAnalysts maintains a library of current taxonomic references. These are used for taxonomic identification purposes when such need arises. Taxonomists are responsible for using current references and publications.

B10. DATA MANAGEMENT

As described in section B4.1, data is directly entered into the custom built LIMS database and user interface. With several features built into it, including steps for taxonomic identification of a specimen, the number of specimens in each taxon, life stage information, taxonomic notes, etc., the data entry program successfully eliminates the need for handwritten bench sheets, the secondary step of data entry, and the errors associated with it. Additionally, a running tally of invertebrates and taxonomic groups are displayed on the screen, therefore allowing the taxonomist to quickly identify low or high counts as a flag for potential discrepancies.



Throughout the project and sample analysis, data entry is double checked for accuracy, and validated by the laboratory coordinator. Using our networked computer systems, the appropriate data are combined for each sample to obtain the sorting statistics and comprehensive taxa lists and counts.

Various metrics calculations are offered as output from the LIMS, with EcoAnalysts standard deliverables including (but not limited to) abundance, richness, and community measures. Additional metrics calculations, including more detailed Benthic Invertebrate Indices, may be provided upon request. Other supplemental reports, such as QA/QC results and data analysis and/or interpretation, can be provided dependant on project requirements.

Data are delivered in an electronic format specified by the client and emailed to the technical contact(s). Hard copies and/or copies on compact disc can be mailed to the client upon request. The delivery schedule is agreed upon by the client and EcoAnalysts, Inc. in advance, specifying the sample lots, dates, and components. EcoAnalysts, Inc. retains all raw data files used and derived in our projects.

Quality assurance data sheet checks are part of the sample validation process, and include scanning for apparent entry errors, measurement errors, omissions, and anomalies. Suspect data are flagged and/or excluded from use. Data may be presented in table, graph, and chart format. Unusual data are rechecked to verify their accuracy.

GROUP C: ASSESSMENT AND OVERSIGHT

C1. ASSESSMENT AND RESPONSE ACTIONS

The project manager, Gary Lester, is responsible for all reporting, tracking, and overall project management including field activities, reviewing the data, reporting, and forwarding all data to the client for inspection. Megan Payne and Bill LaVoie are responsible for laboratory operations involving processing benthic macroinvertebrate indicator samples for projects. Robert Bobier, EcoAnalysts QAM, is authorized to oversee all activities as required for quality assurance.

C2. REPORTS TO MANAGEMENT

Draft reports of project findings will be prepared for the client on a regular basis, as requested. Problems that arise during the project are corrected and reported to client and EcoAnalysts staff via this report. The project manager will submit a final report prior to the conclusion of the task order. All data are tracked through use of EcoAnalysts' LIMS. The data compiled during this project are incorporated into spreadsheets and sent to the client and, if requested, will be uploaded to the client's database.



GROUP D: DATA VALIDATION AND USABILITY

D1. DATA REVIEW, VERIFICATION, AND VALIDATION

All raw data are transcribed into EcoAnalysts' LIMS. Any hard copies of raw data are organized and filed. Statistical analyses of replicate samples are recorded so that the degree of certainty can be estimated, when requested. All laboratory analytical results are cross-checked to ensure data are complete and error free. Data are archived using EcoAnalysts' LIMS on EcoAnalysts' servers, with multiple data backups in place.

D2. VERIFICATION AND VALIDATION METHODS

Project staff follows the EPA *Guidance on Environmental Verification and Validation* (EPA QA/G-8) whereby the data are reviewed and accepted or qualified by project staff.

D3. RECONCILIATION WITH USER REQUIREMENTS

Upon receipt of results of each sample group, calculations and determinations of precision and accuracy are made and, if needed, corrective action is implemented. If data quality does not meet project specifications, the deficient data are flagged and the cause of failure evaluated. For the data to be considered valid, data collection procedures, the handling of samples, and data analysis must be monitored for compliance with all the requirements described in this QAPP. Data are flagged and qualified if there is evidence of habitual violation of the procedures described in this QAPP. Any limitations placed on the data are reported to the data end user in narrative form. Any limitations on data use are detailed in the project reports and other documentation.

