METHOD 1640:

EXTRACTION OF TRACE METALS IN SEAWATER AND SEDIMENT

REFERENCES:

Battelle Pacific Northwest Laboratories, Marine Sciences Laboratory. Standard Operating Procedure MSL-M-034-01. APDC Extraction of Metals in Seawater.

Bloom, N.S. and Crecelius, B.A. 1984. Determination of Silver in Seawater by Coprecipitation with Cobalt Pyrrolidine Dithiocarbamate and Zeeman Graphite-Furnace Atomic Absorption Spectrometry, *Analytica Chimica Acta*, 156, pp.139-145.

Danielsson, L., Magnusson, B., and Westerlund, S. 1978. An Improved Metal Extraction Procedure for the Determination of Trace Metals in Sea Water by Atomic Absorption Spectrometry with Electrothermal Atomization, *Analytica Chimica Acta*, 98, pp. 47-57.

Jan, T.K. and Young, D.R. 1978. Determination of Microgram Amounts of Some Transition Metals In Seawater by Methyl Isobutyl Ketone-Nitric Acid Successive Extraction and Flameless Atomic Absorption Spectrophotometry. *Analytical Chemistry*, 50, No. 9, pp. 1250-1253.

U.S. Environmental Protection Agency, Office of Water Engineering and Analysis Division (4303): Method 1640.

EPA Methods for Chemical Analysis of Water and Wastes, Method 200.8, Revision 5.4 (May, 1994); NOAA Sampling and Analytical Methods of the National Status and Trends Program, Volume III, (1993)

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for the extraction and preconcentration of dissolved and particulate elements from aqueous samples using chelation and precipitation for subsequent analysis by inductively coupled plasma mass spectrometry (ICP-MS). It includes stringent quality control (QC) and sample handling guidelines necessary to avoid contamination and ensure the validity of analytical results

during sampling and analysis. The method contains QC procedures that will ensure that any possible contamination will be detected when blanks accompanying samples are analyzed.

Table 1.

ANALYTE	SYMBOL	AMU	CASRN
Aluminum	(Al)	27	7429-90-5
Antimony	(Sb)	123	7440-36-0
Arsenic	(As)	75	7440-38-2
Beryllium	(Be)	9	744-38-2
Cadmium	(Cd)	111	7440-43-9
Chromium-total	(Cr)	52	7440-47-3
Cobalt	(Co)	59	7440-48-4
Copper	(Cu)	63	7440-50-8
Iron	(Fe)	56 or 57	7439-89-6
Lead	(Pb)	206, 207, or 208	7439-92-1
Manganese	(Mn)	55	7439-96-5
Mercury	(Hg)	202	7439-97-6
Molybdenum	(Mo)	98	7439-98-7
Nickel	(Ni)	60	7440-02-0
Selenium	(Se)	82	7782-49-2
Silver	(Ag)	107	7440-22-4
Thallium	(Tl)	205	7440-28-0
Tin	(Sn)	118	7440-31-5
Titanium	(Ti)	48	
Vanadium	(V)	51	7440-62-2
Zinc	(Zn)	66	7440-66-6

Table 1. Target analytes in seawater samples extracted from both the APDC and iron-palladium procedures. The CASRN is the Chemical Abstract Services Registry Number.

Table 2.

PARAMETER	METHOD DETECTION LIMIT (ug/L)	ACCEPTANCE RANGE (%)	REPORTING LIMIT (ug/L)
Aluminum (Al)	0.01	52-149	0.025
Antimony (Sb)	0.01	44-97	0.025
Arsenic (As)	0.01	71-112	0.025
Barium (Ba)	0.5	70-130	1

Beryllium (Be)	0.005	62-113	0.01
Boron (B)	0.5	70-130	1
Cadmium (Cd)	0.005	69-100	0.01
Calcium (Ca)	0.5	70-130	2
Chromium (Cr)	0.005	85-133	0.01
Cobalt (Co)	0.005	75-124	0.01
Copper (Cu)	0.005	72-108	0.01
Iodine (I)	0.5	70-130	1
Iron (Fe)	0.01	35-97	0.025
Lead (Pb)	0.005	56-116	0.01
Lithium (Li)	0.01	70-130	
Magnesium (Mg)	5	70-130	10
Manganese (Mn)	0.005	64-120	0.01
Mercury (Hg)	0.005	68-117	0.01
Molybdenum (Mo)	0.005	59-125	0.01
Nickel (Ni)	0.005	68-118	0.01
Potassium (K)	5	70-130	10
Selenium (Se)	0.01	55-110	0.025
Silver (Ag)	0.005	66-125	0.01
Sodium (Na)	5	70-130	10
Strontium (Sr)	0.01	70-130	0.025
Thallium (Tl)	0.005	66-92	0.01
Tin (Sn)	0.005	68-110	0.01
Titanium (Ti)	0.005	95-143	0.01
Vanadium (V)	0.005	95-140	0.01
Zinc (Zn)	0.005	62-108	0.01

Table 2. The Approximate detection limits, acceptance range, and reporting limits for the target analytes in aqueous samples.

2.0 SUMMARY OF METHODS

2.1 An aliquot of a well-mixed, homogeneous sample is accurately measured for sample processing. Target metals are chelated out of the aqueous sample using ammonium pyrrolidine dithiocarbamate (APDC). The chelated precipitate is filtered onto a membrane filter and then digested in a nitric acid solution.

2.2 An aliquot of a well-mixed, homogeneous seawater sample is accurately measured for sample processing. Target metals are co-precipitated out of the sample using borohydride Iron-Palladium reductive precipitation. The precipitate is centrifuged out of the seawater matrix. The metal-containing pellet is then digested with nitric acid.

2.3 An aliquot of sample is diluted from 10-100 times before it is acidified using HNO3 until the pH is <2. The diluted, acidified sample must sit for a minimum of 16 hours before it can be analyzed.

2.4 The sample is then analyzed using Inductively Coupled Plasma Mass Spectrometry (ICPMS) by pumping the sample through a nebulizer producing a fine spray. An argon carrier gas atomizes the sample which is ionized and detected with a mass spectrometer. Qualitative identification is based on the mass to charge ratio for each element. It is recommended that samples be analyzed within 1 day of digestion.

3.0 PREVENTION OF INTERFERENCE

3.1 Samples may be contaminated by numerous routes. Contamination by trace metals can occur due to the use of metallic or metal-containing labware (e.g., talc gloves which contain high levels of zinc), containers, sampling equipment, reagents, and reagent water during sampling. Contamination also results from improperly cleaned and stored equipment, labware, and reagents, as well as from atmospheric inputs such as dirt and dust.

3.2 The avoidance of contamination can be achieved by carrying out the following procedures:

3.2.1 Clean sample containers, nucleopore filters, and filtration apparatus with acid and rinse with Milli-Q water. Upon cleaning, store the labware in clean zip-type bags and place them in a plastic box.

3.2.2 Keep samples and glassware covered when possible.

3.2.3 Ensure all materials that come into contact with the sample are nonmetallic. Only the following materials should come into contact with samples: fluoropolymer (FEP, PTFE), conventional or linear polyethylene, polycarbonate, polypropylene, polysulfone, or ultrapure quartz. All materials that will directly or indirectly contact the sample must be cleaned or must be known to be clean and metal free before proceeding.

3.2.4 Minimize exposure of sample to an uncontrolled atmosphere.

3.3 ICPMS Interferences

3.3.1 Chemical interferences are characterized by molecular compound formation, ionization effects and solute vaporization effects. These

effects are not usually pronounced with the ICPMS technique due to the high temperature of the torch.

3.3.2 Isobaric interferences are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer. All elements determined by this method have one isotope free of isobaric elemental interference except Selenium-82, which has isobaric interference from the Krypton impurities in the Argon gas supply. This interference can be minimized by using high purity argon. All data must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest.

3.3.3 Wing overlap interferences may occur when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

3.3.4 Polyatomic interferences are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer. The ions may be formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified and are listed within the instrument data system along with the elements affected. All data shall be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. Equations for the correction of the data are presented below in Table 3.

Table 3.

ELEMENT	MASS	EQUATION
Vanadium	51	(51)*1 - (53)*3.127 + (52)*0.353
Arsenic	75	(75)*1 - (77)*3.132 + (82)*2.736 - (83)*2.761
Selenium	82	(82)*1 - (83)*1.0087
Molybdenum	98	(98)*1 - (99)*.0146
Cadmium	111	(111)*1 - (108)*1.073 + (106)*0.764

3.3.5 Physical interferences are effects associated with the sample nebulization and transport processes. Properties such as the change in viscosity and surface tension can cause significant inaccuracies, especially in samples that may contain high dissolved solids and/or acid concentrations. These interferences are greatly

reduced in this procedure by the use of mass flow controllers for the control of the argon flow rate, the use of a peristaltic pump for sample introduction, and the use of internal standards.

4.0 SAFETY

4.1 It is mandatory to wear a laboratory coat, closed-toe shoes, and safety glasses in the Laboratory. Gloves shall be worn while working with samples and acids.

4.2 All steps involving the use of concentrated acids shall be performed in a fume hood.

4.3 Material Safety Data Sheets (MSDS) are on file and available at all times to personnel using hazardous materials. It is the responsibility of everyone using these materials to be familiar with the potential hazards to the chemicals in their work area. If the analyst is uncertain of the potential hazards of specific chemicals, contact a supervisor prior to using these chemicals.

4.4 Extreme caution, awareness and knowledge of the location and safe use of fire extinguishers, eye wash fountains, and safety showers are required.

4.5 Personnel performing this procedure shall be instructed in the safe use of acids, the requirements for protective equipment, and acid spill cleanup procedures.

5.0 APPARATUS AND MATERIALS

5.1 APDC procedure

5.1.1 250 ml polyethylene screw cap extraction bottles (Nalgene)

5.1.2 Teflon forceps

5.1.3 Polycarbonate filters; 47 mm, 0.45 µm pore size (Nucleopore)

5.1.4 15 ml polyethylene screw cap centrifuge tubes

5.1.5 Sonicator with heated water bath maintained at 65 ± 2 °C

5.2 Iron-palladium procedure

5.2.1 50 ml polycarbonate tapered centrifuge tubes with caps.

5.2.2 Centrifuge system

5.2.3 Sonicator with heated water bath maintained at 65 ± 2 °C

6.0 REAGENTS

6.1 APDC

6.1.1 Cobalt nitrate stock solution, 2000 mg/L- Dissolve 2.0 g cobalt metal (Fisher Scientific, certified) in 950 ml of Milli-Q water and 50 ml HNO_3 (Optima)

6.1.2 Cobalt nitrate, 200 mg/L – Dilute 10 ml stock solution (6.1) to 100 ml with Milli-Q water.

6.1.3 Ammonium Pyrrolidine Dithiocarbamate (APDC), 2% solution (Fisher Scientific)- Dissolve 2.0 g APDC in 100 ml Milli-Q water. Store solution at 4 °C; but use at room temperature.

6.1.4 Nitric acid 10%, ultrapure (Optima, Fisher Scientific)- Mix 10 ml HNO_3 into 90 ml Milli-Q water.

6.1.5 Milli-Q water

6.2 Iron-palladium

6.2.1 Nitric acid 20%, ultrapure (Optima, Fisher Scientific)- Mix 20 ml HNO_3 into 80 ml Milli-Q water.

6.2.2 Pure iron and palladium solution made 1:1, 1000 µg/ml (SPEX).

6.2.3 Ammonium hydroxide, concentrated, ultrapure (Optima, Fisher Scientific).

6.2.4 Sodium borohydride, 5% (Fisher Scientific)- Dissolve 0.5 g sodium borohydride in 10 ml Milli-Q water. A fresh solution is made on day of extraction.

6.2.5 Ammonium Pyrrolidine Dithiocarbamate (APDC), 2% (Fisher Scientific)- Dissolve 2.0 g APDC in 100 ml Milli-Q water. Store solution at 4 °C; but use at room temperature.

6.2.6 Milli- Q water

6.3 Reagent water-Water that is free from the metal(s) that would potentially interfere at the MDL for the metals listed in Tables 1 and 2. The water is prepared by distillation, deionization, reverse osmosis, anodic/cathodic stripping voltammetry, or other techniques that remove the metals and potential interferants.

6.4 Standard Stock Solutions- purchased from a reputable commercial source (Claritas ppt, SPEX CertiPrep Inc; Plasma Cal, SPC Science).

7.0 CALIBRATION AND MAINTENANCE OF THE ICPMS

7.1 Trace metal concentrations are determined by comparing the response of a known standard obtained from a certified source traceable to NIST.

7.2 An initial calibration curve is performed on the instrument covering the expected range of concentrations in the samples before each batch of samples is run and every 12 hours during sample analyses.

7.2.1 Two different commercially available standard solutions are run at three or more concentrations. The calibration standards are diluted to the appropriate levels of the operating range using reagent water containing 1% (v/v) nitric acid.

7.2.2 The standard solutions are prepared the day the samples are run. All calibration solutions are spiked with 1 ml of internal standard solution containing 1000ng/ml of Rhodium and Thulium.

7.3 The response factor is computed as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

A_s: height or area of the response at the m/z for the analyte
C_{is}: concentration of the internal standard in the solution
A_{is}: height or area of the m/z for the internal standard
C_s: concentration of the analyte in the standard or blank solution

7.3.1 Compute the mean RF for each analyte using the individual response factors at each concentration.

7.3.2 If the RF value range is constant (<20%), the RF value is assumed to be invariant and thus used for calculations. If the range varies significantly, the results can be used to plot a calibration curve of response ratios, A_s/A_{is} vs. RF.

7.4 Calibration Verification- Initial calibration verification is performed immediately following calibration. The ICPMS is adjusted until verification criteria are met. After the criteria are met, the blanks and samples may be analyzed.

7.5.1 A second-source calibration standard at a mid-level concentration is analyzed before running the samples and then again every 20 samples.

7.5.2 Using the mean RF value, the percent recovery of each metal is obtained using the calibration curve in the initial calibration.

7.5.3 Compare the recovery of each metal with the corresponding limit for the calibration verification in Table 4. The response for the initial calibration can be used for the blanks and the samples if all of the metals meet the accepted criteria. If a value fails to meet the acceptance range, the system's performance is unacceptable for that compound. The problem should be identified and amended, or a new calibration check standard should be prepared and the test repeated.

Table 4.

METAL	CALIBRATION VERIFICATION
Arsenic	85-115
Cadmium	85-115
Copper	85-115
Lead	85-115
Nickel	85-115
Silver	85-115
Zinc	85-115

Table 4. Quality control acceptance criteria for performance tests for freshwater and effluent samples in EPA method 1640. All specifications are represented as percents. The specifications for cadmium, copper, lead and nickel were calculated from validation conducted on ambient, freshwater samples.

7.5 Tuning solution- this solution is used for mass calibration and instrument tuning before analysis. The solution is composed of the same stock solutions that are used to obtain the calibration curve. Internal standards are not added to this solution.

7.6 Continuing calibration verification (CCV)- Aliquots of multi-element stock standard is added to an aliquot of reagent water. The CCV is treated as a

sample and digested as the other samples, when applicable. The internal standards are added to the CCV as well.

7.7 An instrument maintenance schedule is maintained for the Hewlett Packard 4500 ICPMS. Dates and initials are recorded in a notebook located near the instrument.

7.7.1 The instrument is serviced by the manufacturer at least once per year.

8.0 QUALITY CONTROL

8.1 QA/QC records are maintained to document the quality of data generated. If any element falls outside the designated range, that element has failed the acceptance criteria. Failure to meet the stated requirement shall require that corrective action be taken to eliminate the problem prior to the analysis of any samples. Samples from the batch being analyzed at the time the failure is detected shall be reanalyzed after the corrective action has been taken. A batch is defined as 20 or less samples. If any sample cannot be reanalyzed, the result for that element shall be flagged and a detailed report is included with the result.

8.1.1 **Lab Blanks-** Two process blanks (reagent blanks), where Milli-Q water is treated as a sample, are run with each batch of samples (15 or less samples). The process blanks are used to assess if there is any internal contamination in the instrument. No element shall be detected at greater than 3 times the method detection limit. A rinse blank is used to flush out the instrument between samples to avoid contamination between samples.

8.1.2 **Field Blanks-** At least one field blank consisting of distilled water in a similar container as the sample container is transported to the sampling site. The blank is exposed to the environment while the actual samples are being collected.

8.1.3 **Matrix Spikes-** A matrix spike and matrix spike duplicate shall be analyzed with each batch of samples to determine precision for each element. A control chart is generated to document the precision. The relative standard deviation for all elements combined shall be within 15% and no single element shall be greater than 20% for those elements that are greater than 10 times the method detection limit.

8.1.4 **Duplicate Samples-** Each sample is extracted and analyzed in duplicate. If the duplicates are not in agreement, then the sample is re-extracted and reanalyzed.

8.1.5 **CRM/LCM-** Certified reference materials and/or lab control materials shall be analyzed with each batch of samples to evaluate accuracy for each element. The reported value shall be within 15% of the true value.

8.1.6 **Initial Calibration Check-** Prior to analyzing any samples, an initial calibration of the instrument is performed with each batch of samples (15 or less). This calibration shall be within 15% of the initial calibration curve (see Section 7.2).

8.1.7 **Internal Standards-** Internal standards shall be added in known amounts to blanks, calibration standards, continuing calibration verification solutions, and samples to compensate for instrumental drift. Elements that may be used are presented in Table 5. Relative response factors are used to correct responses of the target analytes.

Table 5. Internal Standards

INTERNAL STANDARD	MASS
Scandium (Sc)	45
Yttrium (Y)	89
Rhodium (Rh)	103
Terbium (Tb)	159
Thulium (Tm)	169
Bismuth (Bi)	209

9.0 SAMPLE COLLECTION, PRESERVATION AND HANDLING

9.1 Sampling personnel are required to wear clean, nontalc gloves at all times when handling sampling equipment and sample containers.

9.2 Before samples are collected, all sampling equipment and sample containers are cleaned by soaking in a 10% nitric acid solution for a minimum of 24 hours followed by five rinses with Milli-Q water. The bottles are capped and individually double-bagged and placed in a clean plastic box.

9.3 Sample bottles are opened only to collect the seawater sample and to add acid preservative. Samples are preserved (at the sampling site or upon

return to the lab) with 1 ml of Optima concentrated nitric acid per liter of sample that will bring the pH ≤ 2.

9.3.1 Samples must be acidified within 48 hours of sampling at 4 ± 2 °C until acidified.

9.3.2 Once acidified, the samples must sit for at least 48 hours allowing the acid to completely dissolve the metals absorbed on the walls of the container.

9.3.3 The sample must have a pH<2. If the pH of the samples is >2, more acid will be added and they must sit for 16 hours. The pH must be verified to be <2 before analysis.

9.4 With each sample set, preserve a field blank, a method blank and a CCV (continuing calibration check) in the same way as the sample(s).

10.0 PROCEDURE

10.1 APDC METHOD

10.1.1 Before a sample can be processed, the sample pH should be verified/adjusted to pH ≤ 2. Do not measure the pH directly from the original sample; rather, pour a small aliquot of sample into a separate container to verify the pH.

10.1.2 Transfer a 200 ml aliquot from a well-mixed, acid preserved sample to a pre-calibrated polyethylene bottle.

10.1.3 To the 200 ml sample add 1 ml of 200 mg/L cobalt nitrate solution. Cap the bottle and mix by shaking. Let the solution stand for 2 minutes.

10.1.4 Remove the cap, add 1 ml of 2% APDC and re-cap the bottle and shake gently for 1 minute. This mixture is set aside to react for a minimum of 30 minutes.

10.1.5 The mixture is filtered through an acid cleaned 0.45 µm Nucleopore filter using an acid cleaned Millipore vacuum filtration system. The Nucleopore filter is handled with Teflon forceps.

10.1.6 The empty polyethylene bottle that once contained the sample is subsequently rinsed with 5 ml of Milli-Q water (acidified to pH 2.0 with Optima HNO_3) and this is filtered through the Nucleopore filter.

This is repeated 2 times. Finally, the Millipore vacuum filtration cup is rinsed with an adequate amount of acidified Milli-Q water to ensure any particles sticking to the sides are rinsed onto the filter. This final step also ensures that all seawater matrix has been rinsed through the filter.

10.1.7 The filter containing the filtrate is removed from the filtration system and folded into quarters ensuring no contact is made with the filtrate. This is then inserted into a 15 ml acid cleaned centrifuge tube.

10.1.8 Add 2 ml of 10 % Optima nitric acid into the centrifuge tube containing the filter using a clean pipette.

10.1.9 The tube containing the filter is placed in a sonicator with a water bath maintained at 65 ± 2 °C and allowed to digest for 2 hours.

10.1.10 Dilute sample (with filter) by adding 8 ml Milli-Q water, re-cap and allow to cool. The sample is now ready for analysis by ICP-MS.

10.1.11 The extracts can be stored at 4 ± 2 °C until analysis; however, they should be analyzed as soon as possible after the extraction.

10.1.12 Internal standards are added just prior to analysis by ICP-MS.

10.2 IRON-PALLADIUM METHOD

10.2.1 Before a sample can be processed, the sample pH should be verified/adjusted to pH ≤ 2. Do not measure the pH directly from the original sample; rather, pour a small aliquot of sample into a separate container and verify the pH.

10.2.2 Transfer a 50 ml aliquot from a well-mixed, acid preserved sample to an acid cleaned 50 ml polycarbonate centrifuge tube.

10.2.3

10.2.4 To the 50 ml sample add 0.5 ml 1:1 1000 µg/ml of Fe/Pd solution with a clean pipette.

10.2.5 Next, add 0.3 ml ammonium hydroxide to the sample and mix by shaking.

10.2.6 Then add 0.5 ml 5% $NaBH_4$ solution, 0.25 ml 2% APDC solution and mix by shaking. This sample is set aside to react for a minimum of 1 hour.

10.2.7 After a minimum of 1 hour, the sample is centrifuged at 2500 rpm for 30 minutes. Upon completion, the seawater matrix is carefully decanted and discarded.

10.2.8 To the remaining pellet, add 1 ml 20% Optima nitric acid with a clean pipette.

10.2.9 The centrifuge tube is placed in a sonicator with a water bath maintained at 65 ± 2 °C and allowed to digest for 2 hours or until all precipitate is dissolved.

10.2.10 After the digestion is complete and the extract(s) have reached room temperature, the sample(s) can be stored at 4 ± 2 °C until analysis by ICP-MS; however, they should be analyzed as soon as possible. Internal standards can be added just prior to analysis by ICP-MS.

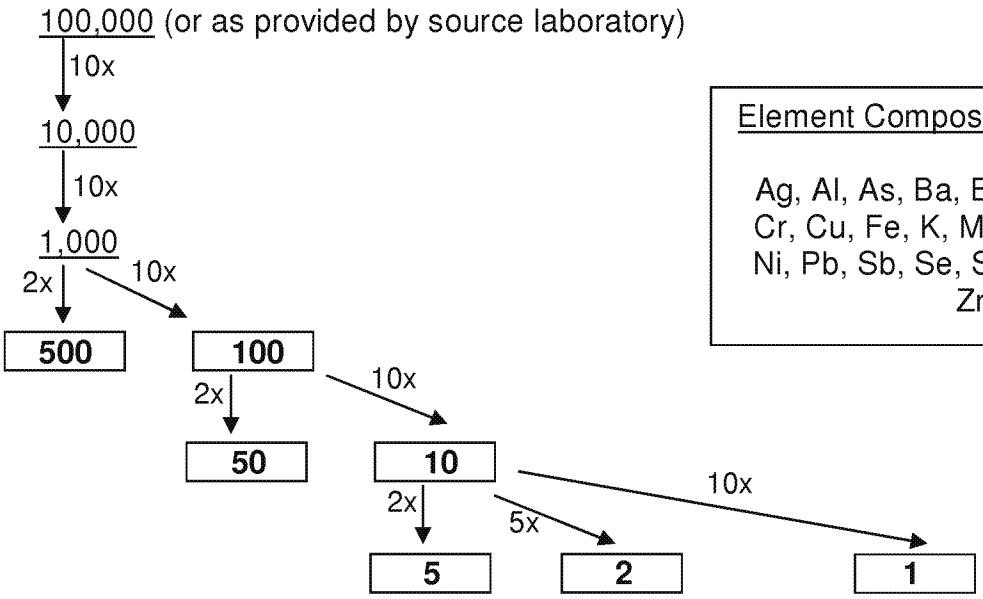
10.3 Sample Analysis- Standards are prepared by serial dilutions before each run on the ICPMS. The pipettes and autosampler tubes used for analysis and standards are calibrated before standards are run. Pipettes are calibrated by weighing 1.000 ml of DI water (=1.000 gram) and calibration marks on the autosampler tubes are checked.

10.3.1 Use volumetric flasks and calibrated pipettors to make calibration standards by diluting 10 mL of a commercially prepared stock solution to 100 mL. The standard source laboratory and lot number of each of the standards used for each ICPMS run are recorded in the laboratory notebook.

10.3.2 Mix standards by inverting and shaking a minimum of 10 times. A dilution stock of 2% HNO_3 and 1% HCl is used for all dilutions. Prepare 5 concentrations of calibration standards ranging from the method detection limit to at or above the maximum expected concentration in the sample. The standards in the boxes below are used in the calibration curves(see diagrams on pages 16 and 17).

GENERAL CALIBRATION MIX (ng/ml) DILUTIONS

FOR LOW CONCENTRATION SAMPLES

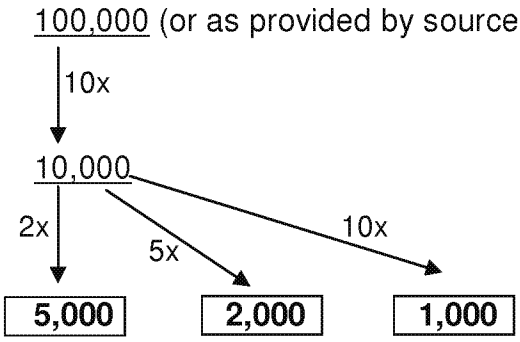


Element Composition of Low Mix:

Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, Zn

GENERAL CALIBRATION MIX (ng/ml) DILUTIONS FOR HIGH CONCENTRATION SAMPLES

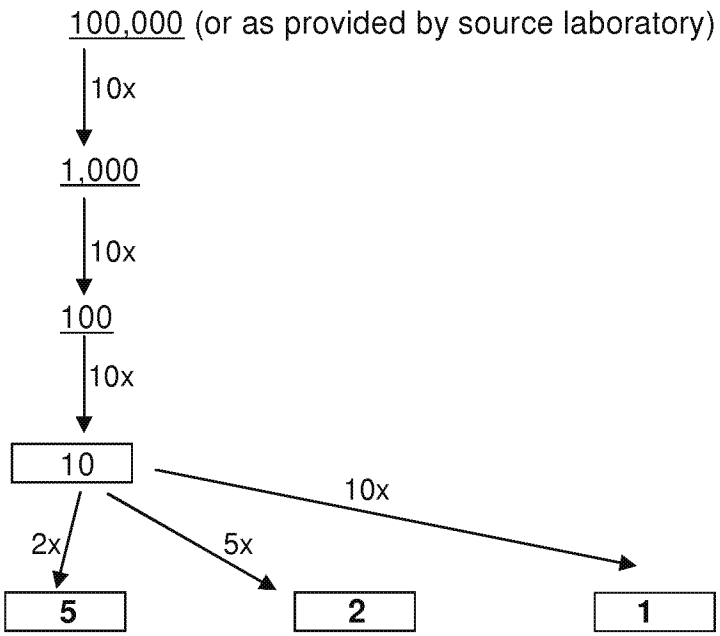
(continuation of calibration mix of low concentrations)



Element Composition of High Mix:

Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sr, Ti, V, Zn

CALIBRATION DILUTION FOR MERCURY STANDARDS (ng/ml)



10.3.3 Instrument parameters are stored in the computer program that operates the ICPMS. These parameters are listed in Table 7.

10.3.4

Table 7. Instrument Parameters

PARAMETER	SETTING
RF Power	1350 watts
Acquisition Mode	Spectrum Analysis
Detector Mode	Auto
Acquisition Points/Mass	3
Acquisition Repetitions	3
Argon Flow Rate	16 L/min
Nebulizer	Concentric
Sample Uptake Rate	0.4 mL/min
Sample Uptake Time	90 sec
Pump Stabilization Time	45 sec
Rinse Time	15 sec
Carrier Gas Flow Rate	1.26 L/min
Auxilliary Gas Flow Rate	1.0 L/min
Spray Chamber Temperature	2 °C
Sample Depth	7.5 mm[18]

10.3.5 Fill the autosampler rinse container with deionized water.

10.3.6 Empty the spray chamber drain bottle and fill to approximately 1/4 full with tap water. It is important that the drain line from the spray chamber be immersed in water to prevent fluctuations in the plasma.

10.3.7 Turn on the argon gas supply.

10.3.8 Ignite the plasma and allow a minimum of 30 minutes for stabilization.

10.3.9 Check the system operating conditions by tuning the instrument according to the parameters listed in Table 8. If parameters do not fall within these limits, retune the instrument per manufacturer's procedures. Once you are satisfied with the tune, save the parameters and print out a copy for the laboratory notebook.

Table 8 Optimal Tune Results

PARAMETER	OPTIMAL RESULT
Sensitivity for AMU 2	7,000 counts
Sensitivity for AMU 89	15,000 counts
Sensitivity for AMU 205	10,000 counts
RSD for AMU 2, 89, & 205	< 5%
Pulse to Analog Factors	100 ± 1
Doubly Charged Ions	< 3%
Oxides	< 1%
Axis	± 0.05 AMU
Peakwidth	0.65 - 0.75 AMU at 10%

10.3.10 Load the appropriate method file into the Chemstation data system. Complete the sample sequence table with the specified sample information and dilution factors. Load the samples into the autosampler according to the order listed in the sequence file. Double check to make sure the standards, blanks, and samples are in the correct autosampler position assigned in the sequence file.

NOTE: The instrument may be set for automatic shutoff at the end of the sequence by adding the following command in the last line of the sequence file:

TYPE = Keyword

KEYWORD = Command

KEYWORD COMMAND = tune ”macro`shutdown.mac’,go”
(this must be typed exactly as written here)

10.3.11 Start the analytical sequence and make sure that it is operating properly.

11.0 CALCULATIONS

11.1 For water samples, concentration factors necessary for the subsequent ICP-MS analyses are calculated by dividing the original seawater volume by the final digestate volume.

Southern California Coastal Water Research Project Toxicology Laboratory Standard Operating Procedure for Mussel Embryo Development Test

I. Overview

This method estimates the toxicity in aqueous samples by a 48 hour exposure of *Mytilus galloprovincialis* embryos. The test endpoint is normal embryo development and survival. The test is based on methods in the EPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (EPA/600/R-95/136). The purpose of this SOP is to detail the test procedure as specifically applied in our laboratory. The SOP is intended to supplement the material in the protocol, not replace it.

II. Supply Checklist

- Deep trays for use as water baths (2)
- Glass bread pan (2)
- Seawater and DIW squirt bottles
- pH, DO and conductivity meter/probes
- Graduated cylinders 50-1000 ml for making gamete and solution dilutions
- Automatic pipets 0.1 ml up to 10 ml
- Water pump
- Tubing
- Thermometer
- 250 ml, 400 ml and 1 L beakers (several)
- Inverted microscope
- Counter, 2 unit
- Sedgwick-Rafter counting chamber
- Perforated plunger to fit 250 ml, 400 ml and 1 L beakers
- Nitex screening 100 µm or smaller openings
- Razor blades
- Eppendorf Pipet tips (100 µl, 1 ml and 10 ml)
- Shell vials with translucent caps, 5 dram
- Formalin, 30% borax buffered (see recipe below)
- Dispenser for formalin to repeatedly deliver 1 ml
- Pasteur pipets and bulbs (both 5 ¾ and 9 in)
- Scintillation vial racks (plastic for exposure, cardboard for storage)
- Spawning and gamete calculation data sheet
- Glass or Fiberglass aquaria tanks (3)
- Air pump
- Pairing knife.
- Air stones
- UV Light –to pass seawater through

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III. Animals Collection and Culturing

Adult Bivalves (*Mytilus galloprovincialis*) are obtained from a commercial supplier. Set up glass aquaria in the cold room. To each tank add about six inches of seawater the day before the mussels arrive. Temperature shock may cause the animals to spawn; therefore once you have received the mussels, the animals should be acclimated to the cold room by opening the travel cooler. After about two hours of acclimation, transfer the mussels equally among the tanks, and add air stones.

The seawater should be changed everyday. Mussels can remain in holding under optimal conditions up to eight weeks from receiving date. No food is given to the mussels while in holding. Water quality measurements (pH, ammonia, DO and salinity) should be made on the system on a weekly basis.

IV. Test Design

Summary of test conditions

Type: Static non-renewal
Salinity: 32 ± 2 g/kg
Temperature: 15 ± 1 °C
Duration: 48 hours
Endpoint: normality of development and survival
Exposure volume: 10 ml
Test containers: 29.35 x 55 mm (5 dram) glass shell vial with snap cap.
Lighting: Ambient laboratory
Photoperiod: 16 hours Light and 8 hours Dark
Salinity adjustment: Hypersaline brine
Dilution water: natural seawater (activated carbon and 0.45 µm filtered)
Water Quality: DO, pH, salinity and ammonia (optional)
Reference toxicant: concurrent with each experimental batch, ammonia chloride or copper chloride

Exposures should be conducted in 5 dram glass shell vials. The vials should be vigorously rinsed with DIW and allowed to dry before use. Vials should be labeled and randomly distributed in vials racks (based on our experiment set-up randomization program).

The sample volume is 10 ml per replicate, with 4 replicates per concentration. Include an additional 5 vials of 32 ‰ seawater to determine the actual embryo density. After the samples are in the vials, the vials should be placed in the 15 °C room for at least ½ hr before starting the exposure. The vials should be kept covered with parafilm whenever possible from the time of labeling through the end of the exposure to prevent cross contamination and evaporation.

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V. Sample Handling

Care should be taken during sample preparation and dilution that cross contamination of glassware used for the samples and for the gametes does not occur. The exposure vials should be covered at all times to prevent contamination.

Samples having a salinity of less than 30 ‰ should be adjusted using hypersaline brine. To make the brine, first place a glass container (usually a 1 L beaker or 1 Gal jar) of seawater in a freezer for at least 18 hr. Remove the container from the freezer and allow the ice to thaw at room temperature. During the thawing process, occasionally pour off the thawed brine to a clean beaker. When the salinity of the brine is close to the desired level, or the volume needed is achieved, final dilution of the brine to the desired level should be made using seawater. The salinity of the brine used for sample adjustment should never exceed 80 ppt, as higher levels have been known to cause toxicity. When testing samples that have no saline content (stormwater, sewage effluent, etc) it is usually desirable to make the brine at 64 ‰ so that a 50:50 mixture of sample and brine has a final salinity of 32 ‰. We have found that brine may be stored in the refrigerator for up to a week.

Water quality measurements are made at the beginning and end of the testing time. Separate sub-samples for water quality analysis of each test sample or dilution should be taken at the time the samples are prepared. Samples should be measured for pH, DO and salinity. Ammonia analysis should be considered optional.

VI. Reference Toxicant

Each test of field or laboratory samples should include a concurrent reference toxicant exposure to ammonia. Copper can be used as an alternative reference toxicant. The reference toxicant exposure should include a control (0 µg/L) and five concentrations of ammonia.

The ammonia concentrations are prepared with ammonium chloride. The ammonia concentrations tested should be 0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L. First prepare a stock solution of 1000 mg/L ammonia with 0.297g of NH_4Cl and 100 mL DIW. Then use the stock solution to achieve these concentrations by adding 0.2, 0.4, 0.6, 0.8, and 1.0 mL of stock solution to seawater to make 100 ml of each concentration. An ammonia sample will also be measured from each concentration on day 0 in order to calculate the actual total and unionized ammonia concentrations. An extra vial for each concentration should be included at test initiation for water quality analysis at test termination.

The copper reference toxicant concentrations are prepared by first making a stock solution of 10,000 µg/L copper. This stock solution consists of 0.0268 g $CuCl_2 \cdot 2 \cdot H_20$ in 1 L DIW. A working stock is prepared by diluting 10 ml of stock solution into 90 ml of seawater to produce a concentration of 1,000 µg/L. These concentrations are achieved by

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adding 0.45, 0.65, 0.95, 1.39, 2.04, and 3.00 mL of working stock to seawater to make 100 ml of each concentration. An approximately 40 ml sample of the highest concentration should be saved in a plastic container for copper concentration verification. This sample should be preserved by adding two drops of concentrated, redistilled nitric acid then storing it in the refrigerator.

VII. Test Procedure

A. Before Spawning Mussels

Fill about half full with 32‰ seawater two deep trays and heat with an aquarium heater to 20 °C. Place both bread pans and the pump with tubing in one of the trays. With seawater, rinse about fifteen 250 ml beakers and fill with 75 mL of seawater at 15 °C.

Gently scrap off the barnacles and other encrusting organisms with a pairing knife from twenty mussels. Then rinse animals with 32 ‰ seawater.

B. Mussel Spawning

Place the animals into bread pans in the 20 °C seawater bath. Turn on the water pump so that there is flow in each pan. Note initial time of mussel addition, look for spawning mussels, after 30 min. stop the pump. Wait 15 min. If no spawning occurs place the mussels in a 15 °C, 32 ‰ seawater bath for 15 min. then start the process again. At least two animals of each sex with good gamete quantity and quality are necessary.

C. Gamete Collection

When individual animals are observed shedding gametes, remove them from the pan. Rinse each animal individually thoroughly with 32 ‰ seawater and place in their own 250 mL beaker that has enough seawater to cover the animal at 15 °C.

Early in the spawning process, using a clean Pasteur pipet mix up the eggs in the beaker from one female and transfer about 0.5 ml of egg solution to the rafter cell. Check the eggs on the microscope at 100X power. Greater than 90% of the eggs should be round, of average size, not clumped, and not containing germinal vesicles. If the eggs appear to be of good quality, add a very small amount of sperm to the eggs in the Rafter cell. Watch for motility of the sperm and the ability to fertilize. Continue checking so that all of the males and females are tested in this manner.

D. Egg Counting

Allow the eggs of the females that were deemed to be in good condition to settle to the bottom of their collection beakers. Pour off most of the water from each

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beaker, then pour the remaining water with the eggs through the 100 um nitex screen into a 1 L beaker. After adding the eggs from all the "good" females, bring the water level in the beaker up to about 600 ml. Allow the eggs to resettle (about ½ hr. After the eggs have settled, again pour off most of the water, then again pour the eggs through the nitex into a clean 1 L beaker. Again bring the water up to about 600 ml.

Put 9 ml of seawater into each of two scintillation vials, labeled A and B. Using the perforated plunger mix the egg solution well and take a 1 ml sample and place it into vial A. Mix vial A well and take 1 ml sample from it and place in vial B. Mix vial B well and place a 1 ml sample onto the Rafter cell. Count all of the eggs on the Rafter cell on a microscope a 100X. If total count is less than 30, then use vial A for counting. Record the count in the appropriate place on the egg and sperm count form. Take a second sample from vial B and count. Record the second count. If the two counts are within 20% calculate the mean. If the counts are not within 20%, count one more sample before calculating the mean. The egg density target should be about 5000-8000 eggs/ml. This is a stock solution, so if the egg density is higher or lower it is ok; just use the actual value when calculating the embryo density. Density must not be less than 1500. If the density of the eggs is less than 1500, let the eggs settle and decant excess water. Recount the eggs as described above.

E. Sperm suspension

Filter high quality sperm through a 100 um nitex screen into one beaker and make a note as to which animals were used on the mussel spawning data sheet.

F. Trial fertilization test

A trial fertilization must be performed with each spawning event. A series of sperm dilutions will be performed to achieve final sperm to egg ratio. Use a 10 mL pipet with the tip cut off to place10 mL of egg suspension into three scintillation vials. Add 0.1, 0.3, and 1.0 mL of sperm suspension using pipets. Let these solutions sit for 1.5 –2.5 hours in the lab. Transfer about 0.5 ml of egg solution to the rafter cell. Check the eggs on the microscope at 100X power. Fertilized eggs will have a single polar body, a very small clear circle attached to an egg, or they will have multiple cells that look like Mickey Mouse ears. Use the ratio of egg to sperm that has the lowest amount of sperm to achieve >90% fertilization.

While the eggs are being fertilized, finish the egg counts and determine the eggs/mL concentration. (See Mussel Spawning Datasheet)

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To calculate the sperm suspension volume necessary to add to the egg solution, take the volume of the egg suspension prepared in section D and multiply by the sperm to egg ratio determined in the trial fertilization.

G. Test Initiation

Add sperm to eggs (embryo suspension), and use the perforated plunger to mix the suspension. Adjust the embryo suspension density to 1500 – 3000/ ml. Our target density and volume for the embryo suspension is 2500 embryos/mL in 300mL of 32 ‰ seawater. (See Mussel Spawning Datasheet) Achieve this by measuring out the needed amount of embryo stock solution and add 15 °C seawater to a total value of 300mL. Use the perforated plunger to mix the suspension. Cover the beaker with parafilm and set aside until ready to use (do not let stand for more than one hour).

On the mussel spawning record form record the time that you will add the embryo solution to the first vial. Using the perforated plunger, continually agitate the embryo solution while adding 0.1 ml to each exposure container. Be careful to insure that the embryo solution is added to the liquid in the exposure containers and does not contact the side of the vials first. Record the time that you finish the embryo addition. Recover the vials with the parafilm. Record the temperature at which the exposure is being performed.

The 5 additional vials of seawater will serve as the initial embryo density sub-samples. One mL of 30% borax buffered formalin will be added to each vial within minutes of the embryo solution addition. These will be used to determine the survival in the controls and the other treatments. Record the counts on the embryo count form. Calculate the actual embryo density by averaging the 5 sub-samples.

48 hours after the start of the addition of embryos, transfer the racks of exposure vials to the Biology Lab. Terminate the test by adding 1 ml of 30% borax buffered formalin to each vial. This should be done inside a fume hood. The formalin should be dispensed from the re-pipettor. Secure a snap cap on each vial and give the vial a quick swirl to insure that the formalin is evenly distributed. This task is made easier with two people; one adding the formalin and the other capping and swirling the vials.

VIII. Microscopic Evaluation

The samples can be evaluated whenever convenient. There is not a known maximum holding time for preserved samples.

The samples are evaluated by placing the entire vial in a small petri dish and placing this over the objective port on the stage of the inverted microscope. The embryos are easily viewed at 100 X. Start at the top of the vial and move across to the opposite side, scoring

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all "D" shape embryos as normal and those without the "D" shape as abnormal. Move the stage down one field of view and make another complete pass of the vial; continue this process until the entire vial has been counted. Record the results on the mussel embryo development examination data sheet and put a colored dot on the cap to designate it as counted.

IX. Data Analysis

There are three endpoints that can be analyzed. One endpoint is the percent normal. In this case the number of normal embryos is divided by the total number of normal and abnormal embryo present in a vial then multiplied by 100. A second endpoint is percent normal alive, which is the number of normal embryos present in the vial divided by the mean of the initial count multiplied by 100. The third endpoint is percent alive. In figuring the percent alive one assumes that if embryos are present, no matter what condition, then they are alive. To calculate percent alive sum of both the normal and the abnormal embryos and divide by the mean of the initial count of embryos multiplied by 100.

Enter the endpoint data into the Excel spreadsheet by container number. The means and standard deviations are calculated automatically by the spreadsheet. For each experiment, run an ANOVA and Dunnett's test using Toxstat. Use a point estimation program (such as Toxstat) to calculate the EC50 using the probit method.

The reference toxicant data are similarly entered in the appropriate Excel spreadsheet. Calculate the EC50 as above and plot this value on the running laboratory control chart for this bioassay.

X. Quality Assurance

Test Acceptability Criteria

Mean normal development in the controls must be at least 90%. Mean survival in the controls must be > 50%. The percent minimum significant difference (MSD) must be less than 25%.

Reference toxicant results

The reference toxicant EC50 should fall within two standard deviations of the mean on the control chart. If the EC50 falls outside this range, results of concurrent tests should be examined carefully. The investigator should include a discussion of the significance of the exceedance in any report of the data.

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Deviations from test conditions

Deviations from acceptable test conditions must be recorded (i.e. temperature out of range). Best professional judgment will be applied to determine whether the deviation was significant enough to render the results of the test questionable. The investigator should include a discussion of the significance of the deviation in any report of the data.

XI. Cleaning procedures

The exposure vials are used as shipped except that they should be vigorously rinsed with DIW and allowed to dry before use. All glassware and plastic ware used in handling the gametes or samples should be processed under the normal toxicology lab cleaning procedure to remove metals and organics.

After it is decided that the embryo samples can be discarded, the vials should be emptied into the sink under a fume hood with running water. The vials should then be rinsed once with tap water and then discarded in the trash. To prevent injuries from broken glass, it best to accumulate the discarded vials in a separate trash bag and then discard directly to the dumpster.

XII. References

USEPA, 1995. "Short-term methods of estimating the chronic toxicity of effluents and receiving water to west coast marine and estuarine organisms. National Exposure Research Laboratory, Office of Research and Development. Cincinnati, Ohio.

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Mussel Spawning Data Sheet

Experiment No. _____ Animal Source _____
Date _____ Time in Culture _____
Temperature of Water Bath _____

Mussel No.	Induction	Spawn	Sex	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				

Pooled eggs from mussels _____
Pooled sperm from mussels _____

Egg Counts

Sample	Dilution	Count	Eggs/mL

For 300 mL of embryo suspension at 2500 embryos/mL use:
300 x 2500 / (counted eggs/mL) = mL of egg stock

750000 eggs / _____ eggs/mL = _____ mL of egg stock

Time of embryo addition _____

Mussel Bioassay Number_____
EMBRYO EXAMINATION

Initials	Count Date	Vial Number	Sample	Normal	Abnormal	% Normal Alive

MARINA DEL REY HARBOR SITE-SPECIFIC OBJECTIVE (SSO) STUDY

WATER SAMPLING METHODS

Southern California Coastal Water Research Project

April 9, 2015

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BACKGROUND

This document describes methods for the collection and field processing of water samples from Marina del Rey Harbor for toxicity and chemical analyses. The data resulting from these analyses will be used to characterize variations in harbor water quality, calculate Water Effects Ratios for copper, and apply the Biotic Ligand Model to predict copper toxicity.

SAMPLE COLLECTION

Water samples will be collected from single or multiple depths (depending on study task), including one meter above the sediment surface and one meter below the water surface. Provisional sample locations include 11 stations that represent the central areas of each harbor basin and portions of the main access channel (Figure 1, Table 1). Upon station occupation, sampling and processing will occur in the following order: 1) record station location and general conditions in log, 2) collect water samples, 3) process samples, 4) measure water quality parameters (grab samples or profiles), and 5) record sampling data in log.

Water samples will be collected using a peristaltic pump fitted with Teflon-lined tubing. Once the tubing intake is at depth, the pump will be turned on to allow tubing to be flushed. After flushing, two liters of site water will be collected in clean 1 liter fill bottles. One 1 liter bottle will be used for toxicity analysis; this sample should be sealed promptly and transferred to an ice chest for storage. Subsampling for subsequent processing of trace metals and organic carbon will come from the fill bottle. The water in the fill bottle will be swirled to homogenize the sample before transferring to the filtering apparatus or sample containers. A "clean hands-dirty hands" technique (see below) will be employed to minimize contamination of samples. Of the two liters of water that will be collected 50 ml will be filtered for dissolved metals and 50 ml more for DOC analysis. All samples will be placed in dark coolers with wet ice for temporary storage. The field crew will not add any preservatives to the samples.

In order to reduce potential contamination, sampling personnel will adhere to the following rules:

- No smoking.
- Do not eat or drink during sample collection.
- Do not breathe, sneeze or cough in the direction of an open sample bottle.

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- Each person on the field crew will wear clean clothing that is free of dirt, grease, or other substances that could contaminate the sampling apparatus or sample bottle.

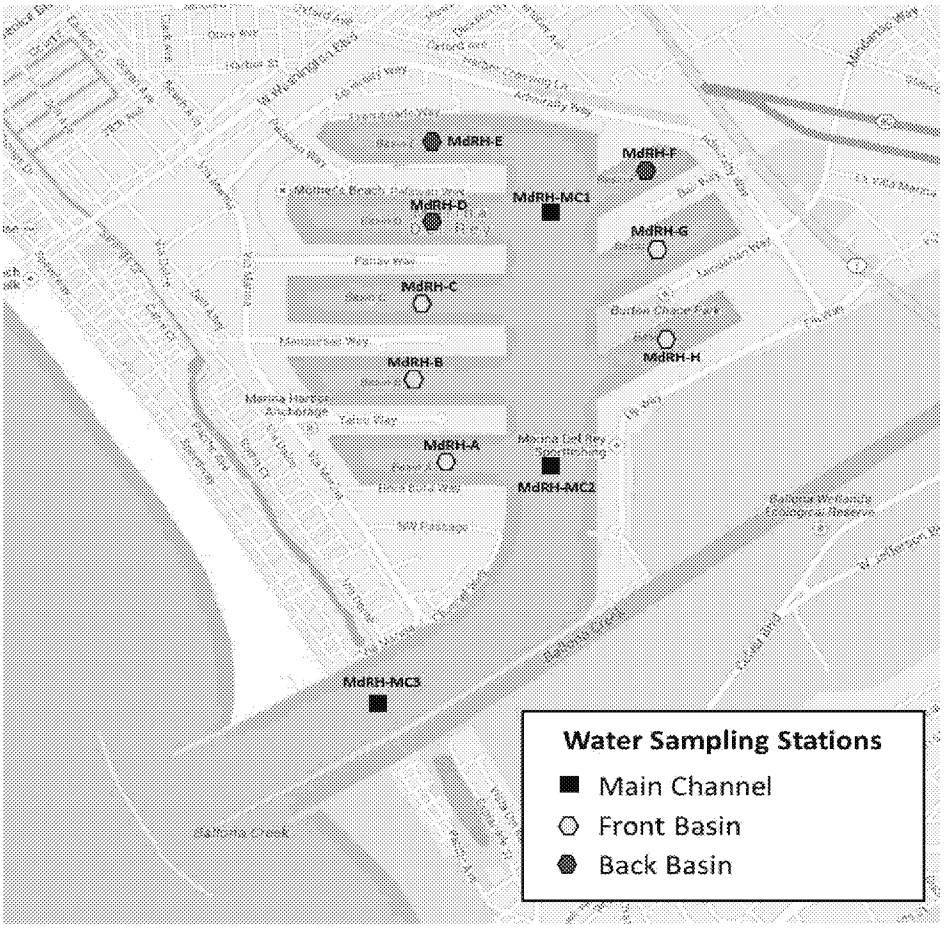

Figure 1. Sampling locations for the study.

Table 1. Sampling locations.

Station ID	Description	Latitude	Longitude
MdRH-MC1	Main Channel, end	33.98054	-118.44819
MdRH-MC2	Main Channel, middle	33.97231	-118.448
MdRH-MC3	Main Channel, entrance	33.96427	-118.455
MdRH-A	Front Basin A, middle	33.97251	-118.45284
MdRH-B	Front Basin B, middle	33.97514	-118.45346
MdRH-C	Front Basin C, middle	33.97773	-118.45372
MdRH-D	Back Basin D, middle	33.98022	-118.45356
MdRH-E	Back Basin E, middle	33.98301	-118.45338
MdRH-F	Back Basin F, middle	33.98198	-118.44502
MdRH-G	Front Basin G, middle	33.97939	-118.44435
MdRH-H	Front Basin H, middle	33.97635	-118.44409

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CLEAN SAMPLE HANDLING TECHNIQUES

To prevent contamination of samples, clean metal sampling techniques using USEPA protocols outlined in USEPA Method 1669 will be used throughout all phases of the sampling laboratory work, including equipment preparation, sample collection, and sample handling, storage, and testing. Filled sample containers will be kept on ice or refrigerated until receipt at the laboratory.

The protocol for clean metal sampling, based on USEPA Method 1669, is summarized below:

- Samples are collected in clean sample vials or bottles with any tubing specially processed to clean sampling standards.
- At least two persons, wearing clean, powder-free nitrile or latex gloves at all times, are required on a sampling crew.
- One person, referred to as "dirty hands", opens only the outer bag of all double-bagged sample bottles.
- The other person, referred to as "clean hands", reaches into the outer bag, opens the inner bag and removes the clean sample bottle.
- Clean hands rinses the bottle at least two times by removing the bottle lid, filling the bottle approximately one-third full, replacing the bottle lid, gently shaking and then emptying the bottle. Clean hands then collects the sample by filling the bottle and replacing the bottle cap.
- After the sample is collected, the sample bottle is double-bagged in the opposite order from which it was removed from the same double-bagging.
- Clean, powder-free gloves should be changed whenever something not known to be clean has been touched.
- The time of sample collection is recorded on the field log sheet.

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SAMPLE PROCESSING

Sample Filtration

A 50 ml plastic syringe with a 0.45 µm filter attached will be used to collect and filter the dissolved metals sample in the field. Each filter apparatus is placed in zip-lock plastic bags and double bagged for storage. The filter equipment will be tested for Cu contamination with a field blank. Use one syringe per station. Maintain clean sampling techniques at all times. Filter the dissolved organic carbon and dissolved Cu samples first before collecting the total metals sample. Double bag each sample container after collection and place it in wet ice for storage until delivery to the analyzing laboratory. The samples will be preserved by the analyzing laboratory.

Dissolved Organic Carbon

To collect the sample for dissolved metals first filter the water sample into the clean container provided. Collect the dissolved metals sample using clean sampling techniques. Remove the syringe and filter from the bag screw the filter tightly onto the tip of the syringe. Next, mix the water sample, fill the syringe with sample, and put the tip of the syringe with the filter into the dissolved metals container. Push the sample through the filter taking care not to touch the inside surface of the sample container with the apparatus.

The sample volume for dissolved organic carbon analysis needs to be 50 ml (Table 2). If the filter becomes clogged prior to generating 50 ml of sample, remove and dispose of the used filter and replace it with a new clean filter. Continue to filter the sample. When 50 ml have been collected, cap the sample container tightly and store on ice for delivery to the analysis laboratory. This sample needs to be kept in the dark to prevent sample degradation from exposure to sunlight. The analysis method and detection limits for dissolved organic carbon can be found in Table 3.

Dissolved Metals

A 50 ml sample is needed for dissolved metals analysis (Table 2). Filter the sample using the methods described above. If the filter becomes clogged prior to generating 50 ml of sample,

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remove and dispose of the used filter and replace it with a new clean filter. Continue to filter the sample. When 50 ml have been collected cap the sample container tightly and store on ice for delivery to the analysis laboratory. Filtration must occur within 15 minutes of sample collection. The analysis method and detection limits for dissolved copper and zinc can be found in Table 3.

Total Metals

The total metals sample does not need to be filtered. Using clean handling techniques, transfer 250 ml of sample to a 250 ml plastic bottle. Double bag the sample and place it on ice after collection. The analysis method and detection limits for total copper and zinc can be found in Table 3.

Table 2. Sample volumes and storage containers.

Analysis Type	Volume (ml)	Container Type
Dissolved organic carbon	50	PE tube (50 ml)
Dissolved metals	50	PE tube (50 ml)
Total metals	250	Pre cleaned HDPE bottle (250ml)
Toxicity	1000	HDPE bottles

Table 3. Analysis methods, method detection limits (MDL) and reporting limits (RL).

Analyte	MDL (µg/L)		RL (µg/L)		Analysis Method
	Total	Dissolved	Total	Dissolved	
Organic Carbon	NA	50	NA	500	SM 5310B
Copper	0.15	0.15	0.15	0.15	EPA 1640 – FePd
Zinc	0.15	0.15	0.15	0.15	EPA 1640 – FePd
Toxicity	NA	NA	NA	NA	USEPA 1995

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FIELD MEASUREMENTS AND OBSERVATIONS

Field measurements will be collected and observations will be made at each sampling site after a sample is collected. Field measurement results and field observations will be recorded on a log sheet similar to the one presented in Table 4. Field measurements will include probe calibration results, water temperature, and station depth. Water quality profile measurements will be saved in digital form and returned to the laboratory for post processing and entry into the sample event records. Profile measurements will be collected using an YSI multi-probe meter at approximately 0.5 m intervals. Prior to each sampling event, water quality sensors for conductivity and pH will be calibrated using fresh calibration solutions. A two-point calibration will be used. After each calibration, the sensor will be checked to verify the accuracy is within 10% of the known value of a standard solution. The calibration process will be repeated until the accuracy is verified. The chlorophyll sensor data will be calibrated against chlorophyll measurements of grab samples of site water collected before and after sampling. These samples will be returned to the laboratory for filtration, extraction, and measurement.

Table 4. Sampling log.

MdRH Water Daily Sampling Log

Date_____ Crew_____

Station_____ Latitude_____ Longitude_____

Time at Start_____ Time at Finish_____

Visual Water Description_____

Picture Numbers_____

Other Notes_____

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QUALITY ASSURANCE / QUALITY CONTROL

Quality assurance and quality control (QA/QC) samples will be collected for analysis to evaluate data quality (Table 3). Field QA/QC for this project includes the following:

Equipment blanks. The use of equipment blanks is intended to test whether contamination is introduced from the collection tubing, filtering equipment and sample collection containers. Two vials, tubes, syringes, filters or bottles from every new box of vials, bottles, syringes or filters will be sent to the analytical laboratory for blank analysis. A pump tubing blank will also be analyzed, consisting of Milli-q water that has passed through the tubing while connected to the peristaltic pump that will be used for water collection in the field. These QA samples will be analyzed once per sampling event. Equipment blanks will be collected prior to the first sampling event, filtered, and analyzed for total and dissolved copper and DOC.

Field blanks. The use of field blanks is intended to test whether contamination is introduced from sample collection and handling, sample processing, or the sample containers. For this sample, laboratory water is processed in the field in the same matter than all the other field samples are collected and processed. Field blanks will be analyzed for total and dissolved copper as well as for DOC. Clean sampling techniques will be used to process these samples. One field blank sample will be processed per sampling event.

Field duplicates. The use of field duplicates is intended to test the precision of sample collection. Field duplicates will be analyzed for all chemistry constituents. Clean sampling techniques will be used to minimize sample contamination. One field duplicate sample will be processed per sampling event. The station to collect the field duplicate will be chosen during each sampling event.

Travel blanks. Travel blanks are sealed bottles that contain laboratory water and are used to test if contamination is introduced by the laboratory or transportation methods. Travel blank bottles will be provided by the analytical laboratory and taken into the field at time of sample collection. These bottles will not be opened during field activities. They will be returned to the analytical laboratory with the other field collected samples. The analytical laboratory will analyze this

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water sample for total and dissolved Cu. One travel blank sample will be processed per sampling event.

Matrix Spike Blanks. Additional water samples will be collected for the analysis of matrix spike samples. The matrix spike sample provides information on the extraction efficiency of the method on the sample matrix. Clean sampling techniques will be used to process these samples. One matrix spike blank sample will be processed per sampling event.

Table 5. Quality assurance sample types and volumes. One of each QA sample type will be collected and analyzed for each sampling event.

Sample Type	DOC (ml)	Total Metals (ml)	Dissolved Metals (ml)
Travel Blank	50	250	50
Field Blank	50	250	50
Field Duplicate	50	250	50
Matrix Spike Blank	100	250	60

Chain-of-custody procedures for this project include the following:

- Proper labeling of samples.
- Use of chain-of-custody (COC) forms for all samples.
- Prompt sample delivery to the laboratory.

All aspects of the sample collection process, including generating field logs at each site and chains of custody (COC) forms, will be documented and tracked. COC forms will accompany all water samples to the laboratory for analysis. SCCWRP will retain a copy of all COCs. Physis will document and track all aspects of sample receipt, analyses, and reporting.

Sample Vial and Bottle Labeling

Each sample will have a waterproof paper label affixed to the container and will be labeled at the time of collection. The following information will be recorded on the container label at the time of collection (Figure 2):

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- Project name
- Sample identification
- Date of sample collection
- Depth
- Analysis to be performed

Project: **MDR SSO**
Station: **MdRH-E**
Sample #: 1
Analysis: Total Copper
Laboratory: Physis
Date: March 3, 2015
Depth: Surface

Figure 2. Sample label example.

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SAMPLE DELIVERY

Samples will be stored and transported at 4±2°C. Water samples will be provided to the toxicity and chemistry testing laboratories on the same day that the sample collection process is completed. The individual sample containers containing the marine water samples for chemical analysis will be picked-up by the analytical chemistry laboratory for analysis. Contacts for the field or laboratory coordinators are shown in Table 5. Each sample must be accompanied by a COC form (Figure 3).

Table 5. Agency contacts.

Coordinator	Agency	Contact Name	Email	Phone
Field	SCCWRP	Dario Diehl	dariod@sccwrp.org	714 755-3212
Toxicity	SCCWRP	Darrin Greenstein	darring@sccwrp.org	714 755-3224
Chemistry	Physis	Rich Gossett	richgossett@physislabs.com	714 602-5320

REFERENCES

USEPA. April 1995. Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels. EPA 821-R-95-034.

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Southern California Coastal Water Research Project
3535 Harbor Blvd. Suite 110
Costa Mesa, CA 92626
(714) 755-3200

Chain of Custody

Date _____ Page ____ of _____

Sample Collection By: _____			Project Name: _____				Project Number: _____
Sample ID	Date	Time	Matrix	Container Type	Number of Containers	Comments	Analysis

Relinquished By		Relinquished By		Relinquished By	
(Signature)	(Date)	(Signature)	(Date)	(Signature)	(Date)
(Printed Name)	(Time)	(Printed Name)	(Time)	(Printed Name)	(Time)
(Company)		(Company)		(Company)	
Received By		Received By		Received By	
(Signature)	(Date)	(Signature)	(Date)	(Signature)	(Date)
(Printed Name)	(Time)	(Printed Name)	(Time)	(Printed Name)	(Time)
(Company)		(Company)		(Company)	

Figure 3. Chain of custody.

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APPENDIX B
QUALITY ASSURANCE PROJECT PLAN

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1 QUALITY ASSURANCE PROJECT PLAN OVERVIEW

This Quality Assurance Project Plan (QAPP) establishes quality assurance (QA) objectives for conducting sampling and evaluation activities described in the Work Plan. The methods and QA procedures described herein will be followed by the Southern California Coastal Water Research Project (SCCWRP) and its contractors during various sample collection and data analysis activities beginning in 2015.

Field and laboratory activities will be conducted in such a manner for the results to meet specified data quality objectives (DQOs). Guidance for QA/quality control (QC) is derived from protocols developed for the U.S. Environmental Protection Agency (USEPA) SW-846 (2007), the USEPA Contract Laboratory Program National Functional Guidelines (USEPA 2009, 2010, 2014), and the methods described in Section 3 below.

2 FIELD QUALITY CONTROL

2.1 Data Collection, Processing, and Sampling Forms

All field activities will be recorded on field forms logged by field staff. Field forms will provide a description of sampling activities, a list of sampling personnel, weather conditions, and a record of all modifications to the procedures and plans identified in this QAPP if necessary. Field information will be recorded as shown in Attachment 1.

The following forms, included as Attachment 1, will be used to record pertinent collection, processing, and sampling information:

- Chain-of-custody (COC) form
- Daily log and sampling form
- Water profiling instrument calibration form

2.2 Sample Identification and Labels

Samples will be identified with a sample identifier that specifies the waterbody or site (Marina del Rey Harbor [MdRH]), basin or station location, and sample number.

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An example sample identifier for the first sample collected from Basin E, would be:

MdRH-E1

An example sample identifier for a field blank of the decontaminated sample processing equipment after sample collection of the above sample would be:

FB-20150630

An example sample identifier for a field duplicate sample collected from Basin E, would be:

MdRH-E1-FD

Each sample will have a waterproof paper label affixed to the container and will be labeled at the time of collection. The following information will be recorded on the container label at the time of collection:

- Project name
- Sample identifier
- Date of sample collection
- Analysis to be performed
- Depth

An example label would be:

Project: **MDR SSO**
Sample ID: **MdRH-E1**
Sample #: 1
Analysis: Total Copper
Laboratory: Physis
Date: March 3, 2015
Depth: Surface

2.3 Sample Custody and Shipping Requirements

Samples are considered to be in one's custody if they are in the custodian's possession or view or in a secured location with restricted access.

COC procedures will be followed for all samples throughout the collection, handling, and analysis process. The principal document used to track possession and transfer of samples is

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the COC form (Attachment 1). Each sample will be represented on a COC form the day it is collected. All entries on the COC form will be made using indelible ink pen. Corrections will be made by drawing a single line through the error, writing in the correct information, and dating and initialing the change. Blank lines/spaces on the COC form will be lined-out, dated, and initialed by the individual maintaining custody.

A COC form will accompany each group of samples to the analytical laboratory. Each person who has custody of the samples will sign the COC form and ensure that the samples are not left unattended unless properly secured. Copies of all COC forms will be retained in the project files.

Each cooler containing samples for analysis will be hand-delivered to Physis Environmental Laboratories, Inc. (Physis) the same day of sample collection or the following morning. In the event that Saturday delivery is required, the field coordinator(s) will contact the analytical laboratory on Friday to ensure that the laboratory is aware of the number of samples to be transferred. Following each shipment, the field coordinator(s) will call the laboratory and verify the shipment from the day before was received and is in good condition. Samples will be packed with ice to maintain recommended storage temperatures of 4°C. Ice will be sealed in separate double plastic bags and placed in the transportation coolers. Individual sample containers will be placed in a sealable plastic bag, packed to prevent breakage, and transported in an ice chest or other suitable container. The shipping containers will be clearly labeled with sufficient information (name of project, time and date of collection, and contact person) to enable positive identification.

Upon transfer of sample possession to the analytical laboratory, the persons transferring custody of the sample container will sign the COC form. Upon receipt of samples at the laboratory, the receiver will record the condition of the samples on a sample receipt form. COC forms will be used internally in the laboratory to track sample handling and final disposition.

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2.4 Field Measurement Quality Objectives

Field measurement quality objectives include calibration and measurement accuracy for measurements including dissolved oxygen, pH, temperature, or salinity. Table B-1 summarizes the measurement quality objectives for field measurements. Field QC samples will also be collected and analyzed by the laboratory as indicated in Table B-2.

3 ANALYTICAL CHEMISTRY LABORATORY QUALITY CONTROL

3.1 Summary of Analytical Methods

All sample analyses will be conducted in accordance with approved methods: USEPA 1640 for total and dissolved metals analysis and Standard Method (SM) 5310B for dissolved organic carbon. Chemical testing will be conducted at Physis, which is accredited under California's Environmental Laboratory Accreditation Program ELAP (CA ELAP; Certificate No. 2769). Toxicity testing will be conducted by SCCWRP. The contact person for the toxicity laboratory is Darrin Greenstein, who can be reached via email at darring@sccwrp.org or by phone (714) 755-3224. The contact person for the chemical laboratory is Rich Gossett, who can be reached via email at richgossett@physislabs.com or by phone at (714) 602-5320, ext. 201.

Prior to analysis, all samples will be maintained according to the appropriate holding times and temperatures for each analysis according to Table B-3. Table B-4 presents the analytes, analytical methods, and targeted reporting limits (RLs) for the chemical testing. Each participating laboratory will prepare detailed reports in accordance with Sections 3.5.6 and 4.1 of this QAPP. Those reports will be included as an appendix in the appropriate data report.

Prior to sample analysis, the laboratory will calculate method detection limits (MDLs) and method reporting limits (MRLs) for each analyte of interest, where applicable. MRLs will be at or below the values specified in Table B-4. Results detected above the MDL and below the MRL will be reported with a "J" qualifier. If required RLs are not achieved, some modifications to the methods may be necessary. These modifications from the specified analytical methods will be provided by the laboratory when establishing the laboratory

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contract and must be approved by Los Angeles County Department of Public Works (LACDPW) prior to implementation.

When completing chemical analyses for this project, the contract laboratories are expected to meet the following minimum requirements:

- Adhere to the methods outlined in this QAPP, including methods referenced for each analytical procedure (Table B-4).
- Deliver scanned and electronic data deliverables, as specified.
- Meet reporting requirements for deliverables.
- Meet turnaround times for deliverables.
- Implement QA/QC procedures discussed in this QAPP and its tables including DQOs, laboratory QC requirements, and performance evaluation testing requirements.
- Notify the project manager of any QA/QC problems when they are identified to allow for quick resolution.
- Allow laboratory and data audits to be performed, if deemed necessary.

3.2 Analytical Laboratory QA/QC Overview

Laboratory QC procedures, where applicable, include initial and continuing instrument calibrations, laboratory control materials (LCMs), laboratory control samples (LCSs), laboratory duplicates, matrix spikes (MS), and method blanks. Table B-5 lists the frequency of analysis for laboratory QA/QC samples, and Table B-6 summarizes the DQOs for precision, accuracy, and completeness.

QC sample results from each sample group will be reviewed by the analyst immediately after a sample group has been analyzed (Table B-6). QC sample results will then be evaluated to determine if control limits have been exceeded. If control limits are exceeded in the sample group, the QA/QC Manager will be contacted immediately and corrective action (e.g., method modifications followed by reprocessing the affected samples) will be initiated prior to processing a subsequent group of samples.

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3.3 Laboratory Instrument Calibration and Frequency

An initial calibration will be performed on each laboratory instrument to be used at the start of the project, after each major interruption to the analytical instrument, and when any ongoing calibration does not meet method control criteria. An initial calibration verification (ICV) will be analyzed following each initial calibration and will meet method criteria prior to sample analysis. Continuing calibration verifications (CCVs) will be performed daily prior to any sample analysis to track instrument performance. The frequency of CCVs varies with the method. For metals and inorganic methods, one CCV will be analyzed for every 10 field samples, or daily, whichever is more frequent. If the ongoing continuing calibration is out of control, the analysis will be halted until the source of the control failure is eliminated or reduced to meet control specifications. All project samples analyzed while instrument calibration was out of control will be reanalyzed. Instrument blanks or continuing calibration blanks (CCBs) provide information on the stability of the baseline established. CCBs will be analyzed immediately prior to or following CCV at the instrument for each type of applicable analysis.

3.4 Laboratory Quality Control

3.4.1 Laboratory Duplicates/Replicates

Laboratory duplicates provide information on the precision of the analysis and are useful in assessing potential sample heterogeneity and matrix effects. Laboratory duplicates are subsamples of the original sample that are prepared and analyzed as a separate sample.

3.4.2 Matrix Spikes and Matrix Spike Duplicates

Analysis of MS samples provides information on the extraction efficiency of the method on the sample matrix.

3.4.3 Method Blanks

Method blanks are analyzed to assess possible laboratory contamination at all stages of sample preparation and analysis. The method blank for all analyses must be less than the MRL of any single target analyte/compound. If a laboratory method blank exceeds this criterion for any analyte/compound, and the concentration of the analyte/compound in any

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of the samples is less than five times the concentration found in the blank (10 times for common contaminants), analyses must stop and the source of the contamination must be eliminated or reduced.

3.4.4 Laboratory Control Samples

LCSs are analyzed to assess possible laboratory bias at all stages of sample preparation and analysis. The laboratory control sample is a matrix-dependent spiked sample prepared at the time of sample preparation along with the preparation of samples and MS samples. The laboratory control sample will provide information on the precision of the analytical process and, when analyzed in duplicate, will provide accuracy information as well.

3.4.5 Laboratory Control Material

Laboratory Control Materials (LCMs) are substances of the same or similar matrix to the project samples. In this study, the LCM will be Physis seawater, which will be used as a reference for background concentrations in clean, natural seawater. The LCM will be prepared and analyzed in the same manner as routine samples and in the same preparation and analytical batch. The recovery of the target analyte(s) provides information on interferences caused by the sample matrix.

3.4.6 Laboratory Deliverables

Data packages will be checked for completeness immediately upon receipt from the laboratory to ensure that data and QA/QC information requested in Section 3.5.6 are present.

3.5 Data Quality Objectives and Criteria

The DQOs for this project are to ensure that data collected are of known and acceptable quality. The quality of laboratory data is assessed by precision, accuracy, and completeness. Definitions of these parameters and the applicable QC procedures are given below. Frequency of QC samples is listed in Table B-5. Applicable quantitative goals for these data quality parameters are listed or referenced in Table B-6.

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3.5.1 Precision

Precision is the ability of an analytical method or instrument to reproduce its own measurement. It is a measure of the variability, or random error, in sampling, sample handling, and laboratory analysis.

In the laboratory, "within-batch" precision is measured using replicate sample or QC analyses and is expressed as the relative percent difference (RPD) between the measurements. "Batch-to-batch" precision is determined from the variance observed in the analysis of standard solutions or LCSs from multiple analytical batches.

Field precision will be evaluated by collecting blind field duplicates for chemistry samples. Field chemistry duplicate precision will be screened against a RPD of 35 percent. However, no data will be qualified based solely on field duplicate precision.

Precision measurements can be affected by the nearness of a chemical concentration to the MDL, where the percent error (expressed as RPD) increases. The equation used to express precision is as follows:

$$RPD = \frac{(C_1 - C_2) \times 100\%}{(C_1 + C_2)/2}$$

Where:

RPD	=	relative percent difference
C_1	=	larger of the two observed values
C_2	=	smaller of the two observed values

3.5.2 Accuracy

Accuracy is a measure of the closeness of a measurement to the true or expected value. Accuracy is determined by calculating the mean value of results from ongoing analyses of laboratory-fortified blanks, LCMs, and standard solutions. In addition, laboratory-fortified (i.e., MS) samples will be measured; this sample type indicates the accuracy or bias in the actual sample matrix. Accuracy is expressed as percent recover (%R) of the measured value, relative to the true or expected value. If a measurement process produces results which are

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not the true or expected value, the process is said to be biased. Bias is the systematic error either inherent in a method of analysis (e.g., extraction efficiencies) or caused by an artifact of the measurement system (e.g., contamination). Analytical laboratories use several QC measures to eliminate analytical bias, including systematic analysis of method blanks, LCSs, and independent calibration verification standards. Because bias can be positive or negative, and because several types of bias can occur simultaneously, only the net, or total, bias can be evaluated in a measurement.

Laboratory accuracy will be evaluated against quantitative laboratory control sample, MS, and surrogate spike recovery performance criteria provided by the laboratory. Accuracy can be expressed as a percentage of the true or reference value, or as a %R in those analyses where reference materials are not available and spiked samples are analyzed.

The equation used to express accuracy is as follows:

$$\%R = 100\% \times (S-U)/Csa$$

Where:

%R	=	percent recovery
S	=	measured concentration in the spiked aliquot
U	=	measured concentration in the unspiked aliquot
Csa	=	concentration of spike added

Field accuracy will be controlled by adherence to sample collection procedures outlined in the sample collection sections of this QAPP.

3.5.3 Completeness

Completeness is a measure of the amount of data that is determined to be valid in proportion to the amount of data collected. Completeness will be calculated as follows:

$$C = [(\text{Number of acceptable data points}) \times 100]/ (\text{Total number of data points})$$

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The DQO for completeness for all components of this project is 95 percent. Data qualified as estimated because QC criteria were not met will be considered valid for the purpose of assessing completeness. Data qualified as rejected will not be considered valid for the purpose of assessing completeness.

3.5.4 Sensitivity

Analytical sensitivities must be consistent with, or lower than, the values listed in Table B-4 in order to demonstrate compliance with this QAPP. When achievable, target reporting limits specified will be at least a factor of 2 less than the analyte's corresponding target criteria.

The MDL is defined as the minimum concentration at which a given target analyte can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero. Laboratory RLs are defined as the lowest level that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions. Laboratory MDLs and RLs will be used to evaluate the method sensitivity and/or applicability prior to the acceptance of a method for this program.

The sample-specific MDL and RL will be reported by the laboratory and will take into account any factors relating to the sample analysis that might decrease or increase the RL (e.g., dilution factor, percent moisture, sample volume, or sparge volume). In the event that the MDL and RL are elevated for a sample due to matrix interferences and subsequent dilution or reduction in the sample aliquot, data will be evaluated to determine if an alternative course of action is required or possible. The sample-specific RL will be provided in the project database.

3.5.5 Field Data Management

Field data forms will be checked for completeness and accuracy by the field coordinator(s) prior to delivery to the project manager(s) and disbursement to rest of the project team. Original forms will be retained and filed in a project binder after data entry and checking are complete.

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3.5.6 Analytical and Chemistry Records and Deliverables

Analytical data records will be retained by the laboratory and in the project files. For all analyses, data reporting requirements will include items necessary to complete data validation. Laboratory analytical reports will be provided in electronic format, including the scanned PDF of the report and the Electronic Data Deliverable (EDD). The analytical laboratory will be required, where applicable, to report the following:

- Project Narrative. This summary, in the form of a cover letter, will discuss problems, if any, encountered during any aspect of analysis. This summary should discuss, but is not be limited to, QC, sample shipment, sample storage, and analytical difficulties. Any problems encountered, actual or perceived and their resolutions will be documented in as much detail as appropriate. The narrative should also include final dilution volumes for all samples analyzed at a dilution in which one or more analytes is reported as not detected.
- COC Records. Legible copies of COC forms will be provided. This documentation will include the time of receipt and condition of each sample received by the laboratory. Additional internal tracking of sample custody by the laboratory will also be documented on a sample receipt form. The form must include all sample shipping container temperatures measured at the time of sample receipt.
- Sample Results. Results for each sample analyzed will be provided. The summary will include the following information when applicable:
  - Field sample identifier and the corresponding laboratory identification code
  - Sample matrix
  - Date of sample preparation
  - Date and time of analysis
  - Identification of the instrument used for analysis
  - Analytical results with reporting units identified
  - Data qualifiers and their definitions
- QA/QC Summaries. Results of the laboratory QA/QC procedures will be provided. Each QA/QC sample analysis will be documented with the same information required for sample results (see above). No recovery or blank corrections will be made by the laboratory. The required summaries are listed below; additional information may be requested.

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- Method Blank Analysis. The method blank analysis associated with each sample and the concentration of all compounds of interest identified in these blanks will be reported.
- MS Recovery. MS recovery data will be included. The name and concentration of all compounds added, %R, and range of acceptable recoveries will be listed. The recoveries and RPD for all MS duplicate analyses will be reported.
- Laboratory Duplicate. The RPD for all laboratory duplicate analyses will be included.
- Laboratory Control Sample. All laboratory control sample recovery data will be included. The name and concentration of all compounds added, %R, and range of acceptable recoveries will be listed. The recoveries and RPD for all laboratory control sample duplicate analyses will be reported.

All instrument data will be fully restorable at the laboratory from electronic backup. Laboratories will be required to maintain all records relevant to project analyses for a minimum of 7 years. Data validation reports will be maintained in the project files with the analytical data reports.

3.5.7 Data Reduction

Data reduction is the process by which original data (analytical measurements) are converted or reduced to a specified format or unit to facilitate data analysis. Data reduction requires that all aspects of sample preparation that could affect the test result, such as sample volume analyzed or dilutions required, be taken into account in the final result. It is the laboratory analyst's responsibility to reduce data, which are subjected to further review by the laboratory manager, the project manager, and independent reviewers. Data reduction may be performed manually or electronically. If performed electronically, all software used must be demonstrated to be true and free from unacceptable error.

3.6 Data Validation and Usability

3.6.1 Data Review, Validation, and Verification

During the validation process, analytical data will be evaluated for method QC and laboratory QC compliance, and their validity and applicability for program purposes will be

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determined. Based on the findings of the validation process, data validation qualifiers may be assigned. Validated project data, including qualifiers, will be entered into the Excel project database, thus enabling this information to be retained or retrieved as needed.

3.6.2 Validation and Verification Methods

Data validation includes review for completeness and accuracy by the field coordinator(s) and laboratory manager; review by the QA/QC manager (or designee) for outliers and omissions and the use of QC criteria to accept or reject specific data. All data will be entered into the Excel project database.

Laboratory data will be reviewed and verified to determine whether all DQOs have been met and that appropriate corrective actions have been taken, when necessary. Calculations will be verified by the laboratory. The project manager or designee will be responsible for the final review of all data generated from sample analyses.

The first level of review will take place in the laboratory as the data are generated. The laboratory manager (or designee) will be responsible for ensuring that data generated meet minimum QA/QC requirements and that the instruments were operating under acceptable conditions during data generation. DQOs will also be assessed at this point by comparing the results of QC measurements with pre-established criteria as a measure of data acceptability.

Data packages will be checked for completeness immediately upon receipt from the laboratory to ensure that data and QA/QC information requested are present. A Stage 2A data quality review will be performed in accordance with EPA National Functional Guidelines (USEPA 2010, 2014) by considering the following:

- Holding times
- Method blanks
- Detection limits
- RLs
- LCS/LCMs
- MS/MSD samples

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Data will be validated in accordance with the project-specific DQOs described above, analytical method criteria, and each laboratory's internal performance standards based on their SOPs.

3.6.3 Reconciliation with User Requirements

The data will be reviewed after each survey to determine if DQOs have been met. If data do not meet the project's specifications, the project manager or designee will review the errors and determine if the problem is due to calibration/maintenance, sampling techniques, or other factors and will suggest a corrective action. It is expected that the problem would be correctable by retraining, revising techniques, or replacing supplies/equipment; if not, the DQOs will be reviewed for feasibility. If specific DQOs are not achievable, the project manager or designee will recommend appropriate modifications.

4 TOXICITY TESTING LABORATORY QUALITY CONTROL

All biological tests will incorporate standard QA/QC procedures, the tests are based on methods in the USEPA's Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (USEPA 1995). Standard QA/QC procedures include the use of negative controls, reference toxicant samples, replicates, and water quality measurements during testing.

The negative control is used to establish the health of test organisms and ensure acceptability criteria are met. Control material will consist of filtered seawater adjusted to the appropriate salinity, if appropriate. Positive control or reference toxicant tests will be used to establish the sensitivity of test organisms. The reference toxicant test median lethal concentration (LC_{50}) or median effective concentration (EC_{50}) should fall within two standard deviations of the historical mean for the laboratory, indicating sensitivity is normal.

Proper water quality conditions will be maintained for all tests to ensure that organisms do not experience undue stress unrelated to test samples. If water quality measurements are outside protocol ranges, corrective action will be taken immediately. Laboratory equipment will be maintained, and all instruments will be calibrated regularly. All laboratory work will be documented on approved datasheets.

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4.1 Toxicity Laboratory Reporting

Toxicity test reports will be retained by the laboratory and stored electronically in the project files. The laboratory will be required, where applicable, to report the following:

- Test Methods. A summary of test conditions for each test will be included. All methods should be in accordance with guidelines described in the Work Plan and other guidance or as otherwise noted in the Work Plan.
- Test Results. Results will include a summary of the following information:
  - Test dates
  - Source of control material
  - Source of organisms
  - Water quality measurements
  - Appropriate lethal or sublethal endpoint results for each species
  - LC_{50} or EC_{50}
  - Control acceptability statement
  - Summary of reference toxicant test results
- Statistical Analyses. Statistical analyses will be performed to determine the median effective concentration (EC_{50}), or the statistically derived concentration indicative of toxic effects in 50% of test organisms under specific test conditions.
- QA/QC Summaries. The results of a QC review, with any protocol deviations and corrective actions taken, will be provided.
- Raw Data. Legible copies of raw datasheets used in testing, including water quality, daily observations, and final lethal or sublethal endpoint results, will be provided.
- Reference Toxicant Test Data. Raw datasheets, statistical analyses, and control charts comparing current test results with historical test results will be provided.
- COC Records. Legible copies of the COC forms will be provided. Forms will include the time of receipt and condition of each sample received by the laboratory. Additional internal tracking of sample custody by the laboratory will also be documented on a sample receipt form. The form must include all sample shipping container temperatures measured at the time of sample receipt.

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5 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE REQUIREMENTS

5.1 Field Instruments/Equipment

In accordance with the QA program, an inventory of field instruments and equipment will be maintained. The frequency and types of maintenance will be based on the manufacturer's recommendations and/or previous experience with the equipment.

The field coordinator(s) will be responsible for the preparation, documentation, and implementation of the preventative maintenance program. The equipment maintenance information will be documented in the instrument's calibration log. The frequency of maintenance is dependent on the type and stability of the equipment, the methods used, the intended use of the equipment, and the manufacturer's recommendations. Detailed information regarding the calibration and frequency of equipment calibration is provided in specific manufacturer's instruction manuals.

All maintenance records will be verified prior to each sampling event. The field coordinator(s) will be responsible for verifying that required maintenance has been performed prior to using the equipment in the field.

5.1.1 Laboratory Instruments/Equipment

In accordance with the QA program, the laboratory will maintain an inventory of instruments and equipment and the frequency of maintenance will be based on the manufacturer's recommendations and/or previous experience with the equipment.

The laboratory preventative maintenance program, as detailed in their QA plan, is organized to maintain proper instrument and equipment performance and to prevent instrument and equipment failure during use. The program considers instrumentation, equipment, and parts that are subject to wear, deterioration, or other changes in operational characteristics; the availability of spare parts; and the frequency at which maintenance is required. Any equipment that has been overloaded, mishandled, gives suspect results, or has been determined to be defective will be taken out of service, tagged with the discrepancy noted,

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and stored in a designated area until the equipment has been repaired. After repair, the equipment will be tested to ensure that it is in proper operational condition.

Each laboratory will be responsible for the preparation, documentation, and implementation of the preventative maintenance program. All maintenance records will be checked according to the schedule on an annual basis and recorded by the responsible individual. The laboratory manager (or designee) shall be responsible for verifying compliance.

5.2 Instrument Calibration

Proper calibration of equipment and instrumentation is an integral part of the process that provides quality data. Instrumentation and equipment used to generate data must be calibrated at a frequency that ensures sufficient and consistent accuracy and reproducibility.

5.2.1 Field Instrument/Equipment Calibration

Field equipment will be calibrated prior to each sampling event according to the manufacturer's recommendations using the manufacturer's standards. A calibration check will be performed at the end of the day. The equipment, calibration, and maintenance information will be documented in the instrument calibration log. The frequency of calibration is dependent on the type and stability of the equipment, the methods used, the intended use of the equipment, and the manufacturer's recommendations. Detailed information regarding the calibration and frequency of equipment calibration is provided in specific manufacturer's instruction manuals.

Equipment that fails calibration or becomes inoperable during use will be removed from service and tagged (time and date of action) to prevent inadvertent use. Such equipment will be satisfactorily recalibrated or repaired and tagged (date and time of return to service) prior to use.

5.2.2 Laboratory Instrument/Equipment Calibration

As part of their QA/QC program, laboratories perform two types of calibrations. A periodic calibration is performed at prescribed intervals (i.e., balances, drying ovens, refrigerators, and thermometers), and operational calibrations are performed daily, at a specified frequency, or

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prior to analysis (i.e., initial calibrations) according to method requirements. Calibration procedures and frequencies are discussed in the laboratory's QA plan. Calibrations are discussed in the laboratory's standard operating procedures (SOPs) for analyses.

The laboratory manager (or designee) will be responsible for ensuring that laboratory instrumentation is calibrated in accordance with any specifications. Implementation of the calibration program shall be the responsibility of the respective laboratory group supervisors. Recognized procedures (USEPA, ASTM, or manufacturer's instructions) will be used when available.

Physical standards (i.e., weights or certified thermometers) will be traceable to nationally recognized standards, such as the National Institute of Standards and Technology (NIST). Chemical reference standards shall be NIST standard reference materials or vendor-certified materials traceable to these standards.

Calibration requirements for each method and respective corrective actions will be accessible, either in the laboratory's SOPs or the laboratory's QA Plan for each instrument or analytical method in use. All calibrations will be preserved on electronic media.

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6 REFERENCES

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USEPA, 2010. USEPA Contract Laboratory Program National Functional Guidelines for Inorganic Superfund Data Review. USEPA Office of Superfund Remediation and Technology Innovation. USEPA 540-R-10-011. January 2010.

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TABLES

Table B-1
Field Measurement Quality Objectives

Parameter	Calibration Adjustment Frequency	Measurement Accuracy
Conventionals		
Salinity (g/kg)	Per manufacturer's instructions	0.1
Temperature (°C)		0.1
pH (su)		0.1
Dissolved Oxygen (mg/L)		1.0

Notes:
g/kg = grams per kilogram
mg/L = milligrams per liter
su = standard unit

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Table B-2
Field Quality Control Samples

Sample Type	Dissolved Organic Carbon volume (mL)	Total Metals Volume (mL)	Dissolved Metals Volume (mL)
Travel Blank	50	250	50
Field Blank	50	250	50
Field Duplicate	50	250	50
Lab Duplicate	50	250	50
Matrix Spike	100	250	60
Pump Tubing Blank	0	250	50

Notes:
Dissolved samples will be field filtered
mL = milliliters

Table B-3
Sample Containers, Holding Times, and Preservation Methods

Parameter	Sample Size	Container Size and Type	Holding Time	Preservative
Dissolved Organic Carbon	50 mL	Centrifuge tube (50 mL)	28 days	Cool/4°C; H_2SO_4[c] to pH<2
Total Metals	250 mL	Pre-cleaned HDPE bottle (250 mL)	6 months	Cool/4°C; HNO_3 to pH<2
Dissolved metals	50 mL	Centrifuge tube (50 mL)	6 months	Cool/4°C; HNO_3[c] to pH<2 after filtration

Notes:
Dissolved samples will be field filtered
mL = milliliters