# Sinclair Inlet Toxicity Assessment :

# Sampling Plan for Puget Sound Naval Shipyard & Intermediate Maintenance Facility Surface Water Copper Bioavailability and Toxicity Study

**FINAL DRAFT**
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# INTRODUCTION

This document describes plans for performing a study to assess the bioavailability of copper in surface water from Sinclair Inlet, adjacent to Puget Sound Naval Shipyard & Intermediate Maintenance Facility (PSNS & IMF). Physical and chemical characteristics of seawater influence the bioavailability and toxicity of metals to aquatic organisms (Stauber et al. 2000, Knezovich et al. 2001, Eriksen et al. 2001, Lorenzo et al. 2002). Therefore, individual water bodies will differ in their potential to buffer against metal toxicity. One way of assessing relative bioavailability at a site is to spike site water samples with various concentrations of copper in the laboratory and compare resulting EC50 values in site water to those observed in concurrent exposures using laboratory water. This is the approach used in calculation of water effect ratios (WER), which are currently used for derivation of site-specific water quality criteria (WQC) for National Pollution Discharge Elimination System (NPDES) permits (USEPA 1994a).

Metal bioavailability can also be determined by measuring the complexation capacity of the water body. Copper complexation capacity (CuCC) is a chemical measurement determined with a copper ion selective electrode (CuISE), in response to systematic addition of copper in ambient water. The response of the CuISE is indicative of the concentration of aqueous free copper ion (Cu(II)aq), which has been shown to be a better predictor of toxicity than total or dissolved measurements (Sunda and Guillard, 1976; Sunda and Ferguson, 1983; Eriksen et al, 2001; Rivera-Duarte et al, 2004). Copper bioavailability in Sinclair Inlet will be evaluated using both of the above-mentioned approaches.

# BACKGROUND

Sinclair and Dyes Inlets, Washington were listed on the 1998 303(d) list of impaired waters because of fecal coliform (FC) contamination in the marine waters and metals and organic contaminants in bottom sediments and fish tissues (WDOE 1998). The Puget Sound Naval Shipyard & Intermediate Maintenance Facility, Department of Ecology, U.S. Environmental Protection Agency and local stakeholders are working together on Project ENVVEST (an acronym for ENVironmental InVESTment) to address contamination issues and develop water cleanup plans for the watershed (Navy, EPA, and Ecology 2000, ENVVEST 2002). Significant progress has been made on the FC Total Maximum Daily Load (TMDL) study for Sinclair and Dyes Inlets (Ecology 2004)(Figure 1), which has benefited from the collaboration and cooperation of many stakeholders within the watershed. Currently, the FC model verification sampling for the TMDL study is being planned (Johnston et al. 2004) and storm water flow monitoring is being initiated for representative storm water outfalls within the study area (TEC 2003a, b).


**Figure 1. The location of PSNS & IMF in Sinclair and Dyes Inlets, WA. (Photo from WDFW).**

Dry dock and storm water discharges from the Shipyard to Sinclair Inlet require NPDES permits issued by the U.S. Environmental Protection Agency (U.S. EPA). The current dry dock permit has an average monthly total recoverable copper concentration limit of 19 ppb while the storm water permit has a total recoverable copper limit of 33 ppb (U.S. EPA 1994b). In addition, the permit also contains loading limits expressed in pounds per day. Although acute and chronic water quality criteria are not exceeded in Sinclair Inlet (Katz et al., 2004), the potential for adding to elevated copper levels in sediments adjacent to the Shipyard is a concern.

The objective of this study is to assess the assimilative capacity of surface water at sites adjacent to PSNS & IMF in Sinclair Inlet. In general, water bodies have a greater capacity to reduce a metal's bioavailability than the laboratory water used for derivation of national ambient water quality criteria (WQC), upon which discharge permits are generally based. This is because laboratory water is typically low in metal-binding particulate matter and dissolved organic matter compared to most ambient waters (USEPA 1994a, c). These differences in bioavailability are accounted for by USEPA's water effect ratio (WER) procedure in which metal-spiked site and laboratory waters are evaluated for toxicity in side-by-side exposures. The site water LC50/EC50 is then divided by the lab water LC50/EC50, resulting in a multiplier that can be used to adjust the national WQC. This work will follow the WER guidance to the extent that EC50 values for very sensitive marine invertebrates will be obtained in both site water and lab

water to characterize the differences in bioavailability between the Sinclair Inlet sites and lab water, as well as among the selected Sinclair Inlet sites.

Copper complexation capacity (CuCC) is a chemical measurement that could be used as a surrogate for the types of toxicity tests described above. CuCC is defined as the capacity of ambient water to assimilate inputs of copper without associated adverse effects upon aquatic organisms. It is measured through a copper ion selective electrode (CuISE), in response to systematic addition of copper in ambient water. The response of the CuISE is indicative of the concentration of aqueous free copper ion ($Cu(II)_{aq}$) in solution, which according to the free-ion model (Buffle et al., 1990), and substantiated by experimental evidence (Sunda and Guillard, 1976; Sunda and Ferguson, 1983; Campbell, 1995, Ericksen et al, 2001; Rivera-Duarte et al., 2004), is the fraction of copper that is available to organisms, making it a better predictor of its potential toxicity than either the total or dissolved copper concentrations. Therefore, CuCC is a chemical measurement indicative of the toxicity of the water to organisms, and, as shown in Figure 2, is very much related to the results from toxicity tests (larval development EC50). As this figure indicates, larval development success of the Mediterranean mussel, *M. galloprovincialis,* decreases with the addition of copper. Similarly, the response of the CuISE indicates a change in slope that coincides with the EC50.

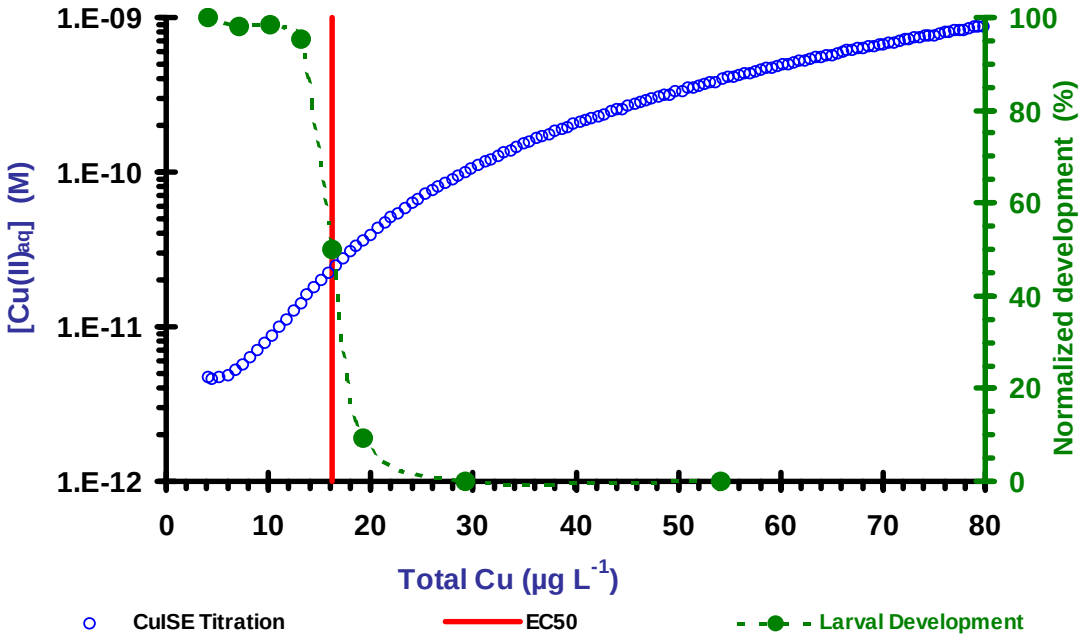


**Figure 2. Relationship between toxicity and complexation capacity (Rivera-Duarte et al. 2004)**

## STUDY GOALS

The goals of this study are to measure the potential for surface water in Sinclair Inlet to buffer against copper associated toxicity. This will be achieved through laboratory-based toxicity exposures following EPA's water effect ratio procedure for developing site-specific water quality criteria (USEPA 1994a), as well as via chemical measurements to determine the complexation capacity at the site.

## STUDY SITE

### Ambient Sampling Locations

Three ambient water samples from Sinclair Inlet will be collected for the toxicity assessment (Figure 3). These stations will be collocated with the sampling sites planned for the fecal coliform model verification study (Johnston et al. 2004). These stations will sampled for dissolved and particulate metals, total suspended solids, total organic carbon, dissolved organic carbon, salinity, alkalinity, and pH (Johnston et al. 2004).

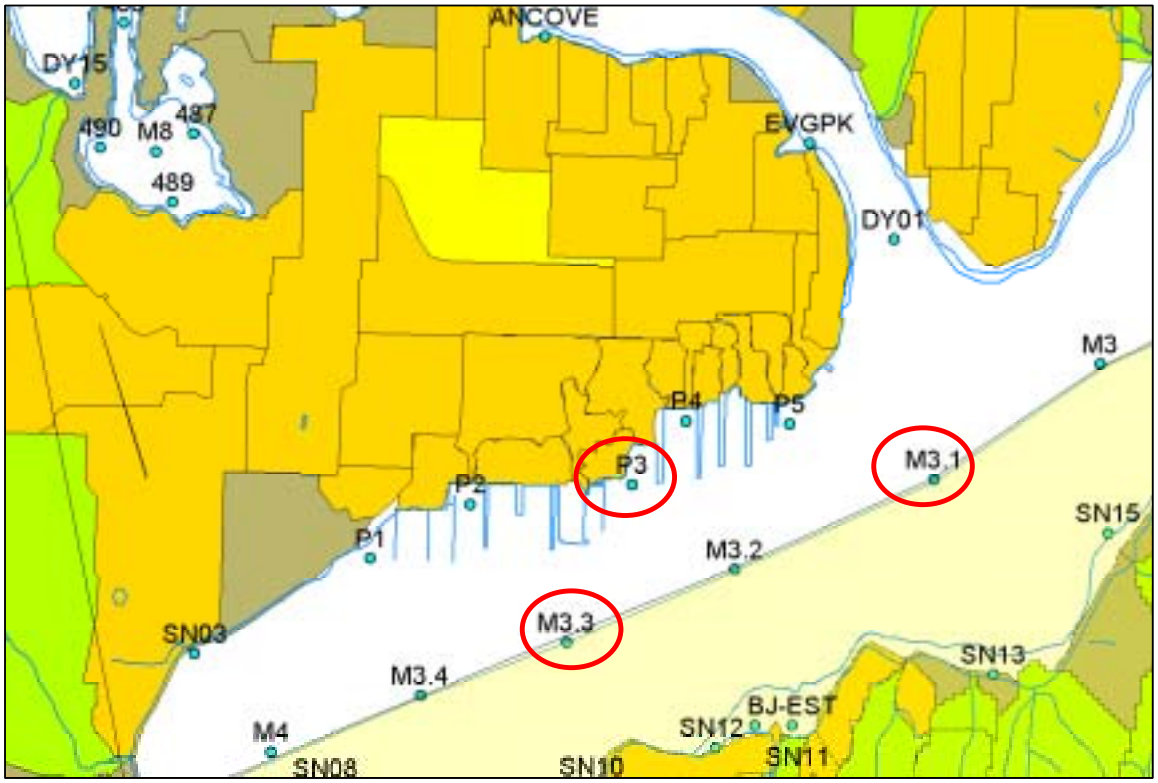


**Figure 3.** Marine stations near the Naval Station and Shipyard for the BREMERTON sampling event. The stations with red circles are the stations to be sampled to determine site-specific toxicity and assimilative capacity of copper.

# METHODS

## Toxicity Assessment

*Sample Collection and Storage*

Site water will be collected from the water surface (depth of approximately 1 m) using clean techniques (US EPA 1995c). Samples will be stored in pre-cleaned 1-L HDPE containers, and shipped overnight to SSC. Bottles will be held at 4 °C during shipping and upon arrival at SSC. Testing will be initiated within 48 h of sample collection, as recommended (US EPA 1994a). Additional samples will be collected for copper analysis (see Copper Measurements below), as well as total suspended solids (TSS) and dissolved organic carbon (DOC).

*Site Water Preparation*

If predators are suspected, passing the sample through a 50 μm mesh screen will be considered. Because site water salinities are expected to be within the range of that tolerated by the test species (28-34 ppt), samples should not require any salinity adjustment. If it is required, however, hypersaline brine will be used to raise the salinity, and appropriate controls added to the test design. No other manipulation of site water samples is expected to be required.

*Test Species*

Toxicity testing will be conducted with embryos of the Mediterranean mussel, *Mytilus galloprovincialis*. This species is relevant because it is sensitive to copper at very low concentrations (e.g. < 10 ppb)(USEPA 1995b), and is present as a commercially important species in the Puget Sound area (Taylor Shellfish Farms 2004). *Mytilus galloprovincialis* has also been used as a test species for caged mussel deployments in Sinclair Inlet during the installation restoration investigations conducted for the Shipyard (URS 2001).

*Toxicity Tests*

Toxicity tests will be conducted following EPA guidance for whole effluent toxicity (US EPA 1995a) and for determining WERs (USEPA 1994a). Site and laboratory water samples will be spiked with a series of copper concentrations (approximately eight), ranging from 0 to 50 μg/L. In this case, laboratory water will be filtered (0.45 μm), open coastal seawater from the research pier at Scripps Institute of Oceanography (SIO). Copper stock solutions will be made from copper sulfate and confirmed by stabilized temperature graphite furnace atomic absorption (STGFAA) spectroscopy prior to use. The same stock solution will be used for both laboratory and site waters. Test concentrations will be prepared separately in 125 mL Erlenmeyer flasks. From each flask, 10 mL will be distributed to each of five replicate pre-conditioned glass 20 mL

scintillation vials for the bioassay. A sixth replicate from each concentration will be saved for later quantification of total recoverable and dissolved copper by STGFAA. An equilibration period of approximately 1 to 3 h will be allowed following copper additions before addition of embryos.

*M. galloprovincialis* will be obtained from Carlsbad Aquafarm, Carlsbad, CA on the same day tests are to be initiated. Mussels will be induced to spawn by thermal shock. Approximately 200 embryos at or beyond the 2-cell stage (within four hours of fertilization) will be added to each test vial. Vials will then be incubated at 15 ± 1 °C for 48 h under a 16 h light: 8 h dark photoperiod. Water quality (pH, temperature, dissolved oxygen, salinity) will be recorded at test initiation and test end. The proportion of normal D-shaped, straight-hinged larvae relative to the number of normal embryos in a set of initial density vials will be determined. Larvae will be evaluated with the aid of an inverted compound microscope.

*Data Analysis*

The proportion of normal larvae from each test concentration will be used to generate EC50 values for each water sample. EC50s will be calculated with ToxCalc™ version 5.0, using the appropriate point estimate technique for the resulting dataset as recommended by the EPA. EC50 values will be calculated based on nominal, total recoverable, and dissolved copper concentration. Potential ambient toxicity will be assessed by comparison of development success in the controls for each test (site water with no added copper) with test acceptability criteria. Control development in the site waters will also be compared with that in the lab water using one-way ANOVA and multiple comparison techniques.

## Copper Measurements

Concurrent with the toxicity samples, additional water samples will be collected for measurement of total recoverable, dissolved, and free copper ion concentrations, as well as copper complexation capacity. Total and dissolved copper concentrations will be used to support the toxicity assessment by allowing precise EC50 determination for each form. Free copper ion concentrations will be used for copper complexation capacity determinations, which are expected to complement the toxicity assessment, demonstrating the method's usefulness as an alternative to biological testing.

*Total recoverable and dissolved copper*

Sampling protocols for the ambient waters will be those of EPA Method 1669, EPA's Trace Metals Sampling Technique (US EPA, 1995c). These include the use of acid-cleaned apparatus and materials made up of polyethylene, and "clean hands/dirty hands" techniques. Preservation, handling and analysis of the samples will be done in class-100 trace metal clean working areas. Enough ULTREX grade nitric acid will be added to the samples in order to decrease the pH to less than 2. Copper concentrations will be measured by stabilized temperature graphite furnace atomic absorption (STGFAA) spectroscopy either by direct injection (for effluent samples) or after liquid-liquid preconcentration with dithiocarbamates (for ambient samples) following Bruland et al (1985). The standard reference material (SRM) CASS4 (coastal seawater) from the

National Research Council of Canada will be used to quantify the recovery of the preconcentration, and SRM 1643d (trace metals in water) of the National Bureau of Standards will be used to evaluate the precision and accuracy of the STGFAA analysis.

*Free copper ion and copper complexation capacity*

The concentration of the free aqueous copper ion ($[Cu(II)_{aq}]$) will be measured with an Orion 94-29 Cu(II) ion selective electrode (Cu-ISE), following procedures used by Zirino *et al* (1998), and Cu-CC was measured as detailed in Rivera-Duarte and Zirino (2003); however, a brief description of the procedures is provided here. Both measurements will be made in a dark, class-100 working station, with constant stirring at 25 ± 0.1°C, by the electrode potential (mV) between a Cu-ISE and an Orion Ag/AgCl double-junction reference electrode. The electrodes will be calibrated with seawater Cu-activity buffers made with $2\times10^{-4}$ M Cu in filtered (0.45 µm) seawater and either $1\times10^{-3}$ M ethylenediamine or $1\times10^{-3}$ M glycine (Belli and Zirino 1993, Zirino et al. 1998). Since $[Cu(II)_{aq}]$ in each buffer will be calculated with a specific ion-interaction model for the measured pH and the concentrations of major ions (Belli and Zirino 1993), the calibrated response of the Cu-ISE is reported as the pCu (i.e., $-\log [Cu(II)_{aq}]$) of the solution.

The change in the response of the Cu-ISE during a titration with copper will be used for the measurement of the Cu-CC (Rivera-Duarte and Zirino, 2003). The titrations will be performed with a TTT 85 Titrator and an ABU 80 Autoburette, both from Radiometer Copenhagen, connected to a personal computer for continuous automatic recording of the data. First, the electrodes will be calibrated and then allowed to equilibrate overnight in an aliquot of the seawater sample. The next day, an aliquot of 250-300 g of fresh seawater sample will be weighed into a Teflon beaker, and the electrodes allowed to equilibrate in it for several minutes before starting the titration. The titration will proceed automatically by additions of 10 µL each once the potential has stabilized to within 0.1 mV $sec^{-1}$ and will be completed after 99 mL of the titrant are added, which is equivalent to an average change in concentration of $7.6\times10^{-7}$ M (48.2 µg $L^{-1}$, n= 78). The titrant will be made with 200 µL of 1000 ± 3 µg $mL^{-1}$ High Purity Copper Standard added to 1L of 18-MΩ water containing 32 g NaCl. Cu-CC is estimated from the inflection point of the resulting titration curve using a MATLAB routine (Rivera-Duarte and Zirino, 2003).

## Schedule

The toxicity study will be scheduled for early April 2004, in conjunction with the Bremerton FC study. The actual sampling dates will be dependent on logistical considerations within the Shipyard (vessel availability, dry dock operations, docking and undocking operations, etc.) and weather conditions.

# Summary

A toxicity assessment will be conducted to evaluate the relative bioavailability of copper at three sites in Sinclair Inlet, adjacent to PSNS and IMF. Toxicity tests involving addition of copper to surface water from each site will be conducted in parallel with identical exposures with laboratory water as a means of detecting differences in bioavailability. Copper complexation capacity, a chemical measure of bioavailability based on free copper measurements, will also be determined. These studies will be used to evaluate the biological availability of copper in the waters of Sinclair Inlets, WA.

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