FREEPORT-McMoRan
COPPER & GOLD

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February 3, 2014

Certified Mail #70123050000053967459
Return Receipt Requested

Erika Schwender, Director
New Mexico Environment Department
Resource Protection Division
P.O. Box 5469
Santa Fe, New Mexico 87502

Dear Ms. Schwender:

Re: Phytotoxicity and Vegetation Community Study Workplan,
Smelter/Tailing Soils Investigation Unit, Chino Administrative Order on Consent

Freeport-McMoRan Chino Mines Company (Chino) submits under separate cover the *Phytotoxicity and Vegetation Community Study Workplan* for the Smelter Tailing Soils Investigation Unit under the Chino Administrative Order on Consent to the New Mexico Environment Department (NMED). The workplan was submitted today to Mr. Matt Schultz.

Please contact Mr. Ned Hall at (520) 393-2292 with any questions or comments concerning this workplan.

Sincerely,

Sherry Burt-Kested, Manager
Environmental Services

SBK:pp
20140203-001

c Matthew Schultz, NMED
Joseph Fox, NMED
Jerry Schoeppner, NMED
Petra Sanchez, US Environment Protection Agency
Ned Hall, Freeport-McMoRan Copper & Gold Inc. (via email)

Draft

Work Plan:

Smelter Tailing Soils Investigation Unit (STSIU) – Phytotoxicity and Vegetation Community Study

Prepared for:

Freeport-McMoRan Chino Mines Company
Administrative Order on Consent
Smelter/Tailing Soils Investigation Unit
Vanadium, New Mexico

January 30, 2014

Prepared by:

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1.0 Introduction

The objective of this work plan is to evaluate the effects of cupric ion activity (measured as pCu = -log[Cu^{2+}]) on ecologically relevant plant and community endpoints after accounting for other confounding physical and chemical factors. Such information will help inform decisions on remedial goals for pCu and remedial technologies useful for the Smelter Tailing Soils Investigation Unit (STSIU).

Based on laboratory phytotoxicity studies and plant community surveys conducted in the field in 1999, the Site-wide Ecological Risk Assessment (ERA) stated that elevated concentrations of copper and other metals, combined with depressed soil pH, have led to a risk of phytotoxicity for some areas of the Chino Mine Site. Because the field and vegetative effects were best correlated with pCu, risk criteria for remedies based on pCu were proposed (NewFields 2005). Though the pre-Feasibility Study (FS) Remedial Action Criteria (RAC) was established using 1999 pCu results, there were a number of limitations and uncertainties associated with the 1999 phytotoxicity tests and vegetation community study used to evaluate the effects of pCu on vegetation at Chino (Schafer and Associates 1999, NewFields 2005). These challenges generally included the following:

- Phytotoxicity Study of pCu Effects
    - The phytotoxicity tests were conducted using non-native, naïve plant species.
    - Physical and chemical factors that vary among Site soils and between the Site and Reference soils used in the study were not fully taken into account. If correlated to pCu, these factors could be confounding the interpretation of the phytotoxicity results with respect to pCu.
    - Sample size was limited for low pCu treatments.
- Vegetation Community Study of pCu Effects
    - ERA sample locations for soils and vegetation did not represent some of the areas with low pCu, such as bedrock and tops of ridges.
    - Reference locations at the Grant County Airport were not representative for the majority of the STSIU.

Moreover, site conditions have changed since the 1999 soil sampling, resulting in the potential reduction in bioavailability of copper in Site soils. One factor is that the two historical smelter stacks have since been shut down and demolished. The smelter stacks historically emitted acid-generating (thus pH-lowering) emissions and trace copper concentrations; during the 1970s, in compliance with new Clean Air Act amendments, the stacks were permitted and controls were implemented to reduce emissions. Another factor is that a significant shift in pH upward was observed at STSIU following a “white rain” precipitation event on January 7, 2008 (ARCADIS 2008). During the event, a milky alkaline rain containing calcium was deposited on the mine site. The change in pH due to the white rain event has lowered cupric ion activity of the soil (ARCADIS 2013a) and, more importantly, possibly changed the complex soil geochemistry in a manner that alters the relationship between pCu and plant and community endpoints.

The studies described below will supplement the original phytotoxicity and community studies using more representative plant species and soil sample locations. The results will be used in the STSIU FS to provide additional empirical information to address the reliability of the cupric ion pre-FS RAC for plants of pCu ≤ 5 where copper is present at concentrations greater than 327 milligrams per kilogram (mg/kg; NMED 2010). The effect of pCu on the STSIU habitat and rangeland quality will be emphasized in this report because the pre-FS RAC is intended to protect wildlife habitat and rangeland generally, not individual plant species.

The objectives of the community and phytotoxicity studies ÁÒÅ to identify the De Minimus (i.e., negligible) effect level (DEL) and probable adverse effect level (PEL) of pCu.

2.0 Phytotoxicity Study

Studies have shown that, in high concentrations, copper has a toxic effect on plants (Loneragan et al. 1981, Paschke and Redente 2002, Kopittke et al. 2010), and beneficial effects at low concentrations as it is an essential plant nutrient. The objective of the phytotoxicity study is to identify thresholds for adverse effects of pCu on plant endpoints such as emergence, survival, and growth. This study will use species and locations representative of the STSIU habitats, after accounting for confounding physical and chemical factors in the soil.

Similar to the approach used in the 1999 study, a DEL and PEL will be estimated from concentration/stressor-response curves developed from this study and through comparison with soils that likely have De Minimus effects because they are far or upwind from past and present contaminant sources. The study conclusions will be reported in the STSIU FS as a line of evidence to assist the New Mexico Environment Department (NMED) and stakeholders to make final decisions for the STSIU, which will ultimately be documented in the Record of Decision (ROD).

As mentioned above, the 1999 phytotoxicity study, due to its limitations, posed significant uncertainty associated with its results. The changes designed to improve upon the 1999 study are discussed below, including incorporating more representative species and sample locations, accounting for confounding physical and chemical factors, and increasing sample size.

2.01 Incorporating Representative Species

Chino has always maintained the hypothesis that plant species adapt and thrive in unique ecosystems such as high-altitude semi-arid environments as well as in soils with naturally elevated metals concentrations (Chino 2004, 2007). Such adapted plants have more resilience than naïve plants or agricultural species, both of which are generally not adapted to the local environmental conditions. As such, plant toxicity tests on naïve species are unlikely to represent the potential phytotoxic effects experienced by locally adapted plants (Loneragan et al. 1981, Bradshaw et al. 1990, MacNair 1990, Paschke and Redente 2002, MFG 2004, Haque 2008), and instead native species should be evaluated in toxicity tests. Specifically, genetic strains of native plant species growing at the mine site may be more tolerant of local conditions than nursery strains (MacNair 1990, Haque 2008) and agricultural species (Paschke and Redente 2002). Natural populations often have a low frequency of plants with tolerant genes, which natural selection increases when the plant population is exposed to high metals concentrations (MacNair 1997, MacNair et al. 2000). Native species are known to develop this increased tolerance over a relatively short time frame (< 50 years [Bradshaw et al. 1990]; 70 years for copper [Bondada and Qiyingma 2003]).

Sauvé et al. (1998) predicted plant thresholds for calculated pCu toxicity based on copper and pH reported in many phytotoxicity studies. Similar to the 1999 phytotoxicity study by NewFields (2005), this study takes Sauvé's study one step further by evaluating the

measured, rather than calculated, pCu toxicity of the unique STSIU seeds and soils. Therefore, Chino proposes to:

(1) Evaluate if plants native to southwestern New Mexico are more tolerant of low pCu than the agricultural species (alfalfa, ryegrass) used in the 1999 phytotoxicity test; and

(2) Evaluate if native plants growing at the Chino mine site are more tolerant of low pCu than naïve plants of the same species of nursery stock.

If at least one of these conditions is true, the relationship (S-shaped curve) between pCu and either plant germination, survival, or growth endpoints in the Site-wide ERA (Figure 2.5-1 in NewFields 2005) will shift leftward in the lower pCu range before the curve asymptotes. This study will evaluate if a leftward shift in this relationship occurs, supporting the hypothesis that native, adapted plants are more tolerant of low pCu than agricultural or naïve species.

2.02 Representative Soils from Possible De Minimus Effect Areas

The reference sample locations in the original phytotoxicity study introduced uncertainty because they are not representative of the study area for this work plan. Chino has long maintained that the reference samples from near the airport evaluated graphically in the Site-wide ERA to assist in establishing DELs are not truly representative of most of the vegetation types and soils present in Chino investigation units (i.e., STSIU and Hanover/Whitewater Creek). The reference sites near the airport are representative only of the small western portion of the STSIU that falls within the Gila Conglomerate Formation/Plack soil type. Rather than using reference samples, which require certainty of no impacts, Chino proposes to sample soils in eight locations far or upwind from past and present contaminant sources. The soils of these eight locations will be referred to as "de minimus soils", defined as soils expected to have small to negligible impacts from mining because they are far from contaminant sources on the mine site (near or outside the STSIU boundary). These eight soils are considered to be representative of the STSIU soils and geology (three rhyolite/Abrazo Luzena bedrock sourced soils, four non-bedrock Andesite-Basalt sourced soil, and one non-bedrock Gila Conglomerate/Plack sourced soil) and are expected to have de minimus effects from both past and present operations at Chino. Seed germination and plant growth on these De Minimus soils will be compared to Site soils in a manner similar to Figure 2.5-1 in the ERA (NewFields 2005). "Site soils" are defined as soils close to past and present contaminant sources from the mine inside the STSIU boundary (Figure 1).

2.03 Accounting for Confounding Factors

The 1999 phytotoxicity study did not explicitly account for potentially confounding physical and chemical factors in the test soils, though many were measured (shown in Table 1) and Site soils were selected that had similar slope and elevation. Ideally, physical and chemical factors other than pCu should be held constant in the phytotoxicity study, but this is not possible when using soils from a broad heterogeneous area. Even after changing to more representative De Minimus locations, the De Minimus locations still may vary from Site soil conditions in some factors. Additionally, areas of potential concern for remediation occur

across soils on varying topography and soil development, and the objective is to evaluate growth across the range of such soils, rather than focus on a subset with similar conditions.

Because nutrients, soil texture, salinity, and other physical and chemical factors will likely vary and can strongly affect plant growth even in soils without metals contamination (Larcher 1995, Plaster 2009), these factors will be evaluated to determine if they are driving the results and to statistically adjust for their influence on pCu effects on the relevant plant endpoints. The 1999 study evaluated which physical and chemical measures were correlated with phytotoxicity endpoints (Table 2.3-1 in NewFields 2005) but did not statistically adjust the concentration-response relationship in a multiple regression for these confounding factors when assessing pCu effects.

2.04 Increased Sample Size and More Representative Samples

To account for confounding factors statistically and detect small shifts in the concentration-response curves, sample size must be larger than that of the 1999 study. The sample size planned for each concentration-response curve is 33 samples, a size that should achieve the power and precision needed to adequately detect shifts in the curve as described in more detail in Section 2.2.

The following sections describe the specifics of the refined design of the laboratory phytotoxicity study.

2.1 Phytotoxicity Design

Laboratory tests will be conducted to assess the ability of both native and non-native seeds to germinate and become established in Site soils. Native site seeds might have poor germination in the laboratory due to low pure live seed (PLS) percentage relative to bred seeds. Native seeds with known high PLS percentages obtained from a nursery are included in the study design as well as site seeds to ensure a test with native plants will be successful. Three soil types will be evaluated during the phytotoxicity study:

1. "Site Soils" – 0 to 6 inch below ground surface (bgs) soils will be collected from 25 upland locations within the STSIU in areas sampled previously that fall within a range of pCu from 2 to 8. The most samples will be in the pCu ranges most likely to represent the threshold for PEL effects, such as between pCu of 3.5 and 5.5. This range had limited sample sizes during the 1999 study, and produced great uncertainty in identifying the threshold where probable phytotoxic effects begin to occur. Figure 1 shows the 25 proposed sample locations. These locations represent the full range of pCu expected on the STSIU as shown in Table 2.
2. "De Minimus Soils" – 0 to 6 inch bgs soils will be collected from eight locations on or near Chino property that are thought to have De Minimus impacts from mining activities but otherwise share similar characteristics to "Site" soils. Results from phytotoxicity tests using these De Minimus soils will be compared to Site soils to evaluate the DEL for effects on each endpoint measured. Figure 1 shows the proposed De Minimus locations. De Minimus locations 21 to 23 are areas NMED and Chino selected during the bedrock evaluation of richness and cover in 2012; De Minimus

locations 25 through 28 are representative of non-bedrock areas located in the eastern portion of the STSIU; and De Minimus location 24 is representative of conditions present in the western portion of the STSIU[1].

3. "Control Soil" –a manufactured soil (sandy loam with ~3% organic matter, see Table 3) consistent with Association of Official Seed Analysis (AOSA) guidance is included as a laboratory control to test and compare viability of the different seeds sown on the same fertile soil.

Note that only soil, not Site seeds, will be collected at these locations. Site seeds will be collected from a special designated 10-acre area (discussed below) rather than from across the STSIU. This restriction will control for effects of precipitation and other non-pCu factors influencing seed development that vary across the STSIU. The three types of soils (Site, De Minimus, and Control) are shown in Table 4 with their required quantities of pots, seeds, and soil.

At each Site and De Minimus soil sampling location, the soil samples will be homogenized and split into a 1-gallon plastic bag and six 2-gallon canvas bags. The gallon bag of soil will be sent to Energy Laboratories for chemical, physical, and pCu analyses. The six 2-gallon canvas bag will be sent to Wildlife International Laboratory for the phytotoxicity testing. Rocks will be removed in the field, and the soil will be sieved to 2 millimeters prior to potting in the laboratory.

In the 1999 study, the factors listed in Table 1 were measured and found to be weakly correlated with phytotoxicity endpoints, except measured pCu and soluble copper (Table 2.3-1 in NewFields 2005). The physical and chemical constituents to be sampled in the current study are listed in Table 5. This list is a subset of the 1999 list of constituents plus additional parameters that might affect the phytotoxicity results, such as pH (in a saturated paste) and cations (exchangeable calcium, magnesium, sodium, and potassium; and soluble aluminum, iron, and manganese) that might compete for cation exchange sites in the soil (Stevens et al. 2003) and ligands on the root (Kinraide et al. 2004, and see Thakali et al. 2006a and 2006b soil biotic ligand model). Salinity (as measured by electrical conductivity [EC] in a saturated paste) can decrease or increase toxicity (Stevens et al. 2003, Warne et al. 2008). Nutrients that may be limiting (nitrogen [represented as nitrate + nitrite], phosphorus, potassium, base cations), soil texture, and soil organic matter also affect plant growth. Some analytes were included, such as dissolved organic carbon (DOC), to re-evaluate correlations in the 1999 study (shown in Table 2.3-1 in NewFields 2005).

Because pCu will be measured using the same method as the 1999 phytotoxicity study (i.e., in a $CaCl_2$ "soil paste" using a cupric ion selective electrode), soil characteristics (e.g., cation exchange capacity), and total soil concentrations of aluminum, iron, and manganese (when present as oxides) that can bind/sorb the cupric ion and thus decrease its bioavailability, do

[1] STS-PT-2013-24 has been included to represent the conditions present on the western portion of the STSIU. This area has different geology and different grazing practices when compared to the greater STSIU. Thus, the results from this location will be used to evaluate the Site soil collected on the west side of Highway 180. STS-PT-2013-24 will be statistically compared to the other seven De Minimus locations to determine if it should be included in the overall De Minimus dataset for eastern soils.

not need to be measured. These characteristics are not needed because the chemical activity of the free ion (which by definition already takes binding by those solid phases into account) will be directly measured (Appendix A - Cupric Ion Sampling Standard Operating Procedure [SOP]).

Soluble copper and pH will also be measured in the $CaCl_2$ extract used for pCu measurements because the 1999 study found that those two analytes correlated to phytotoxicity endpoint results. EC will also be measured in the $CaCl_2$ extract for comparison to the EC measured in the saturated paste. Total soil copper concentration will be measured because it was incorporated in the pCu-predictor equation in the 1999 study. Total soil $CaCO_3$ concentration will be measured because it is an index of acid-neutralizing capacity of the soil, and soil moisture will be measured to allow dry-weight normalization of total soil analytes.

Dissolved concentrations of alkalinity (representing bicarbonate and carbonate concentrations), chloride, fluoride, sulfate, and DOC in soil extracts will be measured to interpret their potential contributions to soil solution geochemistry that might be needed to explain results and evaluate salinity effects.

The Site-wide ERA (NewFields 2005) suggested that herbaceous species are at higher risk from low pCu than woody species; therefore, in the interest of conservatism, the phytotoxicity tests will evaluate sideoats grama (*Bouteloua curtipendula*) and scarlet globemallow (*Sphaeralcea coccinea)*, which are native grass and forb species, respectively, common to the STSIU. Chino will provide wild site seeds of the sideoats grama and scarlet globemallow to the phytotoxicity laboratory. A 10-acre area (see red box shown on Figure 1) will be protected from grazing in the summer and fall of 2013 to increase the potential for seed availability[2]. Seeds will be collected from the protected location and, if necessary to obtain enough seeds, other adjacent locations (see Appendix B – Seed Collection SOP). Seed collection will occur after the monsoon season, when seeds of these species have ripened.

Wild-collected site seeds will be tested against seeds of the same species obtained from a nursery or seed supplier to evaluate if Site seeds demonstrate greater tolerance to higher copper concentrations. Additionally, the standard agricultural test species, alfalfa (*Medicago sativ*a), the species upon which the PEL was based (had more data in 1999 than ryegrass), will be tested to compare to the 1999 results that used alfalfa seeds. The same alfalfa cultivar used in 1999 will be evaluated in this study (Nitro Plus acquired from Terrestrial Seed Company with a germination of 87 percent). The selected test species at nurseries have PLS of 85 percent for sideoats grama (Bamert Seeds in Texas has cultivars from New Mexico) and 71 percent for scarlet globemallow (Prairie Moon Nursery in Minnesota collected wild seed in South Dakota).

[2] Though the study design has the potential to be improved by adding seed collection areas located in varying pCu areas, the proposed study design has been selected to eliminate confounding factors between seeds while maintaining a reasonable sample size.

The 1999 study was based on ASTM International (ASTM 1998) protocols (with modifications) that only addressed select agricultural species and are now outdated. Thus, the best protocols currently available were evaluated and selected for this study. Phytotoxicity tests will be conducted by Wildlife International Laboratory in accordance with the Organization for Economic Cooperation and Development (OECD) protocols (OECD 2006) with some modifications (specified in Appendix C - Wildlife International SOP) and in accordance with Environment Canada (2007a) for grama grasses and alfalfa unless otherwise specified in this plan and Appendix C. For species not covered under any guidance, information on the species from the literature will guide seed preparation protocol, test conditions for emergence and study time frame (outlined in Appendix B). Because of temperature constraints at Wildlife International greenhouse facilities, all three species will be grown at ~20 degrees C in the same room, which meets alfalfa's requirements and is a compromise for the warm (sideoats grama) and cold (scarlet globemallow) season species (Appendix C). The tests will evaluate seed germination, survival, and early growth with the following endpoints assessed at the end of the test:

- Seedling emergence (i.e., germination/emergence success rate)
- Shoot length and weight (dry weight)
- Root length
- Percent survival

Rhizobium root nodules, which were tested in 1999, are not included because the native species proposed in the work plan do not have such nodules. Root weight is also not included because it is difficult to accurately measure, which reduces power to detect differences. It also produced results similar to root length results (in the DEL and PEL) and was correlated to shoot weight in the 1999 study ($r = 0.92$ and 0.82 for alfalfa and ryegrass, respectively). Moreover, root measurements are not required by OECD testing guidance.

The phytotoxicity test will consist of ten 4-inch-diameter pot replicates for each species and each of the 33 soil samples. Following the 1999 phytotoxicity study (NewFields 2005), each replicate includes 12 seeds of the selected species. Seeds will be planted in Site, De Minimus, and Control soils. Soil pH of each pot for a given location will be sampled and averaged prior to planting and at the end of the test period. Pots will be manually watered from above using filtered tap water with its pH decreased to approximately 6 to mimic rainfall pH on the STSIU prior to irrigation (ARCADIS 2008; see Appendix C for well water chemistry). Following the 1999 phytotoxicity study approach (Schafer and Associates 1999), tests for alfalfa and sideoats grama will be conducted for 14 days past the time when greater than 50 percent of plants in control soils have germinated. Such a time frame (a total of 18 to 21 days if emergence takes 4 to 6 days) is slightly shorter than the 21 days recommended as sufficient for evaluating growth of alfalfa and range grasses (Environment Canada 2007a); however, the 1999 phytotoxicity test produced adequate results to detect differences, and this study is designed to be similar to the 1999 study in order to be able to compare results. The forb tests will be conducted over a similar time frame if enough growth occurs during that time period to facilitate measurements. If not, the number of days of the test will be lengthened. Communication will be maintained with the laboratory during the study to

discuss the need for thinning individual pots if overcrowding becomes a problem in some pots but not others during the study.

2.2 Phytotoxicity Data Analysis

Chino will use phytotoxicity test results to better define the pCu that reduces and/or inhibits recruitment and growth. Following the approach taken in the Site-wide ERA (NewFields 2005), a DEL and PEL threshold will be identified. The 1999 study fit one-variable linear regressions to the phytotoxicity data to identify the strongest predictors of changes in laboratory phytotoxicity results (endpoints). However, models based on nonlinear relationships are typically preferred over linear relationships for describing concentration-response relationships if sample size is sufficient to develop such a curve. An S-shaped (logistic) curve rather than a linear relationship was observed in the 1999 phytotoxicity results and, therefore, Chino will fit three non-linear concentration-response curves (one each for alfalfa and means of nursery species and wild site species[3]) to data relating the seedling endpoint values of each Site and De Minimus soil to the respective soil's pCu. The choice of the equation for the non-linear curve will be determined following the procedures outlined in Environment Canada (2007b). The response variable will be control-normalized (divided by average in potting soil control for same seed type). After standardizing endpoints to percent of control values, Chino will evaluate the hypothesis that seed type will significantly affect the relationship between pCu and endpoints (the null hypothesis is that seed type has no effect). In SAS software, the significance and effect of seed type (independent variable) on the phytotoxicity endpoint (dependent variable) will be evaluated as a categorical factor, with pCu as the continuous independent covariate in a non-linear regression that best fits the data and covariates. Differences in the endpoint curves in the uncertain effects region (e.g., between IC5 and IC95; Environment Canada 2007b) will be considered biologically meaningful if they show at least a 10 percent significant change relative to the alfalfa curve at $p \leq 0.05$. Differences of less than 10 percent are generally not considered biologically relevant even if statistical significance is demonstrated (ASTM 2009).

The DEL is defined as the threshold at which effects begin. Variability in standardized toxicity tests in soils often makes it difficult to calculate precise EC10 or IC10 values (Wentsel and Fairbrother *in press*) as DELs. Consequently, a higher effects value (EC20 or EC25) is often recommended as a threshold if the data are highly variable. After evaluating the adequacy of the data in detecting EC10, EC20, or EC25 values using confidence intervals, a DEL will be identified from one of these thresholds. As was done in the 1999 phytotoxicity tests, differences between the mean of the eight De Minimus soil results for each seed type and each of the 25 Site soil samples (paired with the same seed type) will be tested for significance with a one-tailed two-sample t-test to provide additional information on De Minimus effects of pCu in areas far from contaminant sources.

[3] The forb and grass results will be evaluated separately before averaging the results. If the two species show different responses to the Site and Reference soil, five individual non-linear dose response curves will be evaluated (alfalfa, nursery grass, nursery forb, wild grass, wild forb).

Covariate variables in Table 5 plus other physical variables such as bedrock (presence or absence), slope, aspect, and elevation will be added to the model if necessary. Power to detect differences decreases if too many covariates are added to the non-linear model. If heterogeneity for any soil chemical or physical parameter in Table 5 is high enough to potentially impact results, those parameters will be added as additional covariates in the non-linear model in order to remove them as potential confounding factors. However, if a physico-chemical parameter is found to be relatively constant across all soils, and/or its range of values is unlikely to directly or indirectly affect plant growth or survival based on the literature, it may be eliminated from consideration as a covariate.

Using variance and R^2 estimates from the 1999 phytotoxicity study, the current study was designed to provide high power and confidence in the results for both the t-test and non-linear regression. The design for the t-test (minimum n=10 pot replicates) to detect the DEL provides approximately 95 percent confidence with 80 percent power that a difference of 15 percent between the De Minimus and Site soil means (in DEL range of > IC50 pCu) will be statistically detected for the majority of samples ($\geq$ 2/3). An ability to have high power and confidence to detect a 15 percent difference for the majority of samples is better than most toxicity tests (Denton et al. 2003). The sample sizes for the non-linear regression (e.g., one categorical seed type factor and a 3 variable logistic curve) were designed such that the 33 locations should provide 95 percent confidence that the study will provide at least 80 percent power to detect a relationship with a multiple R^2 of at least 0.32 between predicted (modeled) and observed data. The R^2 for 1999 phytotoxicity endpoints based on a much smaller sample size in the Site-wide ERA (NewFields 2005), fit to non-linear 3-variable logistic curves, was at least 0.23. Excluding root length's R^2 of 0.23, the R^2 for the other endpoints ranged from 0.48 to 0.83 in the 1999 study.

3.0 Vegetation Community Analysis

Vegetation parameters at 17 STSIU and five Reference locations were sampled to calibrate remote sensing image data and ground-truth vegetation maps developed from those images in 2011 during the STSIU FS sampling effort (Figure 2). These 22 locations were sampled for vegetation cover, richness, and rangeland condition as an observed apparent trend (OAT) score (Table 7), following the protocol outlined in the FS Proposal (ARCADIS 2011) and in the more specific ARCADIS vegetation sampling SOP (ARCADIS 2012). Slope position and aspect were also recorded.

To determine if correlations exist among the vegetation community parameters and pCu, soil was collected at these 22 locations in July 2013 and submitted to ACZ Laboratories for total copper (mg/kg) and pH (saturated paste) analysis (methods in Table 5). These same soil samples will also be submitted to Energy Laboratory to measure pCu using the electrode method in Appendix A. Sampling procedures were as follows.

- Soil was sampled in the corners and at the center of the 100 x 100 foot plot (five samples total) in which vegetation had been sampled in 2011 and then composited.
- Copper and pH results were used to estimate pCu applying the "upland with reference" equation in the Site-wide ERA (NewFields 2005). Copper concentrations ranged from 96 to 1,640 mg/kg and pH ranged from 3.9 to 7.8 standard units (SU); these results are consistent with historical STSIU results (Table 8).
- Percent exposed bedrock at each site was recorded because of its strong influence on vegetation cover.
- The calculated pCu and measured pCu results will be plotted against the OAT, richness, and cover results to describe any significant linear or non-linear relationships, while controlling for the influence of aspect and percent exposed bedrock. Because aspect (south and north facing) and percent bedrock influence vegetation richness and cover, they will be added as covariates in the regression.

The 22 locations should provide 95 percent confidence that the study will provide at least 80 percent power to detect a multiple R^2 of at least 0.3 between the vegetation parameter and pCu (and two covariates), if such a relationship exists. Covariates other than bedrock and aspect that might influence the results will be added to the regression only if significant (and do not create multicollinearity). Site characteristics considered will include slope, topographic position, or soil characteristics based on mapped soil type in Site-wide ERA. Differences between the mean of the Reference Site results and each of the 18 Site samples (after adjusting for significant covariates) will be tested for significance with a one-tailed, one-sample t-test to identify those not significantly different and to define the DEL based on community parameters. The data and analysis for these samples will be included in the FS.

4.0 Schedule

Chino plans to implement the proposed seed and soil collection during fall 2013. Soil for the vegetation community analysis was collected and analysed by ACZ Laboratories in July 2013. Immediately after collection, Site seeds are sent to Growing Solutions Restoration Education Institute for drying and storage. Site and De Minimus soil is provided to Energy Laboratories for analysis of all factors in Table 4. Seeds (collected and purchased) and sieved soil are provided to Wildlife International Laboratory for phytotoxicity testing, which will commence in the first quarter of 2014. The results of the phytotoxicity study and vegetation community analysis will be provided and discussed with NMED in the third quarter of 2014. The final results will be presented in an appendix to the STSIU FS Report, with a proposed completion date during 2014.

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TABLES

TABLE 1
SOIL SAMPLE ANALYTICAL ANALYSES CONDUCTED FOR 1999 STUDIES

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO
SMELTER/TAILING SOILS IU PHYTOTOXICITY STUDY

Parameter	Units
Bicarbonate as CaCO3	SPLP--mg/L
Carbonate as CaCO3	SPLP--mg/L
Hydroxide as CaCO3	SPLP--mg/L
Total Alkalinity	SPLP--mg/L
Carbon, dissolved organic	SPLP--mg/L
Chloride	SPLP--mg/L
Nitrate/Nitrite as N	SPLP--mg/L
Phosphorus, ortho dissolved	SPLP--mg/L
Sulfate	SPLP--mg/L
% Solids	%
Nitrogen, total Kjeldahl	%
Organic Matter	%
pH	SU
Phosphorus, total	%
Aluminum	mg/kg and SPLP--mg/L
Antimony	mg/kg and SPLP--mg/L
Arsenic	mg/kg and SPLP--mg/L
Barium	mg/kg and SPLP--mg/L
Boron	mg/kg and SPLP--mg/L
Cadmium	mg/kg and SPLP--mg/L
Calcium	mg/kg and SPLP--mg/L
Chromium	mg/kg and SPLP--mg/L
Copper	mg/kg and SPLP--mg/L
Iron	mg/kg and SPLP--mg/L
Lead	mg/kg and SPLP--mg/L
Magnesium	mg/kg and SPLP--mg/L
Manganese	mg/kg and SPLP--mg/L
Mercury	mg/kg and SPLP--mg/L
Nickel	mg/kg and SPLP--mg/L
Potassium	mg/kg and SPLP--mg/L
Selenium	mg/kg and SPLP--mg/L
Sodium	mg/kg and SPLP--mg/L
Thallium	mg/kg and SPLP--mg/L
Vanadium	mg/kg and SPLP--mg/L
Zinc	mg/kg and SPLP--mg/L
pCu^{2+}	$CaCl_2$ and DI extraction
Soluble copper	$CaCl_2$ extraction mg/L

TABLE 2

SAMPLE SIZES IN EACH CUPRIC ION ACTIVITY RANGE

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO
SMELTER/TAILING SOILS IU PHYTOTOXICITY STUDY

pCu Range	# of Locations
2 to 3	4*
3 to 4	5
4 to 5	7
5 to 6	4
6 to 7	3
7 to 10	2

Notes:
pCu - Estimated Cupric ion activity
Table does not include 8 De Minimus soils

TABLE 3
MANUFACTURED CONTROL SOIL CHARACTERIZATION

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO
SMELTER/TAILING SOILS IU PHYTOTOXICITY STUDY

Parameter	Result	
Sand, Percent	89	
Silt, Percent	3	
Clay, Percent	8	
USDA Textural Class	Loamy Sand	
Bulk Density, disturbed (gm/cc)	1.23	
Cation Exchange Capacity (meq/100 g)	3.9	
Moisture at 1/3 Bar (%)	8.5	
Moisture at 15 Bar (%)	4.5	
Organic Carbon - Walkley Black (%)	0.56	
Organic Matter - Walkley Black (%)	0.96	
pH in 1:1 soil:water ratio	7.2	
pH in 0.01M $CaCl_2$ (1:2)	6.7	
Olsen Phosphorus (ppm)	21	
Total Nitrogen (Analyzer) (%)	<0.01	
Soluble Salts (mmhos/cm)	0.49	
Base Saturation Data by Cation:	**(%)**	**(ppm)**
Calcium	70.4	546
Magnesium	10.3	48
Sodium	1.6	14
Potassium	3.3	50
Hydrogen	14.5	6

TABLE 4
SOIL SAMPLE SIZE AND QUANTITIES

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO
SMELTER/TAILING SOILS IU PHYTOTOXICITY STUDY

		Grama Species		Forb		Alfalfa	Total per Sample	Total	Total Needed Soil (gallons)
		Wild	Nursery	Wild	Nursery	Nursery			
Site Soil	Pots (#)	10	10	10	10	10	50	1250	--
	Seeds (#)	120	120	120	120	120	600	15000	--
	Soil (cups)	30	30	30	30	30	150	3750	201
Reference Soil	Pots	10	10	10	10	10	50	400	--
	Seeds	120	120	120	120	120	600	4800	--
	Soil (cups)	30	30	30	30	30	150	1200	64
Control Soil	Pots	10	10	10	10	10	50	50	--
	Seeds	120	120	120	120	120	600	600	--
	Soil (cups)	30	30	30	30	30	150	150	8
Total	Pots	30	30	30	30	30	150	**1700**	--
	Seeds (Collected)	360	--	360	--	--	720	**8160**	--
	Seeds (Purchased)	--	360	--	360	360	1080	**12240**	--
	Soil Collected (no artificial soil)	90	90	90	90	90	450	**5100**	**274**

Notes:
Assumes 25 site locations, 8 reference locations, and 1 replicate for control soil
4.5" pots will require 3 cups of soil each
12 seeds will be planted per pot

TABLE 5
SOIL SAMPLE ANALYTICAL ANALYSES

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO
SMELTER/TAILING SOILS IU PHYTOTOXICITY STUDY

Parameter	Extraction Method	Analytical Method
Alkalinity (total)	ASA Mono #9, Part 2, 10-2.3.1	A 2320B
Chloride	ASA Mono #9, Part 2, 10-3.2	E300.0
Fluoride	ASA Mono #9, Part 2, 10-3.2	A 4500 F-C/Technicon 380-7WE
Exchangeable Calcium (NH4Oac)	ASA Mono #9, Part 2, 13-4	6010/6020
Exchangeable Copper (NH4Oac)	ASA Mono #9, Part 2, 13-4	6010/6020
Exchangeable Magnesium (NH4Oac)	ASA Mono #9, Part 2, 13-4	6010/6020
Exchangeable Potassium (NH4Oac)	ASA Mono #9, Part 2, 13-4	6010/6020
Exchangeable Sodium (NH4Oac)	ASA Mono #9, Part 2, 13-4	6010/6020
Sulfate (soluble)	ASA Mono #9, Part 2, 10-3.2	6010/6020
Copper (total)	3050	6010B
Copper (soluble), $CaCl_2$	ARCADIS SOP	ARCADIS SOP
Aluminum (soluble)	ASA Mono. #9, Part 2, Method 19-3.3	6010/6020
Iron (soluble)	ASA Mono. #9, Part 2, Method 19-3.3	6010/6020
Manganese (soluble)	ASA Mono. #9, Part 2, Method 19-3.3	6010/6020
Nitrate/Nitrite, $CaCl_2$	ASA Mono. #9, Part 2, Method 38-8.1	350.1, 353.2, 351.4
pH (saturated paste with saturated %)	ASA Mono #9, Part 2, 10-3.2	9045C
pH, $CaCl_2$	ARCADIS SOP	ARCADIS SOP
Plant Available Phosphorus (Bray/Olsen)	ASA Mono. #9, Part 2, Method 24-5.1	365.1
Phosphate	ASA Mono. #9, Part 2, Method 24-5.3	365.1
Electrical Conductivity, saturated paste	ASA Mono. #9, Part 2,Method 10-3.3	ASA Mono #9 Part 2
Electrical Conductivity, $CaCl_2$	ARCADIS SOP	ARCADIS SOP
Total Organic Matter	ASA Mono. #9, Part 2, Method 29-3.5.2	Handbook 60
DOC	ASA Mono. #9, Part 2, Method 10-3	ASA Mono #9 Part 2
Soil Texture	ASA Mono. #9, Part 1, Method 15-4	NAPT S-10.10[1]
$CaCO_3$	USDA Handbook 60, Method 23C	Handbook 60
measured pCu	ARCADIS SOP	ARCADIS SOP
Moisture (dry basis)	USDA Handbook 60,Method 26	

TABLE 6
STSOI FS VEGETATION COMMUNITY RESULTS

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO
SMELTER/TAILING SOILS IU PHYTOTOXICITY STUDY

Location	Rangeland (OAT)	Mean Cover	Richness
STS-RWU-2011-1	12	5.5	1
STS-RWU-2011-2	8	7.9	0
STS-RWU-2011-3	24	59.0	6
STS-RWU-2011-4	35	63.9	10
STS-RWU-2011-5	33	34.1	10
STS-RWU-2011-6	16	25.0	7
STS-RWU-2011-7	9	10.9	2
STS-RWU-2011-8	37	45.0	22
STS-RWU-2011-9	11	2.5	1
STS-RWU-2011-10	16	24.3	10
STS-RWU-2011-11	6	4.3	2
STS-RWU-2011-12	10	9.2	2
STS-RWU-2011-13	8	26.0	4
STS-RWU-2011-14	26	26.7	8
STS-RWU-2011-15	14	17.6	7
STS-RWU-2011-16	23	22.4	13
STS-RWU-2011-17	10	35.8	5
Wildlife Reference South	--	14.6	11
Wildlife Reference North	--	30.0	13
STS-RWU-2012-B1	17	17.6	13
STS-RWU-2012-B2	11	2.5	14
STS-RWU-2012-B3	15	3.4	10

TABLE 7
VEGETATION COMMUNITY SOIL SAMPLE ANALYTICAL RESULTS

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO
SMELTER/TAILING SOILS IU PHYTOTOXICITY STUDY

Location	Copper (mg/kg)	pH (SU)	pCu (SU)
1# WEST 0-6	372	7.8	7.8
STS-RWU-2011-1 0-6	338	5.2	5.5
STS-RWU-2011-2 0-6	381	4.1	4.3
STS-RWU-2011-3 0-6	998	5.1	4.1
STS-RWU-2011-4 0-6	427	7.2	7.1
STS-RWU-2011-5 0-6	779	4.6	4.0
STS-RWU-2011-6 0-6	1300	7.3	5.9
STS-RWU-2011-7 0-6	529	4.9	4.7
STS-RWU-2011-8 0-6	287	5.6	6.0
STS-RWU-2011-9 0-6	560	4.4	4.2
STS-RWU-2011-10 0-6	96	4.6	6.4
STS-RWU-2011-11 0-6	216	4.3	5.2
STS-RWU-2011-12 0-6	316	3.9	4.3
STS-RWU-2011-13 0-6	305	5.6	6.0
STS-RWU-2011-14 0-6	1640	5.3	3.8
STS-RWU-2011-15 0-6	1640	5.7	4.1
STS-RWU-2011-16 0-6	395	4.9	5.0
STS-RWU-2011-17 0-6	654	4.6	4.2
WILDLIFE REF NORTH 0-6	213	5.9	6.7
WILDLIFE REF SOUTH 0-6	288	4.6	5.1
STS-RWU-2012-B1 0-6	182	4.6	5.6
STS-RWU-2012-B2 0-6	344	4.7	5.0
STS-RWU-2012-B3 0-6	161	4.7	5.9

Notes:
mg/kg - milligram per kilogram
SU - standard unit

FIGURES

OFFICE: LAKEWOOD DB: MLM TM: MB PM: AT
PROJECT# B0063543.0000
I:\FCX\Chino\DT_2013\Fig1_SmelterSampleLocs.mxd

P1
152
356
180
WEST STOCKPILE
SANTA RITA OPEN PIT
LAMBRIGHT STOCKPILE
SOUTH STOCKPILE
SANTA CLARA
BAYARD
HURLEY
TAILING POND B
TAILING POND 4
TAILING POND C
TAILING POND 6W
TAILING POND 6E
TAILING POND 7
SILVER CITY
GRANT COUNTY AIRPORT
ATCHISON, TOPEKA AND SANTE FE RAILROAD
STS-PT-2013-15
STS-PT-2013-31
STS-PT-2013-5
STS-PT-2013-32
STS-PT-2013-16
STS-PT-2013-36
STS-PT-2013-9
STS-PT-2013-10
STS-PT-2013-35
STS-PT-2013-19
STS-PT-2013-17
STS-PT-2013-20
STS-PT-2013-1
STS-PT-2013-3
STS-PT-2013-8
STS-PT-2013-2
STS-PT-2013-29
STS-PT-2013-13
STS-PT-2013-11
STS-PT-2013-6
STS-PT-2013-14
STS-PT-2013-24
STS-PT-2013-12
STS-PT-2013-30
STS-PT-2013-7
STS-PT-2013-21
STS-PT-2013-22
STS-PT-2013-26
STS-PT-2013-25
STS-PT-2013-28
Seed Collection Area Protected From Grazing
STS-PT-2013-4
STS-PT-2013-27
STS-PT-2013-23

LEGEND:

- Site Sample Locations
- De Minimus Sample Locations
- Seed Collection Area Protected From Grazing
- STSIU Boundary

0 6,200 12,400
SCALE IN FEET

Notes:
1) Service Layer Credits: APFO

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO

SMELTER TAILING SOILS IU PHYTOTOXICITY AND VEGETATION COMMUNITY STUDY

PHYTOTOXICITY SAMPLING LOCATIONS

ARCADIS

FIGURE 1

OFFICE: LAKEWOOD DB: MLM TM: MB PM: AT
PROJECT# B0063543.0000
G:\AProject\FCX\Chino\Chino_GIS\Fig2_Vegetation_CommSamples_20140110.mxd

STS-RWU-2011-4
STS-RWU-2011-15
STS-RWU-2011-1
STS-RWU-2011-8
STS-RWU-2011-6
STS-RWU-2011-3
STS-RWU-2011-5
#1 West
STS-RWU-2011-14
STS-RWU-2011-16
STS-RWU-2011-2
HURLEY
STS-RWU-2011-7
STS-RWU-2011-9
Wildlife Reference Plot North
STS-RWU-2011-13
Wildlife Reference Plot South
STS-RWU-2011-17
STS-RWU-2011-11
STS-RWU-2011-10
STS-RWU-2012-B3
STS-RWU-2012-B1
STS-RWU-2012-B2
TAILING POND B
TAILING POND 4
TAILING POND C
STS-RWU-2011-12
TAILING POND 6W
TAILING POND 6E
TAILING POND 7

Legend

- De Minimus Soil Locations
- Sample Location
- Operations Boundary
- Railroad
- Town Roads
- Major Roads
- City Limits

0 3,200 6,400 Feet

GRAPHIC SCALE

Service Layer Credits: APFO

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO

SMELTER TAILING SOILS IU PHYTOTOXICITY AND VEGETATION COMMUNITY STUDY

VEGETATION COMMUNITY SAMPLING LOCATIONS

ARCADIS

FIGURE
2

APPENDIX A

Cupric Ion Sampling Standard Operating Procedure

ARCADIS
Infrastructure · Water · Environment · Buildings

Imagine the result

Freeport McMoRan Copper and Gold
Chino Mines Company
Grant County, New Mexico

Standard Operating Procedures for Measurement of Cu^{2+} Activity in Soil by Ion-Selective Electrode

September 2013

Standard Operating Procedures for Cu2+ Activity in Soil

Prepared for:
Chino Mines Company
Grant County, New Mexico

Prepared by:
ARCADIS U.S., Inc.
Suite 200
Lakewood
Colorado 80401
Tel 303 231 9115

Date:
September 2013

Standard Operating Procedures for Cu^{2+} Activity in Soil

Chino Mines Company
Grant County, New Mexico

1. Scope and Application

This Standard Operating Procedure (SOP) summarizes the laboratory methods used to measure free cupric ion (Cu^{2+}) activity in soil solutions extracted from Chino Mine site soil samples.

2. Methods

2.1 Equipment and Supplies

1. Thermo Scientific Orion ISE meter, such as the 4-Star pH/ISE meter (Catalog Number 1115001 – 1115004) or 5-Star pH/ISE/DO/conductivity meter (Catalog Number 1119001, 1010152, 1119201); an equivalent ISE meter; or a mV meter with a 0.1 mV resolution. Cupric electrodes can be used on any ISE or mV meter with a BNC connection. The electrodes can also be used on meters with a variety of inputs when an adapter cable is used. Visit www.thermo.com/water for details.
2. Thermo Scientific Orion cupric electrode. The 9429BN cupric half-cell electrode shall be utilized and requires a separate reference electrode (Catalog Number 900200).
3. Magnetic stirrer or Thermo Scientific Orion stirrer probe (Catalog Number 096019). The stirrer probe can be used with 3-Star, 4-Star, and 5-Star benchtop meters.
4. Acid-washed plastic (preferably polystyrene) volumetric flasks, graduated cylinders, and beakers. Acid-washed plastic labware is required for low-concentration Cu^{2+} analysis.
5. Distilled/deionized water (d. H_2O).
6. Cupric electrode filling solution. Use inner chamber filling solution (standard calomel electrode saturated KCl buffer), and outer chamber filling solution (10% w/v KNO_3), for the double junction reference electrode that is used with the 9429BN cupric half-cell electrode.
7. 0.1 M $Cu(NO_3)_2$ calibration standard (Catalog Number 942906).
8. Cupric ionic strength adjuster (ISA), (Catalog Number 940011).
9. $Cu(NO_3)_2$, $CaCL_2$ (Aldrich® calcium chloride hydrate 99.99+%), iminodiacetic acid, potassium acid phthalate ($KHC_8H_4O_4$), Na_4EDTA, NaOH, H_2SO_4, and HNO_3. All reagents should be of highest purity possible, including using trace-metal-grade HNO_3.
10. Reciprocal shaker or other device for shaking 50-ml centrifuge tubes.
11. 0.22-micron cellulose acetate membrane filters.
12. Thermometer.

2.2 Sample Preparation

Air dry the soil samples and sieve each sample through a 2-mm sieve before preparing the soil-solution extracts.

2.3 Soil-Solution Extract

Prepare a 0.01 molar (M) $CaCl_2$ solution in d. H_2O. That $CaCl_2$ solution will be used to extract the soil at a 1:2 soil:$CaCl_2$ solution ratio. For each soil sample, place 15.0 g of soil into a 50-ml screw-cap polyethylene centrifuge tube. Add 30 ml of 0.01M $CaCl_2$ solution and shake for 20 min (e.g., on a reciprocal shaker at 250 rpm). Then centrifuge the tube at 5000 rpm for 10 min and filter the supernatant through a 0.22-micron membrane cellulose acetate filter (pre-rinsed with 100 ml of 0.01 M $CaCl_2$ solution). Rinse the walls of a plastic sample container with 5 ml of the filtered soil extract, and discard that water. Then store the remaining filtrate in the rinsed container for Cu^{2+} determination.

2.4 Ion-Selective Electrode (ISE) Setup

Set-up the ISE according to the Thermo Scientific Orion® Cupric Ion Selective Electrode User Guide (User Guide). The methods summarized below are extracted from the User Guide (included as Attachment A). Refer to the manufacturer's User Guide for additional, more-detailed information as needed. Note that products referenced via catalog numbers below can be found at the Thermo Scientific website (www.thermoscientific.com).

2.4.1 Electrode Preparation

9429BN Cupric Half-Cell Electrode – Remove the protective shipping cap from the sensing element and save the cap for storage.

900200 Double Junction Reference Electrode – Prepare the reference electrode according to the reference electrode user guide. Fill the reference electrode with inner chamber filling solution (standard calomel electrode saturated KCl buffer), and outer chamber filling solution (10% w/v KNO_3). The reference electrode's inner chamber filling solution and outer chamber filling solution will be changed daily.

When not in use, store the electrodes in d. H_2O.

2.4.2 Checking Electrode Operation (Slope)

These are general instructions that can be used with most meters to check the electrode operation. Refer to the meter user guide for more specific information. This procedure measures electrode slope. Slope is defined as the change in millivolts observed per ten-fold change in concentration. The slope provides the best means of checking electrode operation.

1. If the electrode has been stored dry, prepare the electrode as described in the Electrode Preparation section (2.3.1).
2. Connect the electrode cable to a meter with a mV mode. Set the meter to the mV mode.
3. Add 100 ml of distilled water and 2 ml of ISA into a 150-ml beaker. Stir the solution thoroughly.
4. Rinse the electrode with distilled water and place the electrode into the solution prepared in step 3.
5. Pipet 1 ml of the 0.1 M $Cu(NO_3)_2$ standard into the beaker (from Step 3) and stir the solution thoroughly. When a stable reading is displayed (i.e., less than or equal to 0.3 mV variation over a 3-minute period), record the electrode potential in millivolts (mV).
6. Pipet 10 ml of the same standard into the same beaker and stir the solution thoroughly. When a stable reading is displayed, record the electrode potential in mV.
7. There should be a 25 to 30 mV difference between the two millivolt readings when the solution temperature is between 20 to 25 °C. If the millivolt potential is not within this range, refer to the Troubleshooting section of the User Guide. Do not proceed with calibration of the electrode and analysis of samples until an acceptable electrode response is obtained.

2.4.3 Sample Requirements

Samples and standards should be maintained at the same temperature (+/-1 °C). Normal room temperature is acceptable, but that temperature must be maintained during an entire set of analyses (e.g., during an entire day, or at the least during the entire time period in which a calibration curve is generated and the batch of samples referenced to that calibration curve are run). A 1 °C difference in temperature for a 10^{-3} M cupric ion solution will give rise to about a 4% error.

2.4.4 Measuring Hints

- Make the electrode measurements under a constant, reduced light intensity.
- Stir all standards and samples at a uniform, moderate rate. Place a piece of insulating material, such as Styrofoam or cardboard, between the magnetic stir plate and beaker to prevent measurement errors from the transfer of heat to the sample.
- Always use freshly prepared standards for calibration.
- Always rinse the electrode with d. H_2O between measurements, and shake the electrode to remove the water and prevent sample carryover.
- Do not wipe or rub the electrode sensing element.

- Allow all standards and samples to reach the same temperature for precise measurements.
- Concentrated samples (greater than 10^{-1} M cupric) should be diluted before measurement.
- After immersing the electrode in a solution, check the electrode sensing surface for air bubbles and remove air bubbles by re-immersing the electrode in the solution and gently tapping it.
- The fill hole cover must be open during measurements to ensure a uniform flow of filling solution.
- If the combination electrode is used and the electrode is used in dirty or viscous samples or the electrode response becomes sluggish, empty the electrode completely, hold the junction open and flush the junction with d. H_2O. Empty any water from the electrode and refill it with fresh filling solution. Press down on the electrode cap to let a few drops of the filling solution flow out of the electrode and then replenish any lost solution.
- The electrode potential plotted against calibration concentration should result in a straight line with a slope of 25 to 30 mV per decade change in concentration.
- The time response of the electrode (the time required to reach 99% of the stable potential reading) varies from several seconds in concentrated solutions to several minutes near the limit of detection; and in soil extracts that have very low Cu^{2+} activities, the electrode equilibration is very slow and might take more than two hours in the lowest-activity samples. To speed up the equilibration time of the electrode and to prevent memory effects, analyze soil extracts in the order of increasing Cu^{2+} activities, as established with either a preliminary pCu determination or estimated from other soil-extraction and Cu analysis data.
- Because Cu can sorb to container walls, rinse the walls of all sample containers with a small volume of the sample (e.g., a calibration standard or a soil extract) before storing the sample in that container.
- For specific details concerning Electrode Response and other electrode characteristics (including Reproducibility, Limits of Detection, Temperature Effects, Interferences, pH Effects, Complexation, and Theory of Operation), refer to the User Guide.

2.4.5 Electrode Maintenance and Storage

Cupric Half-Cell Electrode Storage (9429BN) and Double Junction Reference Electrode Storage (900200)

Between samples, rinse both electrodes with a flow of d. H_2O, soak two minutes in d. H_2O, and wipe dry with laboratory tissues (but do not touch the electrode sensing element). When storing either electrode for long periods of time, cover the sensing element with the protective shipping cap. The filling solution inside the electrode should not be allowed to evaporate, because it will crystallize. For storage longer than one week, drain the reference electrode, flush the inside with d. H_2O, and store the electrode in d. H_2O.

Polishing the Cupric Half-Cell Electrode

The sensing surface of solid state electrodes can degrade over time, which causes drift, poor reproducibility, and loss of response in low-concentration samples. After each day of use, restore the cupric electrode by polishing the sensing surface for 30 seconds with 3 µm aluminum oxide strips. Then successively soak both electrodes for 5 minutes in 0.025 M H_2SO_4 and 0.1 M Na_4EDTA.

1. Cut off about an inch of the aluminum oxide strip.
2. Hold the electrode with the sensing surface facing up.
3. Place a few drops of d. H_2O on the sensing surface.
4. With the frosted side of the aluminum oxide strip facing down, use light finger pressure to place the polishing strip on top of the sensing surface.
5. Rotate the electrode for about 30 seconds.
6. Rinse the electrode with d. H_2O and store it in d. H_2O.

Cupric Combination Electrode and Double Junction Reference Electrode Flushing

If the area between the electrode sleeve and inner cone becomes clogged with sample or precipitate, flush the area with filling solution or d. H_2O.

1. Hold the electrode body with one hand and use your thumb to push down on the electrode cap to drain the electrode. Push down on the cap until all the filling solution is drained from the chamber.
2. Fill the electrode with d. H_2O and then push down on the cap until all the water is drained from the chamber.
3. Fill the electrode with fresh filling solution up to the fill hole. Push down on the cap to allow a few drops of filling solution to drain out of the electrode and replenish the lost filling solution.

2.5 ISE Analytical Techniques – Electrode Calibration

The electrode calibration described herein follows the methodology in Sauvé et al. (1995) (Attachment B). In that method, the electrode response is calibrated using a series of pH-adjusted, buffered $Cu(NO_3)_2$

solutions. The Cu^{2+} activity in those calibration standards is calculated using the geochemical-speciation program MINEQL+ modified by inclusion of the stability constants listed in Table 1 in Sauvé et al. (1995).

Electrode Calibration Steps

1. Prepare a stock calibration solution containing 1 mM iminodiacteic acid (IDA), 0.1 mM $Cu(NO_3)_2$, 6 mM NaOH, 2.5 mM $KHC_8H_4O_4$, and 0.01 M $CaCl_2$.
2. Each day before starting a new batch of calibration standards and samples, analyze the concentration of dissolved Cu in the stock calibration solution. The dissolved Cu concentration should be determined in stock calibration solution filtered through a 0.22-micron cellulose acetate membrane filter that was pre-rinsed with 100 ml of the stock calibration solution.
3. Prepare 5 calibration standards by adjusting the pH of separate 100-ml volumes of the stock calibration solution to approximately pH 2, 4, 6, 8, and 10 using trace-metal-grade HNO_3. Record the amount of HNO_3 added to each standard. The pH values in the calibration standards only need to approximately equal the nominal pH values, because the exact pH of each calibration standard will be measured after the mV reading on the ISE placed in that standard is recorded. Along with the dissolved Cu concentration of the stock solution, the measured pH will be used to calculate the Cu^{2+} activity of that calibration standard.
4. Set the meter to the mV mode.
5. Rinse the walls of a 20-ml plastic beaker with 10 ml of the first standard [i.e., the least-concentrated standard (highest pH) standard], then discard that water.
6. Add 10 ml of the same standard to the rinsed 20-ml plastic beaker.
7. Rinse the ISE electrodes with distilled water, blot them dry, and insert them into the calibration standard. When a stable mV reading is displayed (i.e., less than or equal to 0.3 mV variation over a 3-minute period), record the mV value.
8. Remove the ISE electrodes from the calibration standard, and then measure and record the temperature and pH of the calibration standard with an Orion® pH meter (SOP-26, SOP-11).
9. From the pH and dissolved Cu concentrations in the stock calibration solution, read the corresponding pCu from Table 1 (interpolating when necessary). Record that pCu value.
10. Repeat steps 5 through 9 for each of the remaining calibration standards, proceeding through consecutively higher Cu^{2+} activity (i.e., through consecutively lower pCu values, which corresponds to consecutively lower nominal pH of the calibration standards).
11. Prepare a pCu calibration curve from the calibration data by plotting the pCu values of the calibration standards on the vertical axis and the corresponding millivolt values on the horizontal axis. In concept, the calibration curve should be linear; however, in practice it might be slightly curvilinear in the high pCu range. If the calibration curve is consistently curvilinear, add intermediate Cu^{2+} activity standards in the curvilinear range to more precisely define the curvilinearity.

12. If the calibration curve is linear, fit a linear-regression equation to the data [with pCu as the dependent variable and mV as the predictor (independent) variable]. If the calibration curve is nonlinear, fit a nonlinear (e.g., polynomial) regression equation to the data. Record the regression equation.

2.6 ISE Analytical Techniques – Measurement of pCu in Soil Extracts

1. After successfully calibrating the ISE electrode, rinse the walls of a 20-ml plastic beaker with 5 ml of the soil extract that is anticipated to have the lowest Cu^{2+} activity (highest pCu), then discard that water.
2. Add 10 ml of the same soil extract to the rinsed 20-ml plastic beaker.
3. Rinse the ISE electrodes with distilled water, blot them dry, and insert them into the soil extract. When a stable mV reading is displayed (i.e., less than or equal to 0.3 mV variation over a 3-minute period), record the mV value.
4. Remove the ISE electrodes from the calibration standard, and then measure and record the temperature and pH of the soil extract with an Orion® pH meter (SOP-26, SOP-11).
5. Calculate the pCu of the soil extract from the electrode calibration curve, and record that value.
6. Repeat steps 1 through 5 for each of the remaining soil extracts, proceeding through consecutively higher anticipated Cu^{2+} activity (i.e., through consecutively lower anticipated pCu of the soil extracts).
7. After every three soil-extract samples, perform a continuing calibration verification (CCV) of the electrode response by measuring one of the intermediate-concentration Cu^{2+}-activity standards used in the calibration procedure (section 2.5). If that mV value has changed by more than 2% from the initial calibration value, recalibrate the electrode and repeat the soil-solution measurements that were conducted since the most recent successful calibration or CCV check.
8. Acidify all the soil extracts that have been analyzed to pH less than or equal to 2 using trace-metal-grade HNO_3. Store those acidified soil extracts and have them analyzed for dissolved Cu concentration.

2.7 ISE Analytical Techniques – Determination of Detection Limit

Determination of an instrument detection limit (IDL) for a Cu-ISE is analogous to the IDL method for other analytical instruments (USEPA 1992). In brief, 7 consecutive pCu analyses are conducted on a sample containing a low concentration of Cu^{2+} (i.e., at or near the expected IDL). The IDL expressed in terms of Cu^{2+} concentration is then calculated as 3 times the standard deviation of those 7 measured Cu^{2+} concentrations; and an IDL can be calculated in terms of pCu by calculating the pCu at that Cu^{2+} concentration. A practical quantitation limit (PQL) that is a lower pCu value (i.e., a higher Cu^{2+} concentration) than the IDL can then be established, if desired.

Detection-Limit-Determination Steps

1. Calibrate the Cu-ISE as instructed in Section 2.5.

2. Rinse the walls of a 20-ml plastic beaker with 10 ml of the least-concentrated Cu^{2+} standard (i.e., the highest pH standard), then discard that water.
3. Add 10 ml of the same standard to the rinsed 20-ml plastic beaker.
4. Rinse the ISE electrodes with distilled water, blot them dry, and insert them into the calibration standard. When a stable mV reading is displayed (i.e., less than or equal to 0.3 mV variation over a 3-minute period), record the mV value.
5. Remove the ISE electrodes from the calibration standard, and then measure and record the temperature and pH of the calibration standard.
6. Using the mV reading recorded in step 4, calculate the pCu of the soil extract from the electrode calibration curve, and record that value.
7. Repeat steps 2 through 6 six more times (i.e., measure the pCu of the least-concentrated Cu^{2+} standard 7 consecutive times, each time using a new subsample of the standard).
8. Convert the 7 measured pCu values to Cu^{2+} activity ($\{Cu^{2+}\}$, in moles/L) using the following equation: $\{Cu^{2+}\} = 10^{-pCu}$.
9. Calculate the standard deviation (S.D.) of the 7 $\{Cu^{2+}\}$ values, and then calculate the IDL in terms of Cu^{2+} activity using the following equation: $\{Cu^{2+}\}$ IDL = 3 × S.D.($\{Cu^{2+}\}$) (also in moles/L).
10. Transform that IDL expressed in terms of Cu^{2+} activity into an IDL expressed in terms of pCu activity using the following equation: pCu IDL = -log($\{Cu^{2+}\}$ IDL).

3. Data Quality

Quality control analyses for the extraction and ion-activity measurement methods should include duplicate extractions for three soil samples and triplicate Cu^{2+}-activity measurements for three extract solutions. Use the results of these quality control analyses to describe the precision and accuracy of the sample measurements.

4. Precision and Accuracy

All quality control measures related to the preparation and use of laboratory containers, reagents, and analysis of extraction solution shall meet guidelines set forth in the Energy Labs Quality Assurance Manual.

5. Reporting

Submit a report that includes:

1. The dates on which the soils were extracted and the dates on which the soil extracts were analyzed for Cu^{2+} activity, pH, and dissolved Cu concentration.
2. For each batch of soil extracts on which the analyses were successfully completed:

   a. Report the temperature, pH, soluble Cu concentration, electric conductivity, and associated Cu^{2+} activity and pCu of all the Cu^{2+} activity standards that were used to construct the calibration curve.
   b. Include a graph of the calibration curve (pCu vs. mV) and the regression equation for that curve.
   c. Report the temperature, dissolved Cu concentration, and the mV reading and corresponding pCu value for each soil extract.
3. A case narrative that lists all deviations from this SOP and any other established sample-processing procedures and laboratory practices.
4. If the IDL was determined:
   a. Report the temperature, pH, soluble Cu concentration, electric conductivity, and associated Cu^{2+} activity and pCu of all the Cu^{2+} activity standards that were used to construct the calibration curve.
   b. Include a graph of the calibration curve (pCu vs. mV) and the regression equation for that curve.
   c. Report the temperature, pH, and the mV reading and corresponding pCu value for each of the 7 consecutive analyses of the Cu^{2+} calibration standard that was used for the IDL determination.
   d. Report the corresponding Cu^{2+} activities calculated from the 7 consecutive pCu values, the calculated standard deviation of those 7 Cu^{2+} activities, and the calculated IDLs expressed as Cu^{2+} activity and as pCu units.

6. References

Sauvé, S., M.B. McBride, and W.H. Hendershot. 1995. Ion-selective electrode measurements of copper(II) activity in contaminated soils. *Archives of Environmental Contamination and Toxicology* 29:373-379.

Sauvé, S.F. 1999. Chemical Speciation, Solubility and Bioavailability of Lead, Copper and Cadmium in Contaminated Soils. Ph.D. Dissertation, Cornell University, Ithaca, NY.

U.S. Environmental Protection Agency (USEPA). 1992. Guidance for Data Useability in Risk Assessment (Part A). Publication 9285.7-09A. U.S. Environmental Protection Agency, Washington, DC.

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Tables

Table 1. pCu [i.e., -log(Cu^{2+} activity)] in iminodiacetic acid (IDA)-based Cu^{2+} calibration standard used by Sauvé et al. (1995), at various pH values (Table 6.2 in Sauvé 1999)

pH	pCu^{2+}	pH	pCu^{2+}	pH	pCu^{2+}	pH	pCu^{2+}
10.00	13.53	8.00	11.43	6.00	8.51	4.00	6.45
9.95	13.52	7.95	11.34	5.95	8.46	3.95	6.40
9.90	13.51	7.90	11.26	5.90	8.40	3.90	6.35
9.85	13.49	7.85	11.17	5.85	8.35	3.85	6.29
9.80	13.48	7.80	11.08	5.80	8.30	3.80	6.24
9.75	13.46	7.75	11.00	5.75	8.24	3.75	6.18
9.70	13.44	7.70	10.91	5.70	8.19	3.70	6.13
9.65	13.42	7.65	10.82	5.65	8.14	3.65	6.08
9.60	13.39	7.60	10.74	5.60	8.09	3.60	6.02
9.55	13.37	7.55	10.65	5.55	8.04	3.55	5.97
9.50	13.34	7.50	10.56	5.50	7.98	3.50	5.91
9.45	13.31	7.45	10.48	5.45	7.93	3.45	5.85
9.40	13.28	7.40	10.40	5.40	7.88	3.40	5.79
9.35	13.25	7.35	10.31	5.35	7.83	3.35	5.74
9.30	13.21	7.30	10.23	5.30	7.78	3.30	5.68
9.25	13.17	7.25	10.15	5.25	7.73	3.25	5.62
9.20	13.13	7.20	10.07	5.20	7.68	3.20	5.56
9.15	13.08	7.15	10.00	5.15	7.63	3.15	5.50
9.10	13.04	7.10	9.92	5.10	7.57	3.10	5.43
9.05	12.99	7.05	9.84	5.05	7.52	3.05	5.37
9.00	12.93	7.00	9.77	5.00	7.47	3.00	5.31
8.95	12.88	6.95	9.70	4.95	7.42	2.95	5.24
8.90	12.82	6.90	9.62	4.90	7.37	2.90	5.18
8.85	12.76	6.85	9.55	4.85	7.32	2.85	5.11
8.80	12.69	6.80	9.48	4.80	7.27	2.80	5.05
8.75	12.63	6.75	9.42	4.75	7.22	2.75	4.98
8.70	12.56	6.70	9.35	4.70	7.17	2.70	4.92
8.65	12.49	6.65	9.28	4.65	7.12	2.65	4.85
8.60	12.42	6.60	9.22	4.60	7.07	2.60	4.79
8.55	12.34	6.55	9.16	4.55	7.02	2.55	4.72
8.50	12.27	6.50	9.09	4.50	6.97	2.50	4.66
8.45	12.19	6.45	9.03	4.45	6.91	2.45	4.60
8.40	12.11	6.40	8.97	4.40	6.86	2.40	4.54
8.35	12.03	6.35	8.91	4.35	6.81	2.35	4.49
8.30	11.94	6.30	8.85	4.30	6.76	2.30	4.43
8.25	11.86	6.25	8.79	4.25	6.71	2.25	4.38
8.20	11.78	6.20	8.74	4.20	6.66	2.20	4.34
8.15	11.69	6.15	8.68	4.15	6.61	2.15	4.29
8.10	11.61	6.10	8.62	4.10	6.55	2.10	4.26
8.05	11.52	6.05	8.57	4.05	6.50	2.05	4.22

Attachment A

Cupric Ion Selective Electrode
User Guide

User Guide

Cupric
Ion Selective
Electrode

Thermo
SCIENTIFIC

Table of Contents

Introduction

This user guide contains information on the preparation, operation and maintenance for the cupric ion selective electrode (ISE). General analytical procedures, electrode characteristics and electrode theory are also included in this user guide. Cupric electrodes measure free cupric ions in aqueous solutions quickly, simply, accurately and economically.

Technical Support Chemists can be consulted for assistance and troubleshooting advice. Within the United States call 1.800.225.1480 and outside the United States call 978.232.6000 or fax 978.232.6031. In Europe, the Middle East and Africa, contact your local authorized dealer. For the most current contact information, visit www.thermo.com/contactwater.

For the latest application and technical resources for Thermo Scientific Orion products, visit www.thermo.com/waterapps.

Cupric ionplus® Sure-Flow® Solid State Combination ISE, Cat. No. 9629BNWP

The cupric combination electrode has the sensing and reference half-cells built into one electrode, which decreases the amount of required solutions and reduces waste. The built-in Sure-Flow reference junction prevents electrode clogging and provides fast and stabile readings. The cupric combination electrode is available with a waterproof BNC connector, Cat. No. 9629BNWP. Electrodes with a waterproof BNC connector can be used on any ISE or mV meter with a BNC connection.

Cupric Solid State Half-Cell ISE, Cat. No. 9429BN and 9429SC

The cupric half-cell electrode must be used with the double junction reference electrode, Cat. No. 900200. The cupric half-cell electrode is available with a BNC connector, Cat. No. 9429BN, and a screw cap connector, Cat. No. 9429SC. Electrodes with a screw cap connector require a separate cable.

Required Equipment

1. Thermo Scientific Orion ISE meter, such as the 4-Star pH/ISE meter or 5-Star pH/ISE/DO/conductivity meter; equivalent ISE meter; or mV meter with a 0.1 mV resolution.

   Cupric electrodes can be used on any ISE or mV meter with a BNC connection. The electrodes can also be used on meters with a variety of inputs when an adapter cable is used. Visit www.thermo.com/water for details.

2. Thermo Scientific Orion cupric electrode.

   The 9429BN and 9429SC cupric half-cell electrodes require a separate reference electrode, Cat. No. 900200.

3. Magnetic stirrer or Thermo Scientific Orion stirrer probe, Cat. No. 096019. The stirrer probe can be used with 3-Star, 4-Star and 5-Star benchtop meters.

4. Volumetric flasks, graduated cylinders and beakers. Plastic labware is required for low level cupric ion analysis.

5. Distilled or deionized water.

6. Cupric electrode filling solution.

   Use Optimum Results™ D filling solution, Cat. No. 900063, for the 9629BNWP cupric combination electrode.

   Use inner chamber filling solution, Cat. No. 900002, and outer chamber filling solution, Cat. No. 900003, for the double junction reference electrode that is used with the 9429BN and 9429SC cupric half-cell electrodes.

7. 0.1 M $Cu(NO_3)_2$ calibration standard, Cat. No. 942906.

8. Cupric ionic strength adjuster (ISA), Cat. No. 940011. ISA provides a constant background ionic strength for samples and standards.

Serial Dilutions

Serial dilution is the best method for the preparation of standards. Serial dilution means that an initial standard is diluted, using volumetric glassware, to prepare a second standard solution. The second standard is similarly diluted to prepare a third standard, and so on, until the desired range of standards has been prepared.

1. **To prepare a 10^{-2} M standard (635.5 ppm) –**
   Pipet 10 mL of the 0.1 M standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

2. **To prepare a 10^{-3} M standard (63.55 ppm) –**
   Pipet 10 mL of the 10^{-2} M standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

3. **To prepare a 10^{-4} M standard (6.355 ppm) –**
   Pipet 10 mL of the 10^{-3} M standard into a 100 mL volumetric flask. Dilute to the mark with deionized water and mix well.

To prepare standards with a different concentration use the following formula:

$$\mathbf{C_1 * V_1 = C_2 * V_2}$$

$\mathbf{C_1}$ = concentration of original standard
$\mathbf{V_1}$ = volume of original standard
$\mathbf{C_2}$ = concentration of standard after dilution
$\mathbf{V_2}$ = volume of standard after dilution

For example, to prepare 1000 mL of a 100 ppm cupric standard from a 6355 ppm cupric standard:

$\mathbf{C_1}$ = 6355 ppm

$\mathbf{V_1}$ = unknown

$\mathbf{C_2}$ = 100 ppm

$\mathbf{V_2}$ = 1000 mL

6355 ppm * $\mathbf{V_1}$ = 100 ppm * 1000 mL

$\mathbf{V_1}$ = (100 ppm * 1000 mL) / 6355 ppm = 15.7 mL

Electrode Setup

Electrode Preparation

9429BN and 9429SC Cupric Half-Cell Electrode – Remove the protective shipping cap from the sensing element and save the cap for storage.

900200 Double Junction Reference Electrode – Prepare the reference electrode according to the reference electrode user guide. Fill the reference electrode with inner chamber filling solution, Cat. No. 900002, and outer chamber filling solution, Cat. No. 900003.

9629BNWP Cupric Combination Electrode – Remove the protective shipping cap from the sensing element and save the cap for storage. Fill the electrode with Optimum Results D filling solution, Cat. No. 900063.

9629BNWP Cupric Combination Electrode Filling Instructions

1. Lift the flip spout on the filling solution bottle to a vertical position.

2. Insert the spout into the filling hole on the outer body of the electrode and add a small amount of filling solution to the reference chamber. Invert the electrode to moisten the top O-ring and then return the electrode to the upright position.

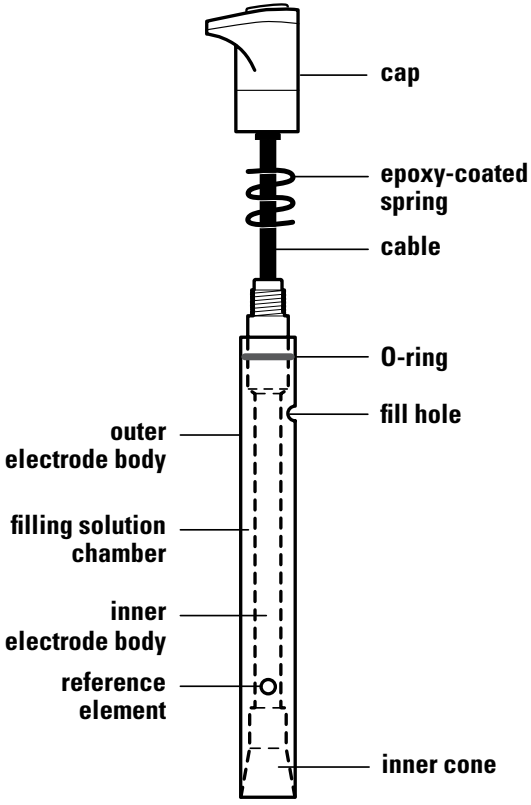
3. Hold the electrode body with one hand and use your thumb to push down on the electrode cap to allow a few drops of filling solution to drain out of the electrode.

4. Release the electrode cap. If the sleeve does not return to its original position, check if the O-ring is moist and repeat steps 2 through 4 until the sleeve returns to the original position.

5. Add filling solution to the electrode up to the filling hole.

Note: *Add filling solution each day before using the electrode. The filling solution level should be at least one inch above the level of sample in the beaker to ensure a proper flow rate. The fill hole should always be open when taking measurements.*

Figure 1
9629BNWP Cupric Combination Electrode



Checking Electrode Operation (Slope)

These are general instructions that can be used with most meters to check the electrode operation. Refer to the meter user guide for more specific information.

This procedure measures electrode slope. Slope is defined as the change in millivolts observed with every tenfold change in concentration. Obtaining the slope value provides the best means for checking electrode operation.

1. If the electrode has been stored dry, prepare the electrode as described in the **Electrode Preparation** section.

2. Connect the electrode to a meter with a mV mode. Set the meter to the mV mode.

3. Add 100 mL of distilled water and 2 mL of ISA into a 150 mL beaker. Stir the solution thoroughly.

4. Rinse the electrode with distilled water and place the electrode into the solution prepared in step 3.

5. Select either a 0.1 M or 1000 ppm cupric standard. Pipet 1 mL of the standard into the beaker and stir the solution thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

6. Pipet 10 mL of the same standard into the same beaker and stir the solution thoroughly. When a stable reading is displayed, record the electrode potential in millivolts.

7. There should be a 25 to 30 mV difference between the two millivolt readings when the solution temperature is between 20 to 25 °C. If the millivolt potential is not within this range, refer to the **Troubleshooting** section.

Measurement Units

Cupric ion concentration can be measured in moles per liter (M), parts per million (ppm) or any convenient concentration unit.

Table 1
Concentration Unit Conversion Factors

Moles/Liter (M)	ppm
1.0	63550
10^{-1}	6355
1.57×10^{-2}	1000
10^{-2}	635.5
10^{-3}	63.55
10^{-4}	6.355
1.57×10^{-5}	1

Sample Requirements

The epoxy body of the cupric electrode is resistant to damage by aqueous solutions. The electrode may be used intermittently in solutions that contain methanol, benzene or acetone. Contact Technical Support for information on using the electrode for specific applications.

Samples and standards should be at the same temperature. A 1 °C difference in temperature for a 10^{-3} M cupric ion solution will give rise to about a 4% error. The combination cupric electrode, Cat. No. 9629BNWP, when used with Optimum Results D filling solution, produces less than a 2% error in the same solution.

The solution temperature must be less than 80 °C.

Cupric samples must be below pH 6 to avoid precipitation of $Cu(OH)_2$. Acidify samples with 1 M HNO_3 if necessary. See the **pH Effects** section to determine the optimum pH working range for your sample.

In all analytical procedures, ISA must be added to all samples and standards before measurements are taken.

Measuring Hints

- Stir all standards and samples at a uniform, moderate rate. Place a piece of insulating material, such as Styrofoam or cardboard, between the magnetic stir plate and beaker to prevent measurement errors from the transfer of heat to the sample.

- Always use freshly prepared standards for calibration.

- Always rinse the electrode with distilled water between measurements and shake the electrode to remove the water and prevent sample carryover. Do not wipe or rub the electrode sensing element.

- Allow all standards and samples to reach the same temperature for precise measurements.

- Concentrated samples (greater than 10^{-1} M cupric) should be diluted before measurement.

- Verify the electrode calibration every two hours by placing the electrode in a fresh aliquot of the least concentrated standard used for calibration. If the value has changed by more than 2%, recalibrate the electrode.

- After immersing the electrode in a solution, check the electrode sensing surface for air bubbles and remove air bubbles by reimmersing the electrode in the solution and gently tapping it.

- For high ionic strength samples, prepare standards with a background composition similar to the sample.

- The fill hole cover must be open during measurements to ensure a uniform flow of filling solution.

- If the combination electrode is used and the electrode is used in dirty or viscous samples or the electrode response becomes sluggish, empty the electrode completely, hold the junction open and flush the junction with distilled water. Empty any water from the electrode and refill it with fresh filling solution. Press down on the electrode cap to let a few drops of the filling solution flow out of the electrode and then replenish any lost solution.

Electrode Storage

Cupric Half-Cell Electrode Storage, Cat. No. 9429BN and 9429SC

The cupric half-cell electrode should be rinsed thoroughly with distilled water and stored dry in the air at all times. When storing the electrode for long periods of time, cover the sensing element with the protective shipping cap.

Double Junction Reference Electrode Storage, Cat. No. 900200

The double junction reference electrode may be stored in the outer chamber filling solution, Cat. No. 900003, between sample measurements and up to one week. The filling solution inside the electrode should not be allowed to evaporate, as crystallization will result.

For storage longer than one week, drain the reference electrode, flush the inside with distilled water and store the electrode dry.

Cupric Combination Electrode Storage, Cat. No. 9629BNWP

For storage between measurements and up to one week, store the electrode in a 4 M potassium chloride solution with cupric. The cupric concentration of the storage solution should be close to the least concentrated cupric calibration standard. Do not add ISA to the storage solution. The filling solution inside the electrode should not be allowed to evaporate, as crystallization will result.

For storage longer than one week, drain the electrode, flush the chamber with distilled water and store the electrode dry with the protective shipping cap covering the sensing element.

Electrode Maintenance

Polishing the Cupric Combination Electrode and Cupric Half-Cell Electrode

The sensing surface of solid state electrodes can wear over time, which causes drift, poor reproducibility and loss of response in low level samples. The electrode can be restored by polishing the sensing surface with a polishing strip, Cat. No. 948201. The polishing strip can also be used if the sensing surface has been etched or chemically poisoned.

1. Cut off about an inch of the polishing strip.

2. Hold the electrode with the sensing surface facing up.

3. Place a few drops of distilled water on the sensing surface.

4. With the frosted side of the polishing strip facing down, use light finger pressure to place the polishing strip on top of the sensing surface.

5. Rotate the electrode for about 30 seconds.

6. Rinse the electrode with distilled water and soak the electrode in a 1 ppm or 10^{-5} M cupric standard for ten minutes.

Cupric Combination Electrode and Double Junction Reference Electrode Flushing

If the area between the electrode sleeve and inner cone becomes clogged with sample or precipitate, flush the area with filling solution or distilled water.

1. Hold the electrode body with one hand and use your thumb to push down on the electrode cap to drain the electrode. Push down on the cap until all the filling solution is drained from the chamber.

2. Fill the electrode with distilled water and then push down on the cap until all the water is drained from the chamber.

3. Fill the electrode with fresh filling solution up to the fill hole. Push down on the cap to allow a few drops of filling solution to drain out of the electrode and replenish the lost filling solution.

Disassembling and Reassembling the Cupric Combination Electrode

Note: *Disassembly is usually not required and should not be done unless a thorough cleaning is required.*

1. Tip the electrode so the filling solution moistens the O-ring on the electrode body. Hold the electrode body with one hand and use your thumb to push down on the electrode cap to drain the electrode.

2. Unscrew the cap counterclockwise and then slide the cap and spring up the cable.

3. Hold the outer sleeve with one hand and firmly push down on the threaded portion with your thumb and forefinger to separate the inner body from the sleeve.

4. Grasp the inner cone with a clean, lint-free tissue and withdraw the body from the sleeve using a gentle twisting motion. Do not touch the pellet above the cone, as it will damage to the pellet. Rinse the outside of the electrode body and the entire sleeve with distilled water. Allow it to air dry.

5. Moisten the O-ring on the electrode body with a drop of filling solution. Insert the screw-thread end of the electrode body into the tapered, ground end of the sleeve.

6. Push the body into the sleeve using a gentle twisting motion until the bottom surface of the inner cone is flush with the tapered end of the sleeve.

7. Place the spring onto the electrode body and screw on the cap. Refill the electrode with filling solution.

Analytical Techniques

A variety of analytical techniques are available to the analyst. The following is a description of these techniques.

Direct Calibration is a simple procedure for measuring a large number of samples. Only one meter reading is required for each sample. Calibration is performed using a series of standards. The concentration of the samples is determined by comparison to the standards. ISA is added to all solutions to ensure that samples and standards have similar ionic strength.

Low Level Calibration is a similar to the direct calibration technique. This method is recommended when the expected sample concentration is less than 0.6 ppm or 10^{-5} M cupric. A minimum three point calibration is recommended to compensate for the electrode's non-linear response at these concentrations. A special calibration standard preparation procedure is the best means of preparing low level calibration standards.

Incremental Techniques provide a useful method for measuring samples, since a calibration is not required. The different incremental techniques are described below. They can be used to measure the total concentration of a specific ion in the presence of a large (50 to 100 times) excess of complexing agents. As in direct calibration, any convenient concentration unit can be used.

Known Addition is useful for measuring dilute samples, checking the results of direct calibration (when no complexing agents are present), or measuring the total concentration of an ion in the presence of an excess complexing agent. The electrode is immersed in the sample solution and an aliquot of a standard solution containing the measured species is added to the sample. From the change in potential before and after the addition, the original sample concentration is determined.

Titrations are quantitative analytical techniques for measuring the concentration of a species by incremental addition of a reagent (titrant) that reacts with the sample species. Sensing electrodes can be used for determination of the titration end point. Ion selective electrodes are useful as end point detectors, because they are unaffected by sample color or turbidity. Titrations are approximately 10 times more precise than direct calibration, but are more time-consuming.

Indicator Titration Method is useful for measuring ionic species where an ion specific electrode does not exist. With this method the electrodes sense a reagent species that has been added to the sample before titration. The cupric electrode may be used in indicator titrations for many different metal ions.

Table 2
Recommended Measuring Techniques

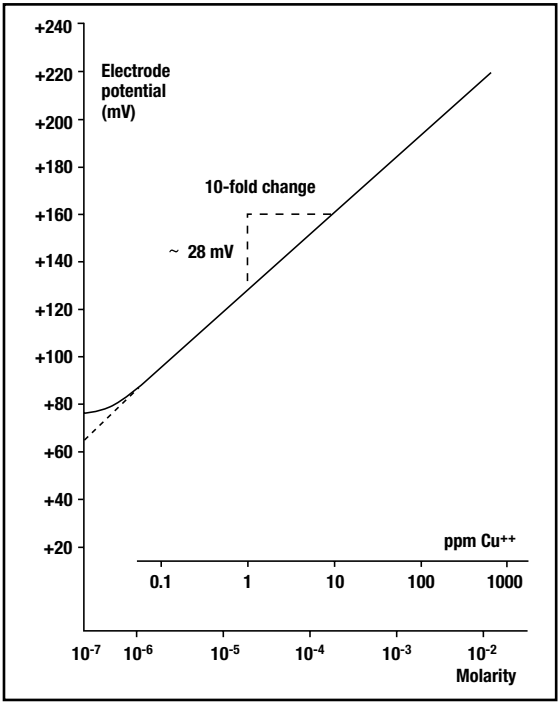
	Direct	Small Volume Direct	Low Level	Known Addition	Titration
$[Cu^{+2}] < 0.6$ ppm			✔		
$[Cu^{+2}] > 0.6$ ppm	✔			✔	✔
$[Cu^{+2}] > 1.0$ ppm		✔			
Increased accuracy					✔
Occasional Sampling				✔	
Small sample volume		✔		✔	
Large number of samples	✔		✔	✔	
Reduce chemical usage		✔			
Field measurement		✔			
Ionic strength greater than 0.1 M	✔			✔	
Other metal analysis					✔ (Indicator Titration)

Direct Calibration Technique

Typical Direct Calibration Curve

In the direct calibration procedure, a calibration curve is constructed either in the meter memory or on semi-logarithmic paper. Electrode potentials of standard solutions are measured and plotted on the linear axis against their concentrations on the log axis. In the linear regions of the curves, only two standards are needed to determine a calibration curve. In non-linear regions, more points must be taken. These direct calibration procedures are given for concentrations in the region of linear electrode response. Low level measurement procedures are given in a following section for measurements in the non-linear electrode region.

Figure 2
Typical Direct Calibration Curve



Direct Calibration Overview

The following direct measurement procedures are recommended for moderate to high level measurements. Samples must be in the linear range of the electrode – greater than 0.6 ppm or 10^{-5} M cupric. A two point calibration is sufficient, although more points can be used. When using an ISE meter, sample concentrations can be read directly from the meter. When using a mV meter, a calibration curve can be prepared on semi-logarithmic graph paper, or a linear regression (against logarithmic concentration values) can be performed using a spreadsheet or graphing program.

Calibration Hints

- Standard concentrations should bracket the expected sample concentrations.
- Always add 2 mL of ISA, Cat. No. 940011, per 100 mL of standard or sample.
- For high ionic strength samples that have an ionic strength of 0.1 M or greater, prepare standards with a background composition similar to that of the samples, or measure the samples using the known addition method.
- During calibration, measure the least concentrated standard first, and work up to the most concentrated standard.

Direct Calibration Setup

1. Prepare the electrode as described in the **Electrode Preparation** section. If using the 9629BNWP combination cupric electrode, fill the electrode with Cat. No. 900063. If using the 9429BN or 9429SC half-cell cupric electrode with the 900200 reference electrode, fill the reference electrode with inner chamber filling solution, Cat. No. 900002, and outer chamber filling solution, Cat. No. 900003.
2. Connect the electrode to the meter.
3. Prepare at least two standards that bracket the expected sample range and differ in concentration by a factor of ten. Standards can be prepared in any concentration unit to suit the particular analysis requirement. See the **Serial Dilution** section for instructions on how to prepare standards. All standards should be at the same temperature as the samples. For details on temperature effects on electrode performance, refer to the **Temperature Effects** section.

Direct Calibration Procedure Using a Meter with an ISE Mode

Note: *See the meter user guide for more specific information.*

1. Add 100 mL of the less concentrated standard and 2 mL of ISA to a 150 mL beaker and stir the solution thoroughly.

2. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. Wait for a stable reading and adjust the meter to display the value of the standard, as described in the meter user guide.

3. Add 100 mL of the more concentrated standard and 2 mL of ISA to a second 150 mL beaker and stir the solution thoroughly.

4. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. Wait for a stable reading and adjust the meter to display the value of the second standard, as described in the meter user guide.

5. Record the resulting slope value. The slope should be between 25 and 30 mV when the standards are between 20 and 25 °C.

6. Add 100 mL of sample and 2 mL of ISA to a clean 150 mL beaker and stir the solution thoroughly.

7. Rinse the electrode with distilled water, blot it dry and place it into the sample. The concentration of the sample will be displayed on the meter.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Direct Calibration Procedure Using a Meter with a mV Mode

Note: *See the meter user guide for more specific information.*

1. Set the meter to the mV mode.

2. Add 100 mL of the less concentrated standard and 2 mL of ISA to a 150 mL beaker and stir the solution thoroughly.

3. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

4. Add 100 mL of the more concentrated standard and 2 mL of ISA to a second 150 mL beaker and stir the solution thoroughly.

5. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

6. Using semi-logarithmic graph paper, prepare a calibration curve by plotting the millivolt values on the linear axis and the standard concentration values on the logarithmic axis.

7. Add 100 mL of sample and 2 mL of ISA to a clean 150 mL beaker and stir the solution thoroughly.

8. Rinse the electrode with distilled water, blot it dry and place it into the beaker. When a stable reading is displayed, record the mV value.

9. Using the calibration curve prepared in step 6, determine the unknown concentration of the sample.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Small Volume Direct Calibration Technique

Take advantage of special design features available with the 9629BNWP ionplus combination cupric electrode to meet your measuring needs. Due to the Sure-Flow reference, this electrode is able to measure sample volumes as small as 5 mL using a modified direct measurement procedure. Because less solution volume is required, the chemical usage of cupric standards and ISA is reduced. This method is also convenient when making field measurements, since the 9629BNWP combination cupric electrode does not require a separate reference electrode. All samples should have a concentration greater than 1 ppm or 1.57×10^{-5} M cupric. A two point calibration is sufficient, although more points can be used. The following procedure recommends using 25 mL of sample. Smaller sample volumes can be used, as long as the final volume of solution is sufficient to cover the bottom of the electrode.

Calibration Hints

- Use the 9629BNWP ionplus combination cupric electrode.
- Standard concentrations should bracket the expected sample concentrations.
- Always keep the ratio of standard or sample to ISA at 50:1.
- For high ionic strength samples that have an ionic strength of 0.1 M or greater, prepare standards with a background composition similar to that of the samples, or measure the samples using the known addition method.
- During calibration, measure the least concentrated standard first, and work up to the most concentrated standard.
- Calibrate with the same volume of standard as the volume of sample that is available for analysis.

Small Volume Direct Calibration Setup

1. Prepare the 9629BNWP combination cupric electrode as described in the **Electrode Preparation** section and fill the electrode with Optimum Results D filling solution, Cat. No. 900063.

2. Connect the electrode to the meter.

3. Prepare at least two standards that bracket the expected sample range and differ in concentration by a factor of ten. Standards can be prepared in any concentration unit to suit the particular analysis requirement. See the **Serial Dilution** section for instructions on how to prepare standards. All standards should be at the same temperature as the samples. For details on temperature effects on electrode performance, refer to the **Temperature Effects** section.

Small Volume Direct Calibration Procedure Using a Meter with an ISE Mode

Note: *See the meter user guide for more specific information.*

1. Add 25 mL of the less concentrated standard and 0.5 mL of ISA to a 50 mL beaker and swirl the solution to mix.

2. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. Wait for a stable reading and adjust the meter to display the value of the standard, as described in the meter user guide.

3. Add 25 mL of the more concentrated standard and 0.5 mL of ISA to a second 50 mL beaker and swirl the solution to mix.

4. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. Wait for a stable reading and adjust the meter to display the value of the second standard, as described in the meter user guide.

5. Record the resulting slope value. The slope should be between 25 and 30 mV when the standards are between 20 and 25 °C.

6. Add 25 mL of sample and 0.5 mL of ISA to a clean 50 mL beaker and swirl the solution to mix.

7. Rinse the electrode with distilled water, blot it dry and place it into the sample. The concentration of the sample will be displayed on the meter.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Small Volume Direct Calibration Procedure Using a Meter with a mV Mode

Note: *See the meter user guide for more specific information.*

1. Set the meter to the mV mode.

2. Add 25 mL of the less concentrated standard and 0.5 mL of ISA to a 50 mL beaker and swirl the solution to mix.

3. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the less concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

4. Add 25 mL of the more concentrated standard and 0.5 mL of ISA to a second 50 mL beaker and swirl the solution to mix.

5. Rinse the electrode with distilled water, blot it dry and place it into the beaker with the more concentrated standard. When a stable reading is displayed, record the mV value and corresponding standard concentration.

6. Using semi-logarithmic graph paper, prepare a calibration curve by plotting the millivolt values on the linear axis and the standard concentration values on the logarithmic axis.

7. Add 25 mL of sample and 0.5 mL of ISA to a clean 50 mL beaker and swirl the solution to mix.

8. Rinse the electrode with distilled water, blot it dry and place it into the beaker. When a stable reading is displayed, record the mV value.

9. Using the calibration curve prepared in step 6, determine the unknown concentration of the sample.

Note: *Other solution volumes may be used, as long as the ratio of solution to ISA remains 50:1.*

Low Level Calibration Technique

These procedures are for solutions that have a cupric concentration of less than 0.6 ppm or 10^{-5} M cupric. For solutions low in cupric but high in total ionic strength (greater than 10^{-1} M), perform the same procedure by preparing a calibrating solution with a composition similar to the sample.

Accurate results require that the following conditions be met:

- Prepare at least three calibration standards that bracket the expected sample concentration.
- Always use low level ISA for standards and samples.
- Plastic labware must be used for all low level cupric measurements.
- Adequate time must be allowed for electrode stabilization. Longer response time will be needed at low level measurements.
- Stir all standards and samples at a uniform rate.

Low Level Setup

1. Prepare the electrode as described in the **Electrode Preparation** section.
2. Connect the electrode to the meter. Set the meter to the mV mode.
3. Prepare the low level ISA by pipetting 20 mL of the ISA, Cat. No. 940011, into a 100 mL volumetric flask and diluting to the mark with distilled water. Use low level ISA for low level measurements only.
4. Select a standard solution. Use either a 10 ppm cupric standard or a 10^{-4} M cupric standard.

   To prepare the 10 ppm standard, pipet 10 mL of the 1000 ppm standard into a 1 liter volumetric flask. Dilute to the mark with distilled water and mix the solution thoroughly.

   To prepare the 10^{-4} M standard, pipet 1 mL of the 0.1 M standard into a 1 liter volumetric flask. Dilute to the mark with distilled water and mix the solution thoroughly.

Low Level Calibration and Measurement

1. Add 100 mL of distilled water and 1 mL of low level ISA to a 150 mL beaker.

2. Rinse the electrode with distilled water, blot it dry and place it into the beaker. Stir the solution thoroughly.

3. Add increments of the 10 ppm or 10^{-4} M cupric standard mixed with low level ISA to the beaker using the steps outlined in **Table 3**. Record the stable millivolt reading after each increment.

4. On semi-logarithmic paper, plot the concentration (log axis) against the millivolt potential (linear axis). Prepare a new calibration curve with fresh standards each day.

5. Measure 100 mL of sample and 1 mL of low level ISA and pour the solutions into a clean 150 mL beaker. Rinse the electrode with distilled water, blot it dry and place the electrode into the sample.

6. Stir the solution thoroughly. When a stable reading is displayed, record the mV value.

7. Determine the sample concentration corresponding to the measured potential from the low level calibration curve.

Table 3
Calibration Curve For Low Level Calibrations
Additions of standard to 100 mL distilled water and 1 mL low level ISA solution.

Step	Pipet Size	Volume Added	Concentration (ppm)
1	0.1 mL	0.01 mL	0.001
2	0.1 mL	0.1 mL	0.011
3	1.0 mL	0.9 mL	0.100
4	10 mL	6.0 mL	0.662

Step	Pipet Size	Volume Added	Concentration (M)
1	0.1 mL	0.01 mL	1.0×10^{-8}
2	0.1 mL	0.1 mL	1.11×10^{-7}
3	1.0 mL	0.9 mL	1.0×10^{-6}
4	10 mL	10 mL	9.9×10^{-6}

Known Addition Technique

Known addition is a convenient technique for measuring samples in the linear range of the electrode (greater than 0.6 ppm or 10^{-5} M cupric) because no calibration curve is required. It can be used to verify the results of a direct calibration or to measure the total concentration of an ion in the presence of a large excess of a complexing agent. The sample potential is measured before and after addition of a standard solution.

Accurate results require that the following conditions be met:

- Concentration should approximately double as a result of the addition.
- Sample concentration should be known to within a factor of three.
- Either no complexing agent or a large excess of the complexing agent may be present.
- The ratio of the uncomplexed ion to complexed ion must not be changed by addition of the standard.
- All samples and standards should be at the same temperature.
- With double or multiple known addition, the final addition should be 10 to 100 times the sample concentration.
- Add 2 mL of ISA to every 100 mL of sample before analysis.

Known Addition Setup

1. Prepare the electrode as described in the **Electrode Preparation** section.
2. Connect the electrode to the meter.
3. Prepare a standard solution that will cause the cupric concentration of the sample to double when added to the sample solution. Refer to **Table 4** for guidelines.
4. Determine the electrode slope by performing the procedure in the **Checking Electrode Operation (Slope)** section.
5. Rinse the electrode with distilled water.

Table 4
Guideline For Known Addition

Volume of Addition	Concentration of Standard
1 mL	100 times sample concentration
5 mL	20 times sample concentration
10 mL*	10 times sample concentration

* Most convenient volume to use

Known Addition Using a Meter with a Known Addition Mode

Note: *See the meter user guide for more specific information.*

1. Set the meter to measure in the known addition mode.

2. Measure 100 mL of the sample and 2 mL of ISA and pour the solutions into a beaker. Rinse the electrode with distilled water and place it into the sample solution. Stir the solution thoroughly.

3. When a stable reading is displayed, set the meter as described in the meter user guide, if required.

4. Pipet the appropriate amount of the standard solution into the beaker. Stir the solution thoroughly.

5. When a stable reading is displayed, record the sample concentration.

Known Addition Using a Meter with a Millivolt Mode

1. Set the meter to the relative millivolt mode. If a relative millivolt mode is not available, use the millivolt mode.

2. Measure 100 mL of sample and 2 mL of ISA and pour the solutions into a 150 mL beaker. Stir the solution thoroughly.

3. Rinse the electrode with distilled water, blot it dry and place the electrode into the beaker. When a stable reading is displayed, set the meter to read 0.0 mV. If the reading cannot be adjusted to 0.0 mV, record the actual mV value.

4. Pipet the appropriate amount of standard solution into the beaker. Stir the solution thoroughly.

5. When a stable reading is displayed, record the mV value. If the meter could not be set to 0.0 mV in step 3, subtract the first reading from the second reading to calculate ΔE.

6. Use **Table 6** to find the Q value that corresponds to the change in potential, ΔE. To determine the original sample concentration, multiply Q by the concentration of the added standard:

   $$\mathbf{C_{sample} = Q * C_{standard}}$$

   $C_{standard}$ = standard concentration
   C_{sample} = sample concentration
   Q = value from **Table 6**

   The table of Q values is calculated for a 10% volume change. The equation for the calculation of Q for different slopes and volume changes is given below.

   $$\mathbf{Q = (p * r) / \{[(1 + p) * 10^{\Delta E/S}] - 1\}}$$

   Q = value from **Table 6**
   $\Delta E = E_2 - E_1$
   S = slope of the electrode
   p = volume of standard / volume of sample and ISA
   r = volume of sample and ISA / volume of sample

Calculating Known Addition for Samples using Lotus, Excel, or Quattro Spreadsheets

If it is more convenient, a simple spreadsheet can be set up to calculate the known addition results, using any ratio of sample to addition. A typical worksheet is shown in **Table 5**. The numbers shown are examples, but the formulas and their locations should be copied exactly.

Table 5
Known Addition Calculations using Lotus, Excel, or Quattro Spreadsheets

A	B	C
1		Enter Value
2	Volume of sample and ISA (mL)	102
3	Volume of addition (mL)	10
4	Concentration of addition	10
5	Volume of sample	100
6	Initial mV reading	45.3
7	Final mV reading	63.7
8	Electrode slope	28.2
9		
10		Derived Values
11	Delta E	+C7 - C6
12	Solution volume ratio	+C3/C2
13	Antilog term	+10^ (C11/C8)
14	Sample volume ratio	+C2/C5
15	Q term	+C12*C14/ (((1+C12)*C13)-1)
16	Calculated initial concentration in same units as addition	+C15*C4

Note: *For Excel, use = instead of + at start of formulas.*

Table 6
Q Values for a 10% volume change, slopes (in column heading) are in units of mV/decade

ΔE	Q Concentration Ratio			
	28.6	29.1	29.6	30.1
2.5	0.2917	0.2957	0.2996	0.3035
3.0	0.2512	0.2550	0.2586	0.2623
3.5	0.2196	0.2230	0.2264	0.2298
4.0	0.1941	0.1973	0.2005	0.2036
4.5	0.1732	0.1762	0.1791	0.1821
5.0	0.1557	0.1585	0.1613	0.1640
5.1	0.1525	0.1553	0.1580	0.1608
5.2	0.1495	0.1522	0.1549	0.1576
5.3	0.1465	0.1492	0.1519	0.1546
5.4	0.1437	0.1463	0.1490	0.1516
5.5	0.1409	0.1435	0.1461	0.1487
5.6	0.1382	0.1408	0.1434	0.1459
5.7	0.1356	0.1382	0.1407	0.1432
5.8	0.1331	0.1356	0.1381	0.1406
5.9	0.1306	0.1331	0.1356	0.1381
6.0	0.1282	0.1307	0.1331	0.1356
6.1	0.1259	0.1283	0.1308	0.1332
6.2	0.1236	0.1260	0.1284	0.1308
6.3	0.1214	0.1238	0.1262	0.1285
6.4	0.1193	0.1217	0.1240	0.1263
6.5	0.1172	0.1195	0.1219	0.1242
6.6	0.1152	0.1175	0.1198	0.1221
6.7	0.1132	0.1155	0.1178	0.1200
6.8	0.1113	0.1136	0.1158	0.1180
6.9	0.1094	0.1117	0.1139	0.1161
7.0	0.1076	0.1098	0.1120	0.1142
7.1	0.1058	0.1080	0.1102	0.1123
7.2	0.1041	0.1063	0.1084	0.1105
7.3	0.1024	0.1045	0.1067	0.1088
7.4	0.1008	0.1029	0.1050	0.1071
7.5	0.0992	0.1012	0.1033	0.1054
7.6	0.0976	0.0997	0.1017	0.1037
7.8	0.0946	0.0966	0.0986	0.1006
8.0	0.0917	0.0936	0.0956	0.0976
8.2	0.0889	0.0908	0.0928	0.0947
8.4	0.0863	0.0882	0.0900	0.0919
8.6	0.0837	0.0856	0.0874	0.0893
8.8	0.0813	0.0831	0.0849	0.0868
9.0	0.0790	0.0808	0.0825	0.0843
9.2	0.0767	0.0785	0.0803	0.0820
9.4	0.0746	0.0763	0.0780	0.0798
9.6	0.0725	0.0742	0.0759	0.0776
9.8	0.0706	0.0722	0.0739	0.0755
10.0	0.0687	0.0703	0.0719	0.0735
10.2	0.0668	0.0684	0.0700	0.0716
10.4	0.0651	0.0666	0.0682	0.0698
10.6	0.0634	0.0649	0.0665	0.0680
10.8	0.0617	0.0633	0.0648	0.0663
11.0	0.0602	0.0617	0.0631	0.0646
11.2	0.0586	0.0601	0.0616	0.0630
11.4	0.0572	0.0586	0.0600	0.0615

ΔE	Q Concentration Ratio			
	28.6	29.1	29.6	30.1
11.6	0.0557	0.0572	0.0586	0.0600
11.8	0.0544	0.0558	0.0572	0.0585
12.0	0.0530	0.0544	0.0558	0.0572
12.2	0.0518	0.0531	0.0545	0.0558
12.4	0.0505	0.0518	0.0532	0.0545
12.6	0.0493	0.0506	0.0519	0.0532
12.8	0.0481	0.0494	0.0507	0.0520
13.0	0.0470	0.0483	0.0495	0.0508
13.2	0.0459	0.0472	0.0484	0.0497
13.4	0.0449	0.0461	0.0473	0.0485
13.6	0.0438	0.0450	0.0462	0.0474
13.8	0.0428	0.0440	0.0452	0.0464
14.0	0.0419	0.0430	0.0442	0.0454
14.2	0.0409	0.0421	0.0432	0.0444
14.4	0.0400	0.0411	0.0423	0.0434
14.6	0.0391	0.0402	0.0413	0.0425
14.8	0.0382	0.0393	0.0404	0.0416
15.0	0.0374	0.0385	0.0396	0.0407
15.5	0.0354	0.0365	0.0375	0.0386
16.0	0.0335	0.0345	0.0356	0.0366
16.5	0.0318	0.0328	0.0337	0.0347
17.0	0.0302	0.0311	0.0320	0.0330
17.5	0.0286	0.0295	0.0305	0.0314
18.0	0.0272	0.0281	0.0290	0.0298
18.5	0.0258	0.0267	0.0275	0.0284
19.0	0.0246	0.0254	0.0262	0.0270
19.5	0.0234	0.0242	0.0250	0.0258
20.0	0.0223	0.0230	0.0238	0.0246
20.5	0.0212	0.0219	0.0227	0.0234
21.0	0.0202	0.0209	0.0216	0.0224
21.5	0.0192	0.0199	0.0206	0.0213
22.0	0.0183	0.0190	0.0197	0.0204
22.5	0.0175	0.0181	0.0188	0.0195
23.0	0.0167	0.0173	0.0179	0.0186
23.5	0.0159	0.0165	0.0171	0.0178
24.0	0.0152	0.0158	0.0164	0.0170
24.5	0.0145	0.0151	0.0157	0.0162
25.0	0.0139	0.0144	0.0150	0.0155
25.5	0.0132	0.0138	0.0143	0.0149
26.0	0.0126	0.0132	0.0137	0.0142
26.5	0.0121	0.0126	0.0131	0.0136
27.0	0.0116	0.0120	0.0125	0.0131
27.5	0.0110	0.0115	0.0120	0.0125
28.0	0.0106	0.0110	0.0115	0.0120
28.5	0.0101	0.0106	0.0110	0.0115
29.0	0.0097	0.0101	0.0105	0.0110
29.5	0.0093	0.0097	0.0101	0.0105
30.5	0.0085	0.0089	0.0093	0.0097
31.5	0.0078	0.0081	0.0085	0.0089
32.0	0.0074	0.0078	0.0082	0.0085
32.5	0.0071	0.0075	0.0078	0.0082

ΔE	Q Concentration Ratio			
	28.6	29.1	29.6	30.1
33.0	0.0068	0.0072	0.0075	0.0079
33.5	0.0065	0.0069	0.0072	0.0076
34.0	0.0063	0.0066	0.0069	0.0072
34.5	0.0060	0.0063	0.0066	0.0070
35.0	0.0058	0.0061	0.0064	0.0067
35.5	0.0055	0.0058	0.0061	0.0064
36.0	0.0053	0.0056	0.0059	0.0062
36.5	0.0051	0.0053	0.0056	0.0059
37.0	0.0049	0.0051	0.0054	0.0057
37.5	0.0047	0.0049	0.0052	0.0055
38.0	0.0045	0.0047	0.0050	0.0052
38.5	0.0043	0.0045	0.0048	0.0050
39.0	0.0041	0.0043	0.0046	0.0048
39.5	0.0039	0.0042	0.0044	0.0046
40.0	0.0038	0.0040	0.0042	0.0045
40.5	0.0036	0.0038	0.0041	0.0043
41.0	0.0035	0.0037	0.0039	0.0041
41.5	0.0033	0.0035	0.0037	0.0040
42.0	0.0032	0.0034	0.0036	0.0038
42.5	0.0031	0.0033	0.0035	0.0037
43.0	0.0029	0.0031	0.0033	0.0035
43.5	0.0028	0.0030	0.0032	0.0034
44.0	0.0027	0.0029	0.0031	0.0032
44.5	0.0026	0.0028	0.0029	0.0031
45.0	0.0025	0.0027	0.0028	0.0030
45.5	0.0024	0.0026	0.0027	0.0029
46.0	0.0023	0.0024	0.0026	0.0028
46.5	0.0022	0.0024	0.0025	0.0027
47.0	0.0021	0.0023	0.0024	0.0026
47.5	0.0020	0.0022	0.0023	0.0025
48.0	0.0019	0.0021	0.0022	0.0024
48.5	0.0019	0.0020	0.0021	0.0023
49.0	0.0018	0.0019	0.0021	0.0022
49.5	0.0017	0.0018	0.0020	0.0021
50.0	0.0017	0.0018	0.0019	0.0020
50.5	0.0016	0.0017	0.0018	0.0019
51.0	0.0015	0.0016	0.0018	0.0019
51.5	0.0015	0.0016	0.0017	0.0018
52.0	0.0014	0.0015	0.0016	0.0017
52.5	0.0013	0.0015	0.0016	0.0017
53.0	0.0013	0.0014	0.0015	0.0016
53.5	0.0012	0.0013	0.0014	0.0015
54.0	0.0012	0.0013	0.0014	0.0015
54.5	0.0011	0.0012	0.0013	0.0014
55.0	0.0011	0.0012	0.0013	0.0014
55.5	0.0011	0.0011	0.0012	0.0013
56.0	0.0010	0.0011	0.0012	0.0013
56.5	0.0010	0.0011	0.0011	0.0012
57.0	0.0009	0.0010	0.0011	0.0012
57.5	0.0009	0.0010	0.0011	0.0011
58.0	0.0009	0.0009	0.0010	0.0011
58.5	0.0008	0.0009	0.0010	0.0010
59.0	0.0008	0.0009	0.0009	0.0010
59.5	0.0008	0.0008	0.0009	0.0010
60.0	0.0007	0.0008	0.0009	0.0009

Cupric Titration Technique

The cupric electrode makes a highly sensitive endpoint detector for titration with EDTA of copper samples. Titrations are more time consuming than direct electrode measurement, but results are more accurate and reproducible. With careful technique, titrations accurate to ± 0.1% of the total cupric ion concentration of the sample can be performed.

EDTA complexes other cations besides cupric ion. Interferences from alkaline earths and other ions, whose EDTA complexes are stable only at high pH, can be eliminated by performing the titration for cupric ion at a low pH. In many cases, other interferences can be eliminated by a suitable choice of sample pH and the addition of masking agents to the sample solution. A comprehensive list of methods is given in the Handbook of Analytical Chemistry, L. Meites, (ed.) McGraw Hill Book Co., New York, (1st edit.), pp. 3-76, 3-225.

Cupric Titration Setup

1. Prepare the electrode as described in the **Electrode Preparation** section. If using the 9629BNWP combination cupric electrode, fill the electrode with Cat. No. 900063. If using the 9429BN or 9429SC half-cell cupric electrode with the 900200 reference electrode, fill the reference electrode with inner chamber filling solution, Cat. No. 900002, and outer chamber filling solution, Cat. No. 900003.

2. Connect the electrode to the meter.

3. Prepare a 1 M EDTA stock solution by adding 38.0 grams of reagent-grade Na_4EDTA to a 100 mL volumetric flask. Dissolve the solids with about 75 mL of distilled water and then dilute to the mark with distilled water.

4. Prepare an EDTA titrant solution 10 to 20 times as concentrated as the sample by dilution of the 1 M EDTA stock solution. For a good endpoint break, the sample concentration should be at least 10^{-3} M in total copper.

Cupric Titration Procedure

1. Place 100 mL of sample into a 150 mL beaker. Place the electrode in the sample and stir the solution thoroughly.

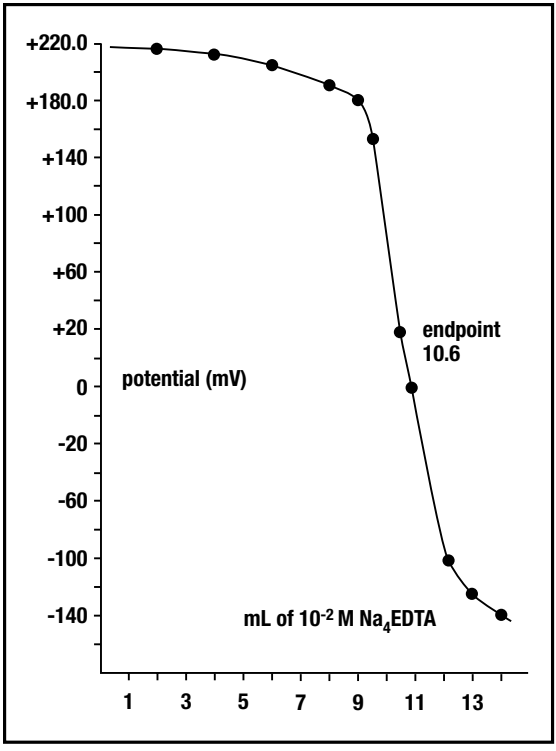
2. Using a 10 mL burette, add increments of titrant and plot the electrode potential against mL of titrant added. The endpoint is the point of greatest slope (inflection point). See **Figure 3**.

3. Calculate the sample concentration before dilution:

$$C_{sample} = C_t (V_t / V_{sample})$$

C_{sample} = sample concentration
C_t = titrant concentration
V_{sample} = sample volume
V_t = titrant volume added at endpoint.

Figure 3
Typical Titration of 10^{-3} M $CuCl_2$ with 10^{-2} M Na_4EDTA



Indicator Titrations

The cupric electrode can be used to detect the endpoint in titrations of other metal ions. A small amount of copper complex is added to the sample, and a complexometric titration is done. The endpoint volume of titrant is used to calculate the sample concentration. The minimum level of sample ion that can be determined by indicator titration is above 10^{-4} M. **Table 7** lists several species that can be titrated, with appropriate reagents and titrants.

1. Prepare the 10^{-2} M reagent by titrating the 0.1 M cupric standard exactly to the endpoint with a 0.1 M solution of the titrant to be used (see **Table 7**). Dilute the solution obtained five-fold, using a volumetric flask, to make the reagent.

2. Prepare a titrant solution about 10 times as concentrated as the sample. Place the titrant in a 10 mL burette.

3. Place the electrode in 50 to 100 mL of the sample. Record the sample volume. Add 1 mL of reagent to the sample. Adjust the sample to pH 9. Stir the solution thoroughly during the titration.

4. Add increments of the titrant and record electrode potential. Plot electrode potential as a function of titrant volume on linear graph paper (see **Figure 4**).

5. Calculate the sample concentration:

   $$\mathbf{C_{sample} = C_t\,(V_t\,/\,V_{sample})}$$

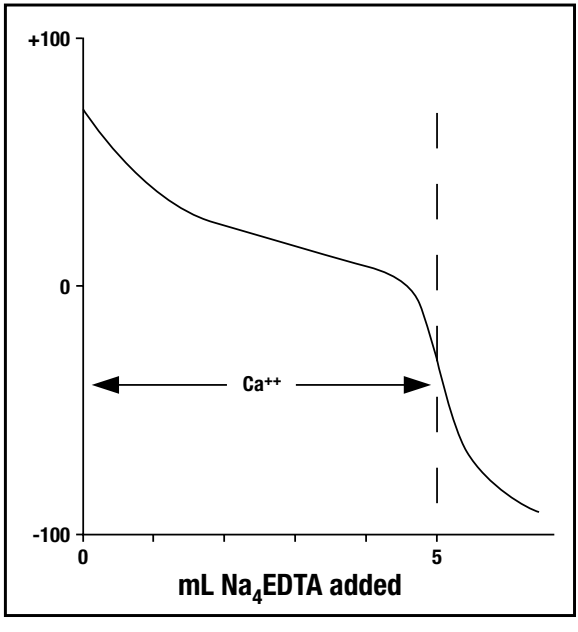
   C_{sample} = sample concentration
   C_t = titrant concentration
   V_{sample} = sample volume
   V_t = titrant volume added at endpoint.

Table 7
Reagents and Titrants for Indicator Titrations

Species	Reagent (10^{-2} M)	Titrant
Barium	CuCDTA	CDTA
Calcium	CuEGTA	EGTA
Cobalt (2+)	CuEDTA	EDTA
Magnesium	CuEDTA	EDTA
Manganese (2+)	CuEDTA	EDTA
Nickel	CuTEPA	TEPA
Strontium	CuEDTA	EDTA
Vanadium	CuEDTA	EDTA
Zinc	CuTEPA	TEPA

Chelometric Indicator Titrations with the Solid-State Cupric Ion Selective Electrode, Ross, J.W., and Frant, M.S.; Anal. Chem., 1969, 41(13), 1900.

Figure 4
Titration of 100 mL of 10^{-3} M Ca^{+2} (CuEDTA indicator added to sample)



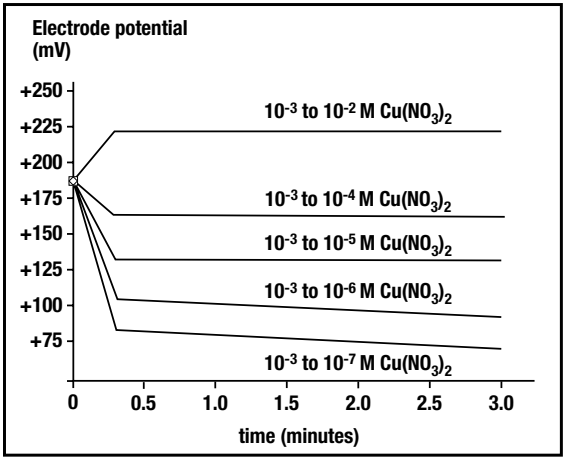
Electrode Characteristics

Electrode Response

The electrode potential plotted against concentration on semi-logarithmic paper results in a straight line with a slope of about 25 to 30 mV per decade change in concentration.

The time response of the electrode (the time required to reach 99% of the stable potential reading) varies from several seconds in concentrated solutions to several minutes near the limit of detection.

Figure 5
Typical Electrode Response to Step Changes in $Cu(NO_3)_2$ Concentration



Reproducibility

Reproducibility is limited by factors such as temperature fluctuations, drift and noise. Within the operating range of the electrode, reproducibility is independent of concentration. With hourly calibrations, direct electrode measurements reproducible to ± 4 % can be obtained.

Limits of Detection

In neutral solutions, cupric ion concentrations can be measured down to 10^{-8} M or 6 x 10^{-4} ppm. Extreme care must be taken in making determinations below 10^{-5} M or 0.6 ppm to avoid sample contamination or adsorption of cupric ion on container walls.

Temperature Effects

Since electrode potentials are affected by changes in temperature, samples and standard solutions should be within ± 1 °C (± 2 °F) of each other. At the 10^{-3} M level, a 1 °C difference in temperature results in errors greater than 4 %. The absolute potential of the reference electrode changes slowly with temperature because of the solubility equilibria on which the electrode depends. The slope of the electrode also varies with temperature, as indicated by the factor S in the Nernst equation. Theoretical values of the slope at different temperatures are given in **Table 8**. If the temperature changes, the meter and electrode should be recalibrated.

The electrode can be used at temperatures from 0 to 80 °C, provided that temperature equilibrium has occurred. For use at temperatures substantially different from room temperature, calibration standards should be at the same temperature as samples. The electrode must be used only intermittently at solution temperatures above 80 °C.

Table 8
Theoretical Slope vs. Temperature Values

Temperature (°C)	Slope (mV)
0	27.1
10	28.1
20	29.1
25	29.6
30	30.1
40	31.1
50	32.1

If sample temperatures vary, use of the 9629BNWP combination cupric electrode is recommended. The Optimum Results D filling solution that is included with the electrode will minimize junction potentials and provide optimum temperature and time response.

Interferences

Mercury and silver ions poison the cupric electrode sensing element and must by absent from the sample solution. Exposure to either of these species at levels greater than 10^{-7} M will require polishing of the electrode sensing surface. Ferric ions affect the sensing element only if the ferric ion level is greater than one tenth of cupric ion level (ferric ion can be eliminated from the sample by adding sodium fluoride and adjusting the sample to pH 4 to 6).

If the electrode is exposed to high levels of interfering ions, it may become unstable and sluggish in response. When this happens, restore normal electrode performance by polishing it. Refer to the **Electrode Maintenance** section.

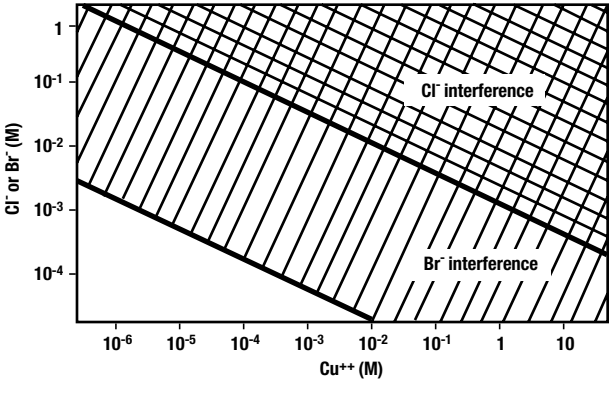
In some cases chloride and bromide ions interfere with the electrode operation. Interference is dependent on the level of chloride or bromide ions relative to the level of cupric ions in the sample and occurs only if the concentrations (in moles per liter) are outside the limits:

$(Cu^{+2})(Cl^{-})_2 > 1.6 \times 10^{-6}$

$(Cu^{+2})(Br)_2 > 1.3 \times 10^{-12}$

Figure 6 shows the regions above the lines in which the cupric ion and chloride or bromide ion levels are high enough to cause electrode malfunction.

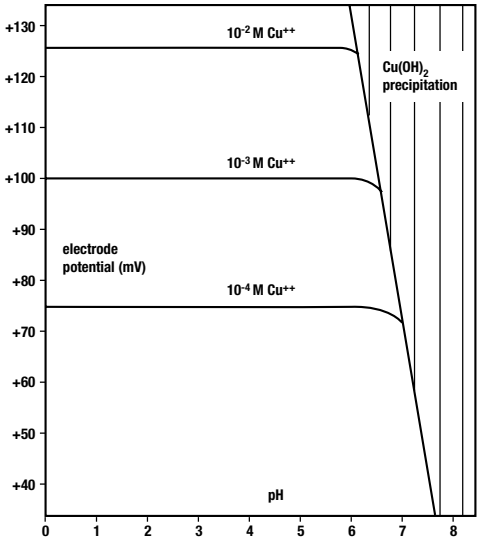
Figure 6
Interference from Chloride and Bromide Ions



pH Effects

The formation of insoluble $Cu(OH)_2$ limits the pH range over which cupric ion measurements can be made. **Figure 7** shows the effects of OH^- in solutions of various cupric ion concentrations. The shaded region indicates the pH range in which the hydroxide ion concentration is high enough to cause precipitation of $Cu(OH)^2$, reducing the level of free cupric ion in the sample. As the figure indicates, the greater the cupric ion concentration, the lower the pH in which cupric hydroxide precipitates. Adjusting sample and standard pH below 6 avoids hydroxide precipitation.

Figure 7
Precipitation of Cupric Ion by Hydroxide Ion



Complexation

Cupric forms complexes with a wide variety of species including acetate, ammonia and organic amines, citrate, amino acids and EDTA. The extent of complexation depends on the concentration of the cupric ion, concentration of the complexing agent and the solution pH. Since the electrode only responds to free cupric ions, complexation reduces the measured concentration. In a large excess (50 to 100 times) of a complexing agent, the total cupric concentration can be measured by known addition.

Soluble cupric salts are precipitated by sulfide, phosphate, hydroxide and other ions. The formation of a precipitate depends on the level of cupric ion, the level of the precipitating ion in the sample solution and the solution pH.

Theory of Operation

The cupric electrode consists of a sensing element bonded into an epoxy body. When the sensing element is in contact with a solution containing cupric ions, an electrode potential develops across the sensing element. This potential, which depends on the level of free cupric ion in solution, is measured against a constant reference potential with a digital pH/mV meter or ISE (concentration) meter. The measured potential corresponding to the level of cupric ion in solution is described by the Nernst equation.

$$\mathbf{E = E_o + S * \log (A)}$$

E = measured electrode potential
E_o = reference potential (a constant)
A = cupric ion activity level in solution
S = electrode slope (about 28 mV per decade)
S = (2.3 R T) / nF
R and F are constants, T = temperature in degrees K and n = ionic charge

The level of cupric ions, A, is the activity or "effective concentration" of free cupric ions in solution. The cupric ion activity is related to free cupric ion concentration, C_f, by the activity coefficient, y.

$$\mathbf{A = y * C_f}$$

The cupric electrode measures cupric ion activity in the same way a pH electrode measures hydrogen ion activity. This can be useful in the study of biological effects and in understanding copper speciation. To measure the cupric ion activity, copper standards are assigned activity values and no ISE or pH adjustments are made to the samples. Estimated cupric ion activities for the cupric nitrate standard are given below. For other cupric solutions, the presence of other species will affect the ion activity.

Table 9
Concentration and activity values of cupric nitrate standardizing solutions at 25 °C

Concentration (M)	Activity (M)
10^{-1}	3.2×10^{-2}
5×10^{-2}	9.6×10^{-3}
10^{-2}	5.5×10^{-3}
5×10^{-3}	1.4×10^{-3}
10^{-3}	7.9×10^{-4}
10^{-4}	9.2×10^{-5}
10^{-5}	10^{-5}

Ionic activity coefficients are variable and largely depend on total ionic strength. The ionic strength of a solution is determined by all of the ions present. It is calculated by multiplying the concentration of each individual ion by the square of its charge, adding all these values up and then dividing by two.

$$\textbf{Ionic strength} = 1/2 \sum (C_i Z_i^2)$$

C_i = concentration of ion i
Z_i = charge of ion i
$\sum$ symbolizes the sum of all the types of ions in solutions

If background ionic strength is high and constant relative to the sensed ion concentration, the activity coefficient is constant and activity is directly proportional to concentration. Ionic strength adjustor (ISA) is added to all cupric standards and samples so that the background ionic strength is high and constant relative to variable concentrations of cupric. For cupric, the recommended ISA is 5 M $NaNO_3$. Other solutions can be used as long as they do not contain ions that would interfere with the electrode response to cupric.

If samples have a high ionic strength (above 0.1 M), standards should be prepared with a composition similar to the samples.

Reference electrode conditions must also be considered. Liquid junction potentials arise any time when two solutions of different composition are brought into contact. The potential results from the interdiffusion of ions in the two solutions. Since ions diffuse at different rates, the electrode charge will be carried unequally across the solution boundary resulting in a potential difference between the two solutions. In making electrode measurements, it is important that this potential is the same when the reference is in the standardizing solution as well as in the same solution; otherwise, the change in liquid junction potential will appear as an error in the measured specific ion electrode potential.

The most important variable that analysts have under their control is the composition of the liquid junction filling solution. The filling solution should be equitransferent. That is, the speed with which the positive and negative ions in the filling solution diffuse into the sample should be nearly as equal as possible. If the rate at which positive and negative charge is carried into the sample solution is equal, then no junction potential can result. Optimum Results filling solutions are specifically designed to meet all reference electrode conditions.

Troubleshooting

Follow a systematic procedure to isolate the problem. The measuring system can be divided into four components for ease in troubleshooting: meter, electrode, sample/application and technique.

Meter

The meter is the easiest component to eliminate as a possible cause of error. Thermo Scientific Orion meters include an instrument checkout procedure and shorting cap for convenience in troubleshooting. Consult the meter user guide for directions.

Electrode

1. Rinse the electrode thoroughly with distilled water.

2. Verify the electrode performance by performing the procedure in the **Checking Electrode Operation (Slope)** section.

3. If the electrode fails this procedure, review the **Measuring Hints** section. Clean the electrode thoroughly as directed in the **Electrode Maintenance** section. Drain and refill the electrode with fresh filling solution.

4. Repeat the procedure in the **Checking Electrode Operation (Slope)** section.

5. It the electrode fails this procedure again and the half-cell cupric electrode is being used, determine whether the cupric or reference electrode is at fault. To do this, substitute a known working electrode for the electrode in question and repeat the procedure in the **Checking Electrode Operation (Slope)** section.

6. If the electrode passes the procedure, but measurement problems persist, the sample may contain interferences or complexing agents, or the technique may be in error.

7. Before replacing a faulty electrode, review this user guide and be sure to thoroughly clean the electrode; correctly prepare the electrode; use the proper filling solution, ISA, and standards; correctly measure the samples and review the **Troubleshooting Checklist** section.

Sample/Application

The quality of results depends greatly upon the quality of the standards. Always prepare fresh standards when problems arise, it could save hours of frustrating troubleshooting! Errors may result from contamination of prepared standards, accuracy of dilution, quality of distilled water, or a mathematical error in calculating the concentrations.

The best method for preparation of standards is serial dilution. Refer to the **Serial Dilution** section. The electrode and meter may operate with standards, but not with the sample. In this case, check the sample composition for interferences, incompatibilities or temperature effects. Refer to the **Sample Requirements**, **Temperature Effects**, **Interferences, pH Effects** and **Complexation** sections.

Technique

If trouble persists, review operating procedures. Review calibration and measurement sections to be sure proper technique has been followed. Verify that the expected concentration of the ion of interest is within the limit of detection of the electrode.

Check the method of analysis for compatibility with your sample. Direct measurement may not always be the method of choice. If a large amount of complexing agents are present, known addition may be the best method. If working with low level samples, follow the procedure in the **Low Level Calibration** section.

Assistance

After troubleshooting all components of your measurement system, contact Technical Support. Within the United States call 1.800.225.1480 and outside the United States call 978.232.6000 or fax 978.232.6031. In Europe, the Middle East and Africa, contact your local authorized dealer. For the most current contact information, visit www.thermo.com/contactwater.

For the latest application and technical resources for Thermo Scientific Orion products, visit www.thermo.com/waterapps.

Warranty

For the most current warranty information, visit www.thermo.com/water.

Troubleshooting Checklist

- No electrode filling solution added –
  Fill the electrode with filling solution up to the fill hole. Refer to the **Electrode Preparation** section for details.

- Incorrect electrode filling solution used –
  Refer to the **Electrode Preparation** section to verify the correct electrode filling solution.

- Electrode junction is dry –
  Push down on the electrode cap to allow a few drops of filling solution to drain out of the electrode.

- No reference electrode present –
  The 9429BN and 9429SC cupric half-cell electrodes require a separate reference electrode, Cat. No. 900200.

- Electrode is clogged or dirty –
  Refer to the **Electrode Maintenance** section for cleaning instructions.

- Sensing element is dirty or etched –
  Refer to the **Electrode Maintenance** section for cleaning instructions.

- Standards are contaminated or made incorrectly –
  Prepare fresh standards. Refer to the **Measurement Hints** and **Analytical Techniques** sections.

- ISA not used or incorrect ISA used –
  ISA must be added to all standards and samples. Refer to the **Required Equipment** section for information on the ISA.

- Samples and standards at different temperatures –
  Allow solutions to reach the same temperature.

- Air bubble on sensing element –
  Remove air bubble by reimmersing the electrode in solution.

- Electrode not properly connected to meter –
  Unplug and reconnect the electrode to the meter.

- Meter or stir plate not properly grounded –
  Check the meter and stir plate for proper grounding.

- Static electricity present –
  Wipe plastic parts on the meter with a detergent solution.

- Defective meter –
  Check the meter performance. See the meter user guide.

Ordering Information

Cat. No.	Description
9629BNWP	Cupric ionplus Sure-Flow combination electrode, waterproof BNC connector
900063	Optimum Results D electrode filling solution, 5 x 60 mL bottles
9429BN	Cupric half-cell electrode, BNC connector (requires separate reference electrode)
9429SC	Cupric half-cell electrode, screw cap connector (requires separate reference electrode)
900200	Double junction reference electrode, pin tip connector
900002	Inner chamber filling solution for the double junction reference electrode, 5 x 60 mL bottles
900003	Outer chamber filling solution for the double junction reference electrode, 5 x 60 mL bottles
942906	0.1 M cupric standard, 475 mL bottle
940011	ISA for cupric measurements, 475 mL bottle
984201	Polishing strips

Specifications

Concentration Range

10^{-8} M to 0.1 M (6.4 x 10^{-4} ppm to 6354 ppm)

pH Range

2 to 12

Temperature Range

0 to 80 °C continuous use, 80 to 100 °C intermittent use

Electrode Resistance

Less than 1 megohms

Reproducibility

± 4%

Minimum Sample Size (9629BNWP)

5 mL in a 50 mL beaker

Size– 9629BNWP

Body Diameter: 13 mm

Body Length: 110 mm

Cap Diameter: 16 mm

Cable Length: 1 meter

Size– 9429BN and 9429SC

Body Diameter: 12 mm

Body Length: 110 mm

Cap Diameter: 16 mm

Cable Length: 1 meter (9429BN only)

** Specifications are subject to change without notice*

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APPENDIX B

Seed Collection Standard Operating Procedures

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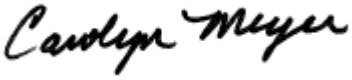
Chino Mines Company
Grant County, New Mexico

Standard Operating Procedures for Seed Collection and Storage

November 2013

ARCADIS

Mary Carroll
Senior Ecologist



Carolyn Meyer
Technical Expert/Ecologist

Standard Operating Procedures for Seed Collection and Storage

Prepared for:
Chino Mines Company
Grant County, New Mexico

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Lakewood
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Date:
September 2013

ARCADIS

Table of Contents

Figure

Standard Operating Procedures for Seed Collection and Storage

Chino Mines Company
Grant County, New Mexico

1. Introduction

This document outlines Standard Operating Procedures (SOPs) for seed collection and storage in support of phytotoxicity studies used to evaluate the effects of copper on native vegetation at the Chino Mines Site located in Grant County, New Mexico (the Site, Figure 1). The Site is located east of the town of Hurley and approximately 12 miles southeast of Silver City; it includes historical smelting facilities, mineral processing facilities, tailing impoundments, and surrounding areas.

This SOP document outlines the quality and quantity of seed material to be collected, documentation procedures, collection procedures, and storage procedures for seed material to be used in the phytotoxicity study.

2. Seed Collection Procedures

Native seeds collected at the Chino Mines Site will be collected as per requirements outlined in the Smelter Tailing Soils Investigation Unit (STSIU) – Phytotoxicity and Vegetation Community Study (ARCADIS 2013). The phytotoxicity tests will evaluate sideoats grama (*Bouteloua curtipendula*) and scarlet globemallow (*Sphaeraclea coccinea*), which are herbaceous species common and native to the Site.

2.1 Seed Collection Localities

Seeds will be collected from one ten-acre location (Seed Collection Area) that has been protected from grazing in the summer of 2013 to increase the potential for seed availability (Figure 1). If sufficient seed of a target species is not present in the Seed Collection Area, additional seed may be obtained in nearby locations within one mile of the Seed Collection Area.

2.2 Seed Viability and Quantity

In order to reduce the variables in the proposed phytotoxicity study, healthy seed should be collected and seed viability tested to ensure standard results. Seeds will be sent to Growing Solutions Restoration Education Institute in Santa Barbara, California to have them cull potentially non-viable seeds (based on appearance), clean, dry, and store the seeds until the phytotoxicity test begins in January 2014. Seed germination viability will be obtained during the phytotoxicity tests on the control pots with potting soil. Methods to ensure mostly pure, healthy seed is collected in the field are described in more detail below in Section 2.4.

The phytotoxicity tests conducted at Wildlife International Laboratory require a minimum of 4,080 seeds per species collected on site (ARCADIS 2013). To protect against loss and account for culling, 8,000 seeds of

sideoats grama and 8,000 seeds of scarlet globemallow will be collected. These species are available from nursery or commercial seed suppliers, have germination requirements compatible with Wildlife International Laboratory capabilities, have high germination rates (≥ 80 percent for the grass and > 70% for the forb) under lab temperatures (~20°C) and are abundant on the seed collection site. Vine mesquite (*Panicum obtusum*) and purple threeawn (*Aristida purpurea*) were common but are difficult to germinate at high rates. Plains bristlegrass (*Setaria macrostachya*) is also common but nursery strains are purportedly often a mix of several species (*S. macrostachya*, S. *leucopila*, S. *texana*) and the species hybridize. Tansy aster (*Machaeranthera tanacetifolia*) is common in the ERA sites sampled in 1999 (Newfields 2005) and was considered for the study, but is not present in abundance on the seed collection site.

2.3 Seed Collection Timing

Seed collection will occur after the monsoon season, when seeds of target species have ripened. In general, seed will be ripe two to five weeks after peak bloom, so field checks should be performed in early September 2013 to track seed maturation. Sudden heat or cold spells may affect seed ripening and dispersal. Seeds should be examined in the field to check for viability, as described below.

2.4 Field Collection Guidelines

Maximum seed viability is achieved when fully ripened, pest-free seed is collected. Seed viability may be affected by lack of pollinators, parasitism, and a range of environmental conditions and there can be a fairly high percentage of unviable seeds. Healthy seeds are generally filled internally from edge to edge with white moist endosperm or embryo tissue (Wall 2012). The following recommendations will enhance the likelihood of collecting viable seed from diverse maternal lines:

- Use a hand lens or bring a microscope into the field to check the condition of the seeds. Fully developed, mature, viable seeds generally turn dark in color with maturity (vs. green), separate from the ovary wall, and/or are easily detached from the plant. A cut test can be used in the field by using a single edge razor, a small wood block, and a hand lens or microscope. Look for plump seeds with the characteristics of mature seeds for each species (Section 4).
- Collect from a minimum of parent plants when possible. Avoid collecting all of the seed from one localized area in the general collection area, in order to allow the species to reseed.
- Do not collect from parent plants with observed pests, fungus, or other illness.
- Fruits and seeds can be hand collected or knocked from the parent plant, or stems bearing fruits can be cut from mature plants with clean by-pass hand pruners and placed in large paper bags for drying. Paper bags should be taped on bottom to prevent seeds from falling through.

- Avoid collecting seed from the ground as this increases the likelihood of mixed species and soil and seed pathogens. If seed is collected from the ground, keep separate from plant collected seed and carefully label. If possible, lay clean clothes or sheets around target plants prior to seed collection if seeds fall and ground collection is necessary.
- Place seeds or fruits loosely in sturdy paper bags. Air circulation is essential to maintain seed health.
- Perform cursory cleaning in the field to maximize the number of seeds in the bags.

Each collection bag should have the initials of the collector, the date, and the species, location, and estimated number of seeds in the bag.

Field data should be recorded to indicate the total number of individuals or area from which seed was collected and proportion of the target population in bloom and/or fruit. Additionally, a list of species growing with these species which look similar to the target species should be recorded or any species that may accidentally be collected. Before going to the Site, soil type, slope exposure, elevation, and global positioning system (GPS) waypoint of the Seed Collection Area should be recorded from the NRCS database and GIS slope/aspect maps. If seeds are collected in other areas, they will be marked with a GPS waypoint and the same information recorded. Field observations should be recorded to confirm the information recorded from the GIS and soil database. Soil and site characteristics observed in the field collection area should be described (presence of A horizon, if armored with rock, percent bedrock in area) and photographs taken. A voucher specimen of each species should be collected, dried, and labeled to demonstrate the correct species was sampled. A voucher specimen should consist of a typical plant or portions of a plant with stems, leaves, and reproductive structures. The dried specimen should be carefully stored in a dry location and sent to a local herbarium.

3. Seed Drying and Storage Guidelines

For all seed drying and storage, the collection identification information should be provided with each seed lot.

Seeds are dried to reduce seed moisture and facilitate seed ripening. Seeds will be sent to Growing Solutions Restoration Education Institute in Santa Barbara, California for drying, cleaning, culling, and processing. Seeds stored for only a few days or less before shipping to this laboratory may be stored in labeled paper bags or envelopes containing a small amount of desiccant (silica gel); desiccant packs may be purchased commercially that have a color indicator showing when conditions are moist or dry. The paper seed containers should be placed in either a cool dry location or a refrigerator. Seeds will be shipped after

collection as soon as possible, which should be after no more than two days. The protocol for the Santa Barbara laboratory is described below.

Green leaves and stems should be removed from the seed heads, where possible, prior to drying. Seeds can be dried on an open seed drying rack or cookie sheet in a warm enclosed area (about 80ºF is ideal) with low humidity until there is no clear sign of moisture. This usually takes 1 to 3 days. An alternative is to dry the seed at 100ºF for six hours to bring down the moisture level in the seeds.

The seed heads then need to be processed to remove as much non-seed material (i.e., "chaff" and/or other plant material) as possible prior to drying. Seed processing is performed manually and involves hand sorting and/or sieving. Larvicides (moth balls) should be avoided because they affect the phytotoxicity test, unless absolutely necessary to present larval growth. If necessary, seeds may be placed in a closed 5-gallon bucket with 3 moth balls for at least two days. Because of the strong odor all work with moth balls will be performed in the open air. After adding larvicide, the buckets are tightly sealed with a lid and stored for two days during the treatment period. After the two days, they are taken outside again to remove the larvicide in open air and transferred to containers for storage as described below.

After seed drying and processing, seeds should be examined for uniformity, health, and plumpness. Malformed or diseased seeds should be culled, and if quantities allow, the largest seeds should be retained and the smallest seeds culled.

Because humidity changes are easily transferred through paper, seeds should be placed in a sealed container such as tightly closing resealable containers. A desiccant pack should be included in each container to avoid decay. Seeds are then placed in a refrigerator until ready for shipment. All stored seeds will be inspected every few weeks for any signs of decay or degradation, and decaying seeds should be removed. Desiccant packs should be changed if necessary.

4. Species Descriptions

Seeds of sideoats grama (*Bouteloua curtipendula*) and scarlet globemallow (*Sphaeralcea coccinea*) have been selected for this study as both species are common and native to the Chino site. A brief description of each species and its seed characteristics is provided below. In addition, a description of alfalfa (*Medicago sativa*), an agricultural species used in previous phytotoxicity studies, is provided,

Sideoats Grama (*Bouteloua curtipendula*)—grass species

Sideoats grama is a widely distributed warm season perennial grass in the Grass Family. Most plants either arise singly, in clumps, or form large patches, depending on the variety. The variety *caespitosa* occurs from the southwestern United States to South America in prairies and arid grasslands, desert scrub, pine-oak and pinyon-juniper woodlands, and Ponderosa pine forests, whereas the variety *curtipendula* extends from the southwestern United States north to Canada in prairies, hardwood savannas, and other habitats; a third variety is confined to Mexico. Sideoats grama occurs at a range of elevations, from near sea level to over 8,000 feet. Due to the importance of this grass in rangelands and habitat restoration, considerable information is available on its biology and several horticultural forms have been developed.

The elongate flower spikes produce pendulous spikelets from mid-summer to fall, with seed ripening following several weeks later. The elliptical seeds (caryopsis) are 4.5 mm long by 1.5 mm wide. There are 160,000 seeds/ per pound (USDA 2013). Seeds are generally collected while still retain surrounding flowering structures, which are removed during

cleaning. Germination rates vary with place of seed origin, temperature, timing of rainfall, and other environmental conditions and have ranged from 18 to 96 percent in various studies, with common values of 30 to 70 percent. Germination is favored when floral parts are removed from the caryopses; when seeds are planted one-inch deep vs. shallower or deeper; when seeds are relatively plump and heavy; and under various experimental temperatures that tended to be warm, between 50 and 86^{o}F (USDA 2013). Germination may occur within 2 to 7 days in moist soil (Wasser 1982, Jordan and Haferkamp 1989).

Scarlet globemallow (*Sphaeralcea coccinea*)—forb species

Scarlet globemallow is a low-spreading, warm season, long-lived perennial forb to half-shrub in the Mallow Family. Stems emerge from a woody caudex located just under the soil surface and reach a height of 10-40 cm (4-16 inches). Plants are densely covered with stellate hairs. Leaves are alternate, palmately lobed, 1-3.7 cm long and 1-5 cm wide. The deep orange to pinkish colored flowers are clustered in dense, short racemes. There are 5 distinct petals, 5 united sepals, and 5 to numerous styles. Stamens are joined by their stalks into a tube and several pistils united in a ring. The fruit is an indehiscent schizocarp with 1-seeded carpels. Plants are rhizomatous. Growth begins in March and April, flowering in May to July and seed matures unevenly between July and August throughout much of its range. There are approximately 500,000 seeds per pound. The seed has a hard seed coat that must be scarified in order for germination to occur.

Eight species of Sphaeralcea occur at Chino (Newfields 2005) and care should be taken to ensure that the seeds of the correct species are collected. About 15 percent of the seeds of a plant are ripe at any one time (indeterminate seed ripener; St. Johns and Ogle 2009); one must ensure ripe seeds are harvested. In addition, seed may be subject to insect predation while still on the parent plant. Globemallow should be harvested when lower capsules begin to dry (St. Johns and Ogle 2009). Seed capsules can be cut from the parent plant and placed in seed collection bags to save time, and cleaning can be done later. Gloves and

safety glasses should be used when handling seed because the stellate hairs on the seed and surrounding capsules can be a severe eye irritant. Fruit is a wedge-shaped capsule held in a ring of ten or more seeds. Avoid collecting seeds exhibiting seed predation.

Scarlet globemallow germinates best after 30-day stratification (cold period) and mechanical (or acid) scarification of the seed coat to germinate (Dunn 2011, St Johns and Ogle 2009). Recommended planting depth is 6.4 mm (Rawlins et. al 2009). Seeds germinate rapidly with scarification, sometimes within 1 day (Deno 1993).

Alfalfa (*Medicago sativa*)—agricultural species

Alfalfa is a deep-rooted herbaceous perennial forb in the Pea Family that is cultivated for forage in many regions of the world. Multiple stems arise from a narrow woody crown and reach up to 1 m (3 feet) in height at maturity, bearing alternate leaves divided into three lance-shaped to ovoid leaflets. Flowers appear in spring, summer, and early fall and range in color from violet to yellow-green; the small legume fruits are spiraled in two to three turns and each contains 10 to 20 seeds. Alfalfa is considered a species complex, with nine facies classified as subspecies and hundreds of cultivars; there are both diploid and tetraploid forms. It originated in Southeast Asia and was first cultivated in Iran.

There are approximately 200,000 seeds per pound; viable seeds are bright olive-green. On average, about 45 to 73 percent of seeds have a hard seed coat that requires scarification for germination (USDA 1982); hard seed coats are produced more frequently on plants in cold climates (northern latitudes or higher elevations) compared with warm climates such as southern California or lower latitudes. Long-lived seeds have exhibited 81 percent germination after 19 years of seed storage (Watts et. al 1992). Seeds can be pretreated by mechanical scarification or by heating in hot water (219^{o}F) for 4 minutes. Recommended planting depth for alfalfa seeds is ¼ to ½ inch (5-10 mm). Optimal germination rates are obtained with ambient temperatures between 65 and 77^{o}F and seedlings appear within three to four days (Horton 1989).

The alfalfa variety Nitro Plus will be obtained from Territorial Seed Company in Cottage Grove OR for this study (Lot # 18041). This variety exhibits germination rates of 87 percent during laboratory testing.

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P1
152
356
180
WEST STOCKPILE
SANTA RITA OPEN PIT
LAMBRIGHT STOCKPILE
SOUTH STOCKPILE
SANTA CLARA
BAYARD
HURLEY
TAILING POND B
TAILING POND 4
TAILING POND C
TAILING POND 6W
TAILING POND 6E
TAILING POND 7
SILVER CITY
GRANT COUNTY AIRPORT
ATCHISON, TOPEKA AND SANTE FE RAILROAD
STS-PT-2013-15
STS-PT-2013-31
STS-PT-2013-5
STS-PT-2013-32
STS-PT-2013-16
STS-PT-2013-36
STS-PT-2013-9
STS-PT-2013-10
STS-PT-2013-35
STS-PT-2013-19
STS-PT-2013-17
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STS-PT-2013-1
STS-PT-2013-3
STS-PT-2013-8
STS-PT-2013-2
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STS-PT-2013-30
STS-PT-2013-7
STS-PT-2013-21
STS-PT-2013-22
STS-PT-2013-26
STS-PT-2013-25
STS-PT-2013-28
Seed Collection Area Protected From Grazing
STS-PT-2013-4
STS-PT-2013-27
STS-PT-2013-23

LEGEND:

- Site Sample Locations
- De Minimus Sample Locations
- Seed Collection Area Protected From Grazing
- STSIU Boundary

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SCALE IN FEET

Notes:
1) Service Layer Credits: APFO

FREEPORT-MCMORAN CHINO MINES COMPANY
VANADIUM, NEW MEXICO

SMELTER TAILING SOILS IU PHYTOTOXICITY AND VEGETATION COMMUNITY STUDY

PHYTOTOXICITY SAMPLING LOCATIONS

ARCADIS

FIGURE 1

APPENDIX C

Wildlife International Standard Operating Procedure

ARCADIS
Infrastructure · Water · Environment · Buildings

Imagine the result

A Test to Determine the Seedling Emergence and Growth of Terrestrial Plants in Various Field-Collected Soils

Submitted to:

ARCADIS U.S., Inc.

Wildlife International
EVANS ANALYTICAL GROUP

8598 Commerce Drive
Easton, Maryland 21601 USA
410-822-8600

January 2, 2014

P R O T O C O L

A TEST TO DETERMINE THE SEEDLING EMERGENCE AND GROWTH OF TERRESTRIAL PLANTS IN VARIOUS FIELD-COLLECTED SOILS

Submitted to

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January 2, 2014

A TEST TO DETERMINE THE SEEDLING EMERGENCE AND GROWTH
OF TERRESTRIAL PLANTS IN VARIOUS FIELD-COLLECTED SOILS

SPONSOR:	ARCADIS U.S., Inc.
SPONSOR'S REPRESENTATIVE:	Matthew Barkley
TESTING FACILITY:	Wildlife International, 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Anne B. Sindermann, Senior Biologist Wildlife International
LABORATORY MANAGEMENT:	John R. Porch Manager of Plant and Invertebrate Toxicology

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: _____ Experimental Termination Date: _____

Project No.: 757P-101

Test Concentrations: N/A

Test Substance No.: N/A Reference Substance No. (if Applicable): N/A

PROTOCOL APPROVAL

_____ _____
STUDY DIRECTOR DATE

_____ _____
LABORATORY MANAGEMENT DATE

_____ _____
SPONSOR'S REPRESENTATIVE DATE

INTRODUCTION

Wildlife International will conduct a toxicity test with three species of plants to determine seedling emergence and growth in various field-collected soils. The test will be conducted at the Wildlife International plant testing facility in Easton, Maryland. The test species will be sideoats grama, scarlet globemallow and alfalfa. The study will be based on procedures in OECD Guideline for Testing of Chemicals, Guideline 208: *Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test* (1), with some modifications to allow use of natural Chino soils and replicate aspects of the 1999 phytotoxicity study in the Ecological Risk Assessment (e.g., measure root length). Raw data for all work performed at Wildlife International and a copy of the final report will be sent to the Sponsor and a copy filed by project number in archives located on the Wildlife International site, or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to determine the seedling emergence and growth of three species of terrestrial non-target higher plants in various field-collected soils.

EXPERIMENTAL DESIGN

For each of the species tested, seeds will be planted in each of thirty-four test soils. There will be eight field-collected de minimus soils, twenty five field-collected test soils, and one commercially obtained potting soil as a negative control. No test substance will be incorporated into the soil used for planting. Field-collected test and reference soils will be provided by the Sponsor. There will be ten replicate pots for each soil type, with twelve seeds planted per replicate. The replicates will be placed on a benchtop in a greenhouse according to a randomized design. The test duration will be 14 days after 50% emergence of control plants (nursery seeds in control soils), during which time possible phytotoxic effects of the test substance on seedling emergence and growth of emerged seedlings will be evaluated (more details on test duration provided below). Data collected from all replicates within a soil type will be pooled for calculating group means.

MATERIALS AND METHODS

Species to be Tested

The three species of plants planned for use in this study are listed below:

Family	Scientific Name	Common Name	Planting Depth
Monocots			
Poaceae	*Bouteloua curtipendula*	Sideoats grama	20 mm
Dicots			
Malvaceae	*Sphaeralcea coccinea*	Scarlet globemallow	10 mm
Fabaceae	*Medicago sativa*	Alfalfa	10 mm

Sideoats grama and scarlet globemallow [*Sphaeralcea coccinea*] will be tested as two distinct populations: one consisting of field-collected seeds and the other consisting of seeds provided by a plant nursery or seed supplier. One seed source of alfalfa will be tested. Seeds will be planted at the species specific depths shown in the above table. Seeds used in this study will not have been treated with fungicides, insecticides or repellents prior to test initiation. Seeds will be provided by the Sponsor. Any documentation provided by the supplier concerning the identification and history of the seeds used will be included in the study data.

TEST SOILS

Test soils will be collected from twenty-five test sites and eight de minimus sites and shipped to the greenhouse facility. A standard potting soil will be manufactured as discussed in the main phytotoxicity study plan (ARCADIS 2013). The soils will be delivered sieved to 2 mm. Prior to planting and at the conclusion of the study, the pH of each soil type will be measured with a soil probe.

Environmental Conditions

The test will be conducted within a greenhouse. Relative humidity, light intensity and temperature within the greenhouse will be measured continuously with a Campbell CR10 or equivalent datalogger. The temperature within the greenhouse will be controlled by a Wadsworth Micro/Step 50 Control System, or equivalent at a set-point temperature of ~20 degrees Celsius. A photoperiod of at least

16 hours of light will be maintained in the greenhouse. Artificial lighting may be used to supplement natural sunlight on short days or on overcast days.

Test Procedure

Test plants will be grown in plastic pots approximately 11 cm in diameter and 10 cm in depth. Fifty growth pots will be filled with soil from each test or de minimus site. Twelve seeds of one species (or specified wild or nursery population) will be planted per replicate, following Table 3 in the Phytotoxicity study plan. Scarification and stratification is not required for the sideoats grama. For scarlet globemallow, the seeds must be scarified and then cold stratified using the following procedure:

1. Scarify using either sand paper by rubbing over seeds until seed embryo if just visible, or nick each seed with a tiny cut through seed coat. Wash.

2. Then cold stratify the scarlet globemallow seeds for approximately 30 days. For small quantities of seeds (6,000 of each species will be provided and the laboratory will determine if this is "small"), mix at a ratio of 1:3 or more with moist peat moss or moist vermiculite, place in a tightly sealed polyethylene bag or glass jar, and store in the refrigerator at a temperature of 35 – 41°F. For bulk seeds, soak in water for a few hours first, then place wet in a sealed container in the refrigerator at temperature of 35 – 41°F. In either case, the seeds must be kept moist during the entire length of the treatment. This will require periodic checking and the addition of water if necessary. If any white root tips are visible, the whole batch should be sown immediately. The longer the radicals are when the seeds are sown, the greater the probability of damage and the greater the mortality rate is apt to be. If the stratification period is inadvertently lengthened, it is usually not detrimental, providing the radicals are still very short or not yet showing. In contrast, to cut the stratification period short by even a few days could be harmful if no radicals are visible. By prematurely discontinuing stratification, primary dormancy may not be broken. Consequently, a secondary dormancy may be induced, which is more difficult to break than the original dormancy. The cold stratification period necessary to break dormancy varies depending upon the species. After stratification, the seeds should be sown promptly before they have a chance to dry out.

The seeds will be planted at the species appropriate depth and will be approximately equally

spaced. Pots will be uniquely identified with a minimum of the species name, project number, designation of soil type (test, de minimus, potting soil negative control), and replicate. For the species other than alfalfa, the pot also will be labeled as wild or nursery seed. After planting, the growth pots will be placed on benches in the greenhouse in a randomized configuration to minimize bias from microclimates that may exist within the greenhouse. Water will be supplied to the growth pots by watering from the top to keep the soil evenly moist, simulate natural conditions and reduce leaching of metals and salts in the soil column. The pH of the tap water will be adjusted to approximately 6 using dilute HCL before it is used to water plants. Records of the days that watering occurs and the source of water used will be kept in the study data.

The control growth pots will be observed for germination daily to be able to record when 50% have emerged following the OECD guidance. The in-life portion of the test will terminate fourteen days after 50% of the control plants grown from nursery seed in potting soil have germinated for the grass and alfalfa species. If sufficient growth is available for measuring the forb (scarlet globemallow), the test will be terminated for that species at 14 days. If not, then it could be extended to twenty-one days after 50% of the seeds have germinated. On the day of test termination, the pH of soil in each replicate will be measured using a soil probe (accurate to +/- 0.2 SU). At the termination of the in-life portion of the test, percent of seeds that germinated will be reported. Also, length and weight measurements of the shoot and length of the root and the condition and survival of the emerged seedlings will be recorded. The height of each living seedling within a replicate will be determined. The method used to measure root length will be described in the raw data and included in the final report but the root length will be based on the longest root. After shoot height and root length measurements are completed, plants will be clipped at the stem/root soil level and the above-ground portion (shoots) of all living plants within each replicate will be weighed. The total dry shoot weight (biomass) of each replicate will be determined.

Overcrowded conditions may occur in plants unaffected by copper in the soil toward the end of the test as the plants grow. If this appears to be occurring, the laboratory will contact the Sponsor to discuss the possibility of thinning the plants in affected pots.

DATA ANALYSES

The mean number of emerged seedlings, surviving seedlings, shoot height, shoot dry weight, and root length for each treatment group (soil type) will be calculated. Compliance with the OECD criteria for control survival will be evaluated. The test for the site seeds will be considered successful if their germination and survival in the negative control soil meets the following minimum requirements:

- Alfalfa: OECD guidance of 80% germination for crop species with a 90% survival rate for the negative control.
- Sideoats Grama: OECD guidance of 65% germination for non-crop species with a 90% survival rate for the negative control.
- Scarlet globemallow: 55% germination with 80% seedling survival for the negative control (ASTM 2009 standards for the carrot).

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International will include but not be limited to:

1. Copy of signed protocol.
2. Dates of initiation and termination of the test.
3. Observations.
4. Test conditions (temperature, humidity, etc.).
5. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International and sent to the Sponsor that will include the raw data. The report will include, but not be limited to, the following, when applicable.

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.

3. A description of the methods used to conduct the test (including, but not limited to description of the test system as pot dimensions, pot material, amounts of soil, soil characteristics as pH, growth conditions as light intensity, photoperiod, temperatures, watering schedule and method, pH of water).
4. A description of the test species, including the source and scientific name.
5. A description of the preparation of the test soils.
6. The methods used to allocate seeds to test substrates and begin the test, the number of seeds and replicates per treatment, and the duration of the test.
7. A description of circumstances that may have affected the quality or integrity of the data.
8. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
9. The raw data in tables for results and a description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical (pH) chemistry data and a statement of the conclusions drawn from the analyses. The report will include photographs of example pots at end of test showing pots of poor, fair, and good growth for each seed/soil type, if applicable).
10. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
11. The location where a copy of the raw data and final report are to be stored.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will not be conducted in accordance with OECD, FDA or EPA Principles of Good Laboratory Practices, and no statement of compliance with Good Laboratory Practices will be included in the report. Raw data for all work performed at Wildlife International and a copy of the final report will be sent to the Sponsor and a copy filed by project number in archives located on the Wildlife International site, or at an alternative location to be specified in the final report.

REFERENCES

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