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Cobalt freshwater toxicity

Acute and Chronic Toxicity of Cobalt to Freshwater Organisms: Using a Species Sensitivity Distribution Approach to Establish International Water Quality Standards

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Abstract: Water quality standards for cobalt (Co) have not been developed for the European Union or United States. The objective of this research was to produce freshwater Co toxicity data that could be used by both the EU and US to develop appropriate regulatory standards (i.e., Environmental Quality Standards [EQS] or Predicted No Effect Concentration [PNEC] in Europe and Ambient Water Quality

Criteria [AWQC] or State Water Quality Standards (WQS) in the US). Eleven species, including algae, an aquatic plant, and several invertebrate and fish species, were used in the performance of acute and chronic Co toxicity tests. Acute median-lethal or median effective concentration (LC_{50} or EC50) values ranged from 90.1 µg Co/L for the duckweed (*Lemna minor*), to 157,000 µg Co/L for the midge (*Chironomus tentans).* Chronic 10% effect concentration (EC_{10}) values ranged from 4.9 µg Co/L for the duckweed, to 2,170 µg Co/L for rainbow trout (*Oncorhynchus mykiss*). Chronic 20% effect concentration (EC_{20}) values ranged from 11.1 µg Co/L for the water flea (*Ceriodaphnia dubia*), to 2,495 µg Co/L for *O. mykiss*. Results indicated that invertebrate and algae/plant species are more sensitive to chronic Co exposures than fish. Acute-chronic ratios (derived as acute LC_{50}s divided by chronic EC_{20}s) were lowest for juvenile *O. mykiss* (0.6) and highest for the snail, *Lymnaea stagnalis* (2,670). Following the European-based approach and using EC_{10} values, species sensitivity distributions (SSD) were developed and a median hazardous concentration for 5% of the organisms ($HC_{5,50\%}$) of 1.80 µg Co/L was derived. Chronic EC_{20} values were used, also in a SSD approach, to derive a US EPA-style final chronic value (FCV) of 7.13 µg Co/L.

Keywords: cobalt, PNEC, water quality criteria, water quality standards, water quality guidelines

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INTRODUCTION

Cobalt is a naturally-occurring element that is ubiquitously distributed throughout the world. It is an anthropogenically important trace metal that is used in a wide range of industrial and technological applications, including use as a component in high-temperature-resistant alloys for jet engines and a component that improves the performance of magnetic alloys and rechargeable batteries (Hamilton 1994). It is also used as a pigment, petroleum industry catalyst, and as a nutrient in fertilizers. Cobalt is primarily found in nickel and nickel-copper deposits and mined in Australia, Canada, Russia, the Democratic Republic of the Congo, or Zambia (Slack et al 2017, BGS 2018). Cobalt is usually rare in freshwater environments, other than in situations where mining, manufacturing, or other activities may produce locally elevated concentrations. Gaillardet et al (2007) estimated an average dissolved Co concentration in major world rivers of 0.148 µg/L, with average values for large rivers around the world ranging from <0.02 to 0.43 µg/L. In Canada, nation-wide water quality monitoring showed dissolved and total Co concentrations ranged from 0.002 to 64 µg/L and 0.002 to 3.9 µg/L, respectively (Environment Canada 2017). Co concentrations reported in surface waters taken from three of the most highly contaminated sites in Canada ranged from 0.025 to 2028 µg/L (Environment Canada 2017). In the United States (US), Co concentrations in surface waters ranged from less than 0.1 to greater than 1000 µg/L where the highest values were from small streams contaminated by mining (Mebane et al 2015). Background levels of dissolved Co in European waters are estimated to be 0.18-0.21 µg/L (Neal et al 1996, Borg 1983). Recently, the Forum of European Geological Surveys (FOREGS; [Salminen et al 2005]) sampled stream waters from random locations within a grid throughout

Europe and determined concentrations of an extensive range of elements, including Co. Mean stream concentrations of dissolved (<0.45µm) Co were determined to be 0.333 ± 1.01 µg Co/L (n=807; range: 0.01 - 15.7 µg Co/L).

Although Co is an essential element found in vitamin B12, only trace amounts are required for life (Eitinger et al 2005, Gruber et al 2011, Blust 2011). It is unclear what environmental concentrations pose a risk to aquatic species; ambient water quality criteria (AWQC; US Environmental Protection Agency [EPA]) and Environmental quality standards (EQS; European Union (EU) under the European Water Framework Directive) do not exist for Co. Canada, however, published a Federal Water Quality Guideline (FWQG) in 2017 that reflected the effect of water hardness (as mg CaCO3/L) on Co bioavailability (Environment Canada 2017); the site-specific value (as dissolved Co µg/L) is calculated from the equation:

$$FWQG = 10^{(0.414[\ln \text{hardness}] - 1.887)}.$$

Few published studies have addressed the toxicity of Co to freshwater organisms. These studies are limited by the number of species tested (i.e., single-species; *Daphnia magna* (Biesinger and Christensen 1972), *Oncorhynchus mykiss* (Marr 1998), *Chlamydomonas reinhardtii* (Lustigman et al 1995) and the exposure duration, investigating only acute or chronic endpoints. Furthermore, few of the extant studies would satisfy current acceptability requirements for derivation of AWQC or EQS/PNEC due to shortcomings in the experimental design (e.g., tests not performed according to standard guidelines, water physicochemical parameters not reported, and results based on nominal concentrations). Although the combination of existing studies provides a useful

indication of Co toxicity to aquatic species, the data are too limited or unsuitable to meet regulatory standards. The paucity of Co freshwater toxicity data warrants a comprehensive analysis to determine safe environmental Co concentrations. Such an analysis should take into consideration international data requirements in order to be useful to all interested global regulatory authorities (e.g., USEPA and the European Chemicals Agency [ECHA]), which may have different toxicity testing criteria and requirements. For example, although the USEPA accepts acute toxicity data for developing AWQC, ECHA focuses their interest predominately on chronic toxicity data. In addition, ECHA requires data for algae and higher plants and considers these results when developing a Species Sensitivity Distribution (SSD; Table 1), while the USEPA only considers algae/plant data when developing an AWQC (CCC, Criteria Continuous Concentration), but not as part of a SSD analysis.

The primary objective of this research was to generate a high-quality freshwater toxicity dataset for Co that can be used to derive both EQS/PNEC and AWQC. To achieve this, we adopted a tiered approach focused on: 1) generation of acute toxicity data needed to derive an USEPA acute AWQC (CMC; criteria maximum concentration) and set exposure concentrations for chronic tests; and 2) generation of chronic toxicity data needed to derive an EU EQS/PNEC and USEPA chronic water quality criteria (CCC).

MATERIALS AND METHODS

The research presented herein was conducted as a cooperative effort between the Oregon State University's Aquatic Toxicology Research Laboratory (OSU, Albany, OR,

USA; formerly Parametrix Environmental Research Laboratory [PERL]) and the University of Ghent's Laboratory of Environmental Toxicology and Aquatic Ecology (Ghent, Belgium).

Chemicals and materials

Oregon State University Studies

Reagent grade cobalt chloride ($CoCl_2$•$6H_2O$; CAS 7791-13-1; supplied by J.T. Baker, Alfa Aesar, or an equivalent supplier) was used as the test material for all studies. For each study, a Co stock solution was prepared in deionized water and diluted to the exposure concentration with either reconstituted laboratory water, standard synthetic freshwater, or laboratory blended water (well water blended with reverse osmosis-treated water with a targeted hardness of 80 – 120 mg/L as $CaCO_3$). For the studies that were conducted using flow-through test procedures (acute and chronic studies with *Pimephales promelas*, *Danio rerio,* and *Oncorhynchus mykiss*), a continuous-flow proportional diluter, designed after Benoit et al. (1982), was employed. The diluter system was constructed of glass, silicone adhesive, and silicone stoppers. Test solutions were delivered to the test chambers through cross-linked polyethylene tubing. A flow-splitting chamber was used for each test concentration to promote mixing of the test solution and to equally allocate the test solution between replicate test chambers. A Marriotte bottle was used to continuously deliver an appropriate volume of Co stock solution to the diluter.

University of Ghent Studies

Cobalt chloride ($CoCl_2$•$6H_2O$, CAS 7791-13-1) was the substance used for all studies. *D. magna* was exposed to cobalt chloride in standardized test medium (ISO-medium; OECD Guideline 211 OECD 1998), which was vigorously aerated prior to spiking. Stock solutions were prepared by dissolving 79.70 or 19.93 mg/L $CoCl_2$•$6H_20$ in 100 mL deionized water for the acute and chronic tests, respectively. The entire amount of stock for the tests was prepared at one time: 200 mL (acute) or 5 L (chronic). Exposure solutions for *Hyalella azteca* acute and chronic tests were mixed in a standardized artificial test medium (Borgmann-medium, Borgmann 1996) and stocks were prepared as described for *D. magna*. *Lymnaea stagnalis* tests were conducted using general ASTM guidelines modified after Gomot (1998), which used artificial freshwater (hardness 140 mg/L as $CaCO_3$). Test medium for *Pseudokirchneriella subcapitata* was prepared as recommended by OECD Guideline 201 (OECD 1984), but with EDTA replaced by dissolved organic matter (DOM); humic acid at 32 µg/L (Sigma-Aldrich) (Heijerick et al 2002). Similarly, *Lemna minor* was tested in Swedish Standard *Lemna* growth medium with EDTA replaced with artificial DOM composed of humic acid at 32 µg/L.

Toxicity tests

A series of acute and chronic toxicity tests were conducted to determine the freshwater toxicity of Co to 10 standard freshwater species. The OSU laboratory conducted tests on *Ceriodaphnia dubia* (water flea), *Aeolosoma* sp. (oligochaete), *P. promelas* (fathead minnow), *D. rerio* (zebrafish), and *O. mykiss* (rainbow trout) and the University of Ghent conducted tests on *D. magna* (cladoceran), *H. azteca* (amphipod), *L.*

stagnalis (great pond snail), *L. minor* (duckweed), and *P. subcapitata* (green algae). While nine aquatic species were subjected to both acute and chronic toxicity tests, *L. minor* and *P. subcapitata* were only used in chronic toxicity testing; however, acute (EC_{50}) and chronic (EC_{10}) values were determined from these tests as described in ECHA (2008). Acute and chronic toxicity data for an additional, twelfth species, *Chironomus tentans*, was obtained from a study conducted and reported by Pacific EcoRisk (2005). The acute tests (Table 2) examining *C. tentans* were initiated with 2^{nd}-3^{rd} instar larvae, while the chronic tests (Table 3) were initiated with 1^{st} instar larvae from an in-house culture. Chronic tests of *C. tentans* were based on the USEPA freshwater sediment chronic toxicity testing guidelines (USEPA 2000), modified to incorporate use of very fine-grained silica sand. The Co toxicant stock was prepared with $CoSO_4$ ("Baker Analyzed," VWR International) rather than $CoCl_2$. Exposures were conducted in a flow-through system using both a natural water (Panther Creek, Idaho, USA; approximate water quality: hardness 35 mg/L as $CaCO_3$, pH 8.0, DOC 2.3 mg/L) and a lab water as controls.

The acute and chronic toxicity tests conducted at OSU and the University of Ghent laboratories followed the general methodological guidance provided by the ASTM International (ASTM 2002a, 2002b), the US Environmental Protection Agency (USEPA 2002a, 2002b) and the Organization for Economic Co-operation and Development (OECD 1992), unless a standardized protocol was not available. Each acute study was designed to yield a median-lethal/effective concentration ($E(L)C_{50}$) following continuous exposure. Details regarding lighting, temperature, species and life stages used at test initiation, number of replicates, exposure duration, type of exposure (i.e., static, flow-

through, etc.), and control/dilution water constitution, for acute toxicity tests are summarized in Table 2.

Chronic tests were designed to derive effective concentrations to reduce endpoint performance by 10% and 20% (EC_{10} and EC_{20}), relative to control. Details regarding lighting, temperature, species and life stages used at test initiation, number of replicates, exposure duration, type of exposure (i.e., static, flow-through, etc.), and control/dilution water constitution, for chronic toxicity tests are summarized in Tables 3 and 4. The chronic tests were conducted using the same species tested in the acute studies, in addition to *P. subcapitata* and *L. minor* (Table 3). The ages at initiation of the chronic tests were the same as those used for acute tests for all species, with the exception that the fish chronic tests were initiated using freshly fertilized eggs (Table 4).

Control and dilution water were run concurrently with all laboratory tests and was comprised of synthetic water prepared by adding salts (e.g., $CaSO_4$, $MgCl_2$) to deionized water. Conductivity (µS/cm), hardness (mg/L as $CaCO_3$), alkalinity (mg/L as $CaCO_3$), total ammonia (mg/L as N), total residual chlorine (mg/L), dissolved oxygen (mg/L), pH, and temperature (ºC) were measured in dilution water at test initiation and during the conduct of the tests. For the Pacific EcoRisk tests, water quality (e.g., temperature, pH, DO, conductivity, alkalinity, and total ammonia) was measured from one replicate per treatment. For the University of Ghent studies, pH, hardness, temperature, and oxygen concentration were measured at the beginning and/or end of each test (acute and chronic) and DOC was measured for all chronic tests.

In all of the OSU studies both total and dissolved Co was measured in all treatments in each toxicity test, and in the University of Ghent tests only dissolved Co was measured. To quantify dissolved Co, treatment water was collected and passed through a 0.45 µm filter (Supor® polyethersulfone (PES) membrane; Acrodisc®, Pall Life Sciences) prior to acidification and analysis. Immediately after collection, total and filtered Co samples were acidified with nitric acid to a pH of <2 (0.14 N HNO_3; ultrapure). Cobalt concentrations were determined using flame or graphite furnace atomic absorption using a PerkinElmer AAnalyst™ 800 high-performance atomic absorption spectroscopy according to USEPA Methods 243.1 and 243.2 (USEPA 1979; method detection level (MDL): 8 µg/L; Limit of quantitation (LOQ): 25.3 µg/L; Graphite furnace-AAS: MDL: 1.0 µg/L; LOQ: 3.1 µg/L). Dissolved Co was similarly analyzed for in the Ghent University studies.

The biological endpoint measured for all acute toxicity tests was survival (mortality was defined as no visible movement and no response to gentle prodding with a blunt probe). Survival was assessed at 48h for *D. magna* (based on immobility), *H. azteca*, and *C. dubia;* and 96h for *C. tentans, L. stagnalis*, and the three fish species. Population growth (EC_{50}) was considered as the acute toxicity endpoint assessed at 48h for *Aeolosoma* sp. The chronic test biological end-points included reproduction, growth, survival, and biomass. Reproduction and population growth were assessed for the rotifer, *Brachionus calyciflorus*, after 48h and *D. magna* after 21d, growth rate was assessed for *H. azteca* and *L. stagnalis* after 28d, growth rate (biomass) was assessed for *P. subcapitata* after 72h and for *L. minor* after 7d. For *P. subcapitata*, exponentially dividing cells were filtered through a 0.45 µM membrane prior to analysis of the cell

concentration. Survival and reproduction of *C. dubia* were assessed after 7d of chronic exposure, population growth of *Aeolosoma* sp. was assessed following a 14d chronic exposure, and survival and growth (biomass) were assessed for *C. tentans*, *P. promelas*, *O. mykiss*, and *D. rerio* following 20, 34, 81, and 33 days of chronic exposure, respectively. Reproductive endpoints for *C. tentans* were measured by inserting a removable emergence trap on day 20 to catch emerged adults. Emergence was recorded beginning on day 22 and adults were transferred to reproduction chambers. In the reproduction chambers, egg masses were examined daily and the number of eggs was recorded.

Toxicity endpoints were assessed relative to the average dissolved Co concentrations. Acute LC_{50} values were calculated using logistic equation and Spearman-Karber or Trimmed Spearman-Karber methods, depending on the data collected for each test (Stephan 1977). Chronic statistical values were calculated using logistic equation, threshold sigmoid regression, or piecewise linear regression analysis, based on best-fit analysis. Exposure concentrations were log(normal)-transformed before calculations of EC_{10} and EC_{20} values. All statistical calculations were carried out using the Comprehensive Environmental Toxicity Information System (CETIS, Tidepool Scientific Software), Toxicity Research Analysis Program (TRAP Ver. 1.2; USEPA), or STATISTICA (StatSoft, Inc.).

RESULTS

Acute toxicity tests

A total of 13 acute toxicity tests were conducted using 11 species. All water quality parameters (hardness, alkalinity, pH, conductivity and temperature) remained within consistent tolerance ranges of the organisms throughout the study and are presented in Table 5. Measured DO concentrations were above 60% of saturation at the average test temperature. Actual concentrations of Co measured in the exposure solutions did not differ substantially from nominal exposure concentrations in static, static-renewal or flow-through tests. In general, the dissolved Co concentrations for all studies were within 20% of the nominal values. Total Co concentrations averaged 76 to 123% of nominal Co concentrations and the mean dissolved Co was approximately 82 to 120% of total. Comparisons of total and dissolved Co measurements indicated that the majority of the Co present in the exposure solutions was in a dissolved form. Control survival was greater than 93% for all acute tests.

There was a significant concentration effect on juvenile and larval organism survival in all tests. Among the 11 species assessed, acute toxicity values (LC_{50}) differed from a low of 90.1 μg Co/L for the duckweed, *Lemna minor*, to a high of 429,000 μg Co/L for the midge, *C. tentans* (Table 5). The acute toxicity species sensitivity ranking, in order from most to least sensitive, was as follows: *L. minor* > *P. subcapitata* > *O. mykiss* > *C. dubia* > *P. promelas* > *H. azteca* > *D. magna* > *D. rerio* > *Aeolosoma* sp. > *L. stagnalis* > *C. tentans*. For the acute studies that included both larval and juvenile life stages of *P. promelas* and *D. rerio*, the larval fish were more sensitive by factors of 18

and 5, respectively, with larval and juvenile LC_{50} values of 3,090 and 54,100 µg Co/L, respectively, for *P. promelas* and 15,980 and 85,290 µg Co/L, respectively, for *D. rerio* (Table 5). The LC_{50} for each species used in acute Co bioassays are summarized in Table 5. All biological response data are presented in the Supplemental Information tables (Tables S1-S10).

Chronic toxicity tests

A total of 11 chronic toxicity studies were conducted using 11 species; one additional chronic test is discussed and included in the analysis that was conducted with the midge, *C. tentans*, and reported separately (Pacific EcoRisk 2005). A summary of water quality characteristics for the control/dilution waters in all of the chronic tests is provided in Table 6. Measured concentrations of Co in the exposure solutions did not vary substantially over the test duration and did not differ substantially from nominal exposure concentrations in either the static-renewal or flow-through tests. The time-weighted averages over the duration of the tests were between 85% and 126%. Total Co concentrations averaged 105 to 119% of nominal Co concentrations and the mean dissolved Co was approximately 89 to 105% of total. Comparisons of total and dissolved Co measurements indicated that the majority of the Co present in the exposure solutions was in a dissolved form, thus all data endpoints are reported on the basis of dissolved Co. Chronic effect levels for 12 species tested were between 4.9 µg Co/L (EC_{10} for *L. minor*) and 2,171 µg Co/L (EC_{10} for *O. mykiss*; Table 7). These values differed by a factor of 443, underscoring the wide-range of sensitivities among the species tested. The chronic toxicity species sensitivity ranking, in order from most to least sensitive, was as follows: *L. minor* > *H. azteca* > *C. dubia* > *L. stagnalis* > *P. subcapitata* > *D. magna* >

Aeolosoma sp.> *C. tentans* > *P. promelas* > *Brachionus calyciflorus* > *D. rerio* > *O. mykiss*. The EC_{10} and EC_{20} values following chronic Co exposure are summarized in Table 7. Acute-chronic ratios (ACR), defined as the acute LC_{50} divided by the chronic EC_{20}, differed substantially, ranging from 0.6 (*O. mykiss*) to 2,670 (*L. stagnalis)* with invertebrate species showing larger ACRs (between 129 and 2,670, n=6) than those noted for fish species (between 0.6 and 10, n=3) (Figure 1). Acute-chronic ratios, defined as the acute LC_{50} divided by the chronic EC_{10}, were similar to the ACRs based on EC_{20} and ranged from 0.7 (*O. mykiss*) to 6,410 (*L. stagnalis*) with invertebrate species showing larger ACRs (between 182 and 6,410; n=6) than those noted for fish species (between 0.7 and 14.7, n=3) (Figure 1).

In addition to the "most sensitive" endpoints summarized in Table 7 (e.g. growth, biomass), mean time-to-hatch (days [d]) and presence or absence of morphological defects (i.e., teratagenocity) was recorded, if observed, for three of the fish species. For *P. promelas*, hatching success was not affected by Co exposure over the series of tested concentrations. The overall median time-to-hatch for five treatments was 3.94 ± 0.4 d compared to 3.5 ± 0.6 d in controls. For *D. rerio*, the overall median time-to-hatch for five treatments was 4.58 ± 0.6 d compared to 4.3 ± 0.5 d in controls; no treatment related differences in hatch time were noted. For *O. mykiss*, no differences in egg mortality or the time-to-hatch were observed. The overall median time-to-hatch for five treatments was 34.7 ± 0.26 d, in comparison to 34.0 ± 0.2 d in the controls. No malformations were observed in any of the controls or Co exposed organisms during any of these studies. All biological response data are presented in the Supplemental Information tables (Tables S11-S22).

DISCUSSION

Evaluation of generated data

To our knowledge, this study represents the first comprehensive report of cobalt's acute and chronic freshwater toxicity to species representing taxa ranging from algae/plants, invertebrates, and fish. Among the organisms tested, *L. minor* showed the greatest sensitivity to Co under acute and chronic exposure. The acute species sensitivity ranking demonstrated that juvenile rainbow trout was the most sensitive non-plant species tested with an LC50 of 1,512 (1,343-1,704) µg Co/L. Interestingly, this species was the least sensitive in chronic toxicity testing. However, it is important to note that the acute test was performed with juvenile rainbow trout, while the chronic test was initiated with newly fertilized eggs. Differences in life-stage sensitivities likely contributed to this discrepancy. De Schamphelaere and Janssen (2004) reported, similarly, that chronic exposures of fish eggs to zinc resulted in lower toxicity than exposures starting with juvenile fish.

Prior to the studies described herein, little reliable chronic toxicity data existed for Co. Biesinger and Christensen (1972) used $CoCl_2$ as the cobalt test compound and reported a *D. magna* 21d EC_{16} (reproduction) value of 10 µg Co/L (nominal) when tested in Lake Superior water (hardness 45 mg/L as $CaCO_3$, pH 7.7). In the present study, we found the 28d *D. magna* EC_{20} to be 65.2 µg Co/L (EC_{20}), which is approximately 5-fold higher than the EC_{16} value reported by Biesinger and Christensen (1972). As a direct comparison between the studies, Biesinger and Christensen (1972) reported an acute (48h) LC_{50} of 1.11 mg Co/L, whereas the present study reported a 48h LC50 of 5.89 mg

Co/L. This reflects an approximate 5-fold difference in acute Co sensitivity between the two studies, which is consistent with the apparent difference in chronic Co sensitivity. This discrepancy could be due to disparities in the *D. magna* clones used previously compared with those used in the present study or other methodological inconsistencies (e.g., 1x versus 3x weekly solution renewals), differences in the statistical endpoints (EC_{16} versus EC_{20}), and possible exposure concentration differences (Biesinger and Christensen (1972) reported data based on nominal concentrations).

Aside from the Biesinger and Christensen (1972) study, other studies published on Co freshwater toxicity differed from the present study in that they focused on acute exposures for which the duration differed from the present study. For example, Diamond et al. (1992) reported 48h NOECs for *P. promelas* of 1.25 (hardness 50 mg/L as $CaCO_3$) to 13.7 mg Co/L (hardness 400 mg/L) and 7d NOECs of 1.23 (hardness 50 mg/L) to 3.83 mg Co/L (hardness 800 mg/L). Diamond et al. (1992) also reported 24h LC_{50} values of 2.35 (hardness 50 mg/L) to 4.20 mg Co/L (hardness 400 mg/L) for *C. dubia*, which is similar to the 48h LC_{50} of 2.39 mg Co/L (moderately hard water, hardness 100 mg/L) reported in the present study. An additional study by Marr et al. (1998) reported a 96h LC_{50} of 1.41 mg Co/L for *O. mykiss*, which was consistent with the 96h LC_{50} of 1.51 mg Co/L reported in the present study (Table 4). The results of the Diamond et al. (1992) study underscore the importance of considering water hardness, as toxicity appears to be a function of this factor. Notably, the present study did not consider bioavailability; however, this is being addressed in subsequent research. Thus, although limited chronic toxicity data are available for comparison (e.g., Norwood et al. 2007, Kimball 1978), acute toxicity results were generally consistent with those studies previously reported in

the literature. Chronic toxicity studies that were identified typically did not meet current scientific standards for acceptability (e.g., lack of measured concentrations, unavailability of raw data with which to do statistical analyses) and were therefore not included in our SSD calculations.

Derivation of a species sensitivity distribution (SSD)

The primary objective of this study was to generate data that could be used to derive a freshwater PNEC ($PNEC_{freshwater}$) in accordance with European criteria (ECHA 2008) and an AWQC-style aquatic reference standard following USEPA methods (USEPA 1985). The $PNEC_{freshwater}$ can be derived using a limited database in conjunction with specific Assessment Factors (AF: 10-100) or through the use of a SSD analysis on a more "robust" dataset (AF: 1-5). A minimum of eight different taxonomic groups should be included in the effects database for SSD analysis and this guidance was adopted in RIP 3.2, Chapter R10 (ECHA 2008) (Table 1). The Co chronic effects studies described herein used organisms representing each of these categories, including a range of algal, invertebrate, fish, and plant species (Table 1).

The 12 chronic freshwater toxicity EC_{10} values generated for 12 different freshwater organisms (Table 5) were used for the construction of a SSD (n = 12 species; Figure 2). Using the RIVM software package ETX (v2.0), a log-normal distribution was fitted to the data set and the hazardous concentration for 5% of the species (HC_5 value) was derived as the 5^{th} percentile of the SSD using a log normal distribution function (Van Vlaardingen et al 2004). According to the ECHA guidelines, we derived the median estimate of the HC_5 value, i.e. the $HC_{5,50\%}$, which was 1.80 μg Co/L (95% CI: 0.16– 7.62

μg Co/L) (Figure 2). The "fit" of the log normal distribution function is not ideal and an analysis of the "best fit" for available functions (@RISK software, Palisade Corporation) indicate that a Pareto distribution fits the data more appropriately, resulting in an HC_5 of 5.62 μg Co/L. A variety of statistical methods exist for determining the acceptability of "goodness-of-fit" for a particular distribution model. The Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), Anderson–Darling goodness of fit test and the Kolmogorov-Smirnov-test have been suggested as criterion to aid in the choice of a parametric distribution (ECHA 2008, ICMM 2016, Wang and Liu 2006). All of these assessment metrics were conducted and all judged the Pareto distribution to provide a superior fit for the SSD data than the logistic model.

According to the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) regulations RIP 3.2 Chapter R10 (ECHA 2008), if the SSD technique is used, an assessment factor (AF) between one and five, to be judged on a case-by-case basis, should be applied to the HC_5 (i.e., $PNEC_{aquatic} = HC_{5,50\%}/AF$). The size of the AF should reflect the degree of uncertainty associated with the robustness and scope of the available data. Selection of an AF should be based upon an uncertainty analysis best-suited to risk assessors, and is therefore not within the scope of this paper.

The present study also provided an acceptable database for use in deriving both an acute (CMC: criteria maximum concentration) and chronic (CCC; criteria continuous concentration) AWQC-style aquatic reference standard following the USEPA prescribed approach (USEPA 1985). Using the acute toxicity data presented in Table 4 (not considering the plant and algal data as per the EPA methodology) the Final Acute Value

(FAV) would be 1,199 µg Co/L; dividing this value by 2 would give a CMC of 599.5 µg Co/L. When sufficient data are available to characterize the effect of a water quality parameter (e.g., water hardness, pH, DOC), this information can be used to normalize the available data to a common condition (e.g., a common water hardness), thereby reducing between test variability within a test species. Insufficient acute toxicity data are currently available to permit this type of correction with Co, although relationships between water hardness and Co acute toxicity have been reported [Blust 2012, Diamond et al 1992, Pourkhabbaz et al 2011, Environment Canada. 2017).

Although use of a SSD-based approach is favored in deriving chronic AWQC, limitations in the availability of chronic toxicity data frequently lead to the derivation of AWQC CCC by dividing the FAV by the acute-chronic ratio. However, the range of acute-chronic ratios observed with Co is too large to apply this method for deriving the CCC (USEPA 1985); therefore, the SSD approach based on chronic data must be employed. Based on the studies reported herein, sufficient chronic data are available to characterize the effect of water quality parameters on Co chronic toxicity. Using this information and following the US EPA's method for AWQC derivation, the USEPA-style final chronic value (FCV) is 7.13 µg Co/L. This is similar to the PNEC derived herein of 5.62 µg Co/L (using a "best fit" procedure). Thus, despite differences in approaches to determining PNECs versus an AWQC-style aquatic reference standard, the outcome was similar.

As noted in Table 1, there are differences in the requirements set forth for Europe (under REACH) versus the US EPA (pursuant to Section 304(a) of the Clean Water Act). For example, whereas REACH requirements do not consider acute toxicity, much of the

data used for establishment of AWQC for most of the metals in the US were derived from acute toxicity data in cases where chronic toxicity data were not available and derivation of chronic AWQC were based upon an acute-chronic ratio approach (USEPA 1985). Additionally, the US does not include plant or algae data in developing the SSD used to derive the Final Chronic Value subsequently used in establishing the AWQC (Table 1). Ideally, both acute and chronic freshwater toxicity data should be considered in deriving aquatic standards. As we demonstrated here, generation of acute toxicity data is an important prelude to chronic toxicity testing. Careful planning so that both acute and chronic toxicity testing follows the requirements of the respective agencies will provide the most useful toxicity information to an international audience.

CONCLUSION

Due to the lack of published data on Co freshwater toxicity, a thorough assessment of acute and chronic toxicity was warranted. We conducted a series of freshwater acute and chronic toxicity assays using 12 species (including one from an external report), ranging from algae to fish and including the eight taxonomic groups required by ECHA for derivation of a PNEC and the USEPA for derivation of an AWQC. All other REACH requirements for chronic toxicity testing were fulfilled, in addition to fulfilling AWQC requirements established by the Clean Water Act in the US, making the data internationally relevant (USEPA 1985). Chronic toxicity EC_{10} values ranged from 4.9 µg Co/L (*L. minor*) to 2,171 µg Co/L (*O. mykiss*), representing a 443-fold difference. A species sensitivity distribution (SSD) was used to calculate a $HC_{5,50\%}$ of 1.80 µg Co/L (or 5.62 µg Co/L using a “best fit” analysis). The existing literature and these data highlight the need for developing both acute and chronic toxicity assessments. Owing to

the effect of site-specific water quality parameters (e.g., hardness, pH) on the bioavailability and toxicity of metals, future assessments of Co toxicity will build upon the data reported herein and will address the effects of these parameters.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (bill.stubblefield@oregonstate.edu).

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Figure 1: EC_{10} or EC_{20}, for 9 freshwater species exposed to $CoCl_2$. Data presented in order of relative chronic cobalt sensitivity (most sensitive on left, least sensitive on right). Fish species have lowest chronic cobalt sensitivity and lowest ACRs.

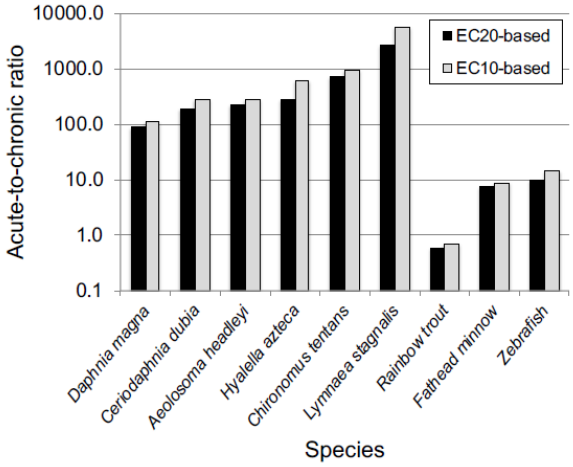

Figure 2: Species sensitivity distribution (SSD) and $HC_{5,50\%}$ fit to chronic EC_{10} values for cobalt in aquatic environments.

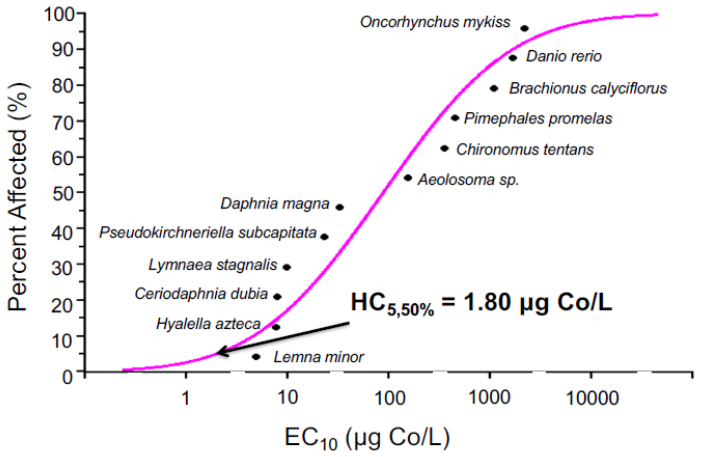

Table 1. Minimum taxonomic group requirements for the EU species sensitivity distribution method (ECHA 2008) and the US EPA method (USEPA 1985) for deriving PNEC or WQC, and species and test methods used in the present study to fulfill these requirements.

EU requirement	US EPA requirement	Test species	Test method
Fish	The family Salmonidae in the class Osteichthyes	*Oncorhynchus mykiss*	Early life-stage
Second family in the phylum Chordata	A second family of fish in the Class Osteichthyes (preferably a commercially or recreationally important warm-water species)	*Pimephales promelas*	Early life-stage
	A third family in the phylum Chordata	*Danio rerio*	Early life-stage
Crustacean	Planktonic crustacean	*Daphnia magna, Ceriodaphnia dubia,*	Life-cycle
Insect	Insect	*Chironomus tentans*	Life-cycle
A family in a phylum other than Arthropoda or Chordata	A family in a phylum other than Arthropoda or Chordata	*Aeolosoma* sp. (phylum: Annelida) *Brachionus calyciflorus* (phylum Rotifera)	Life-cycle
A family in any order of insect or any phylum not already represented	A family in any order of insect of any phylum not already represented	*Lymnaea stagnalis* (phylum: Mollusca)	Life-cycle

	Benthic crustacean	*Hyalella azteca*	Chronic, growth
Algae		*Pseudokirchneriella subcapitata*	Chronic, growth
Higher plant		*Lemna minor*	Chronic, growth

Table 2. Overview of the acute test conditions with cobalt chloride for nine different test species tested at Parametrix/Oregon State University and Ghent University (*C. tentans* tests were conducted in an independent study by Pacific EcoRisk.)

	Cladoceran ***Daphnia magna***	**Amphipod** ***Hyalella azteca***	**Great Pond Snail** ***Lymnaea stagnalis***	**Water flea** ***Ceriodaphnia dubia***	**Midge** ***(Chironomus tentans)***	**Oligochaete** ***(Aeolosoma* sp.*).***	**Fathead minnow** ***(Pimephales promelas)***	**Rainbow trout** ***(Oncorhynchus mykiss)***	**Zebrafish** ***(Danio rerio)***	
Origin	Originally collected clone (K6) from a pond in Kiel (Antwerp, Belgium) and cultured for 15+ years at Ghent University (Belgium)	Aqua Survey (USA) culture maintained at Ghent University (Belgium) since 1996	University of Amsterdam	In-house culture, PERL (Albany, Oregon, USA)	Aquatic Biosystems (Fort Collins, Colorado, USA)	Carolina Biological Supply (Burlington, North Carolina, USA)	In-house culture, PERL (Albany, Oregon, USA)	In-house culture, PERL (Albany, Oregon, USA)	Oregon State University (Corvallis, Oregon, USA)	Aquatica (Plant City, Florida, USA)	Scientific Hatcheries (Huntington Beach, California, USA)
Test protocol	OECD 202 [33]	Modified OECD 202 [33]	General ASTM test guidelines; modified Gomot [13]	USEPA [21]	ASTM [21]	Newman [34]; Niederlehner et al. [35]	ASTM [18]	ASTM [18]	ASTM [18]	ASTM [18]	ASTM [18]
Test duration	48 hours	96 hours	96 hours	48 hours	96 hours	48 hours	96 hours	96 hours	96 hours	96 hours	96 hours
Culture and Test medium	Artificial ISO medium (OECD 1998), 250 mg/L as $CaCO_3$,	Borgmann Medium; 125 mg/L as $CaCO_3$, pH 7.9	Artificial freshwater; 140 mg/L as $CaCO_3$, pH 7.6 – 7.9	Synthetic moderately hard water, 100 mg/L as $CaCO_3$	Lab Blended H_2O (Well water blended with reverse	Soft Reconstituted Lab Water	Moderately Hard Reconstituted Water	Lab Blended H_2O (Well water blended with	Lab Blended H_2O (Well water blended with reverse	Lab Blended H_2O (Well water blended with	Lab Blended H_2O (Well water blended with

	Cladoceran ***Daphnia magna***	**Amphipod** ***Hyalella azteca***	**Great Pond Snail** ***Lymnaea stagnalis***	**Water flea** ***Ceriodaphnia dubia***	**Midge** ***(Chironomus tentans)***	**Oligochaete** ***(Aeolosoma* sp.*).***	**Fathead minnow** ***(Pimephales promelas)***		**Rainbow trout** ***(Oncorhynchus mykiss)***	**Zebrafish** ***(Danio rerio)***	
	pH=7.5				osmosis H_2O)			reverse osmosis H_2O)	osmosis H_2O)	reverse osmosis H_2O)	reverse osmosis H_2O)
Endpoints	Survival (Immobilization)	Survival	Survival	Survival	Survival	Survival	Survival	Survival	Survival	Survival	Survival
Tested life stage	< 24 hour old	6-7 days old	1 month old	< 24 hour old neonates	2nd instar larvae	< 24 hours old	Larval (3-day old)	Juvenile	Juvenile	Larval	Juvenile
Tested concentrations (nominal)	0.56 – 10 mg Co/L	0.56 – 18 mg Co/L	0 – 100 mg Co/L	0 – 8 mg Co/L	0 – 1000 mg Co/L	0 – 50 mg Co/L	0 – 10 mg Co/L	0 – 100 mg Co/L	0 – 10 mg Co/L	0 – 40 mg Co/L	0 – 400 mg Co/L
Renewal frequency	Static	Static	Static	Static	Flow-Through 14 mL/min	Static	Once at 48 hours	Flow-Through	Flow-Through 10 mL/min	Flow-Through 25 mL/min	Flow-Through 50 mL/min
# Replicates / conc	3	3	4	4	3	4	4	2	2	4	2
# Individuals / replicate	10	10	5	5	10	5	10	10	20	15	10
Test conditions	50-mL polyethylene cups; 20 °C, 16L:8D; ~1000 lux	150 mL polyethylene cups; 100 mL/replicate; gauze substrate; 25°C; 16L:8D; ~1000 lux	500 mL glass jars; 400 mL/replicate; 20°C; 12L:12D; ~1000 lux	30-mL plastic cups; 25 mL/ replicate; 20 °C, 16L:8D	1 L glass jars; 200 mL/replicate; sterilized sand substrate; 23°C; 16L:8D	Polystyrene culture well plates; 10 mL/replicate; 25°C; 16L:8D	1 L beakers; 250 mL/replicate; 20°C; 16L:8D	19 L aquarium; 16 L/replicate; 25°C; 16L:8D	19 L aquarium; 15 L/replicate; 12°C; 16L:8D	1-L chamber; 400-600 mL/replicate; 27°C; 16L:8D	19 L aquarium; 15 L/replicate; 25°C; 16L:8D
Feeding regime	None	None	None	None	Daily, Tetrafin slurry	None	Once, 2 hours before 48 renewal	None	None	Daily, Infusoria and encapsulated shrimp diet	None

Table 3. Overview of the chronic test conditions with cobalt chloride for nine different plant and invertebrate test species tested at

Parametrix/Oregon State University and Ghent University. (*C. tentans* tests were conducted in an independent study by Pacific EcoRisk.)

	Cladoceran ***Daphnia magna***	**Amphipod** ***Hyalella azteca***	**Great Pond Snail** ***Lymnaea stagnalis***	**Green Algae** ***Pseudokirchneriella subcapitata***	**Duckweed** ***Lemna minor***	**Water flea** ***Ceriodaphnia dubia***	**Oligochaete** (***Aeolosoma* sp**)	***Chironomus tentans***	**Rotifer** (***Brachionus calyciflorus*****)**
Origin	Originally collected from a pond in Kiel (Antwerp, Belgium) and cultured for 15+ years at Ghent University	Aqua Survey (USA) culture maintained at Ghent University (Belgium) since 1996	University of Amsterdam	Obtained from Culture Collection of Algae and Protozoa (Ambleside, United Kingdom) and maintained at Ghent University 15+yrs	Ghent University standard culture	In-house culture, PERL (Albany, Oregon, USA)	Carolina Biological Supply (Burlington, North Carolina, USA)	In-house culture, Pacific EcoRisk (Martinez, CA, USA)	Florida Aqua Farms (Dade City, FL, USA)
Test protocol	OECD 211 [11]	Modified OECD 211 [11]	General ASTM test guidelines; modified Gomot [13]	OECD 211 [11]	OECD 221 [36]	USEPA [22]	Newman [34]; Niederlehner et al. [35]	US EPA 2000	Snell and Moffat (1992), Grosell et al. (2006)
Test duration	21-days	28-days	28-days	72-hrs	7-days	7-days	14-days	20-days	48 hours
Culture and Test medium	Artificial ISO medium (OECD 1998), 250 mg/L as $CaCO_3$, pH 7.5	Borgmann medium (Borgmann 1996), 125 mg/L as $CaCO_3$; pH 7.9	Artificial freshwater; 140 mg/L as $CaCO_3$; pH 7.6 – 7.9	OECD media (no EDTA; added DOM as Aldrich Humic Acid)	Modified Swedish Standard media (SSI 1995) (no EDTA; added DOM as Aldrich Humic Acid)	Synthetic moderately hard water, 108 mg/L as CaCO3	Synthetic Soft water, 54 mg/L as CaCO3	Panther Creek water, and lab control water at hardness matching that of Panther Creekk	Moderately hard reconstituted water; Hardness 100 mg/L as $CaCO_3$
Endpoints	Survival and Reproduction	Population Growth	Growth Rate	Biomass Growth Rate	Frond #, Biomass (dry weigh/fresh weight)	Survival and Reproduction	Population Growth	Survival, growth, reproduction	Population Growth
Tested life stage	< 24 hour old neonates	6-7 days old	1 month old	1.10^4 cells/mL of an exponential growing culture	Colonies of 2-4 visible fronds	< 24 hour old neonates	< 24 hours old	<24 hours old (1^{st} instar larvae)	< 2 hours old
Tested concentrations (nominal)	5.6 – 320 µg/L Co	3.2 – 100 µg/L Co	3.2 – 1,000 µg/L Co	18 – 560 µg/L Co	3.2 – 10,000 µg/L Co	0 – 200 µg/L Co	0 – 600 µg/L Co	0 – 2,500 µg/L Co	0 – 2,500 µg/L Co
Renewal frequency	Semi-static (renewal 3	Semi-static (renewal 3	Semi-static	Static; shaken 2x daily	Static	Daily	3 times/wee	Flow-through,	Static

	x/week)	x/week)	(renewal 2x/week)				k	0.6 mg/mL (2x daily)	
# Replicates/conc.	10	10	8	3	4	10	4	12	4
# Individuals/replicate	1	3	1	1.10^4 cells/mL of an exponential growing culture	12 fronds	1	Start of 5	12	25
Test conditions	50 mL polyethylene cups; 25 mL/replicate; 20°C; 16L:8D	100 mL polyethylene cups; 100 mL/replicate; 25°C; 12L:12D; ~1000 lux; gauze substrate	100 mL polyethylene vessels; 100 mL/replicate; 20°C; 12L:12D; 100-1000 lux	100 mL Erlenmeyer; 50 mL/replicate; 25°C; continuous light; 120 µmol photons m^{-2} s^{-1}	500 mL glass jars; 100 mL/replicate; 24°C; continuous light; 6000-10000 lux	30 mL Souffle cups; 25 mL/replicate; 25°C; 16L:8D	Polystyrene culture well plates; 10 mL/replicate; 25°C; 16L:8D; 5 foot-candles	Flow-through setup in 300 mL glass beaker with 250 mL overflow, 23ºC, 16L:8D	1-L chamber; 400-600 mL/replicate; 27°C; 16L:8D
Feeding regime	Daily; 3:1 mix (*Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*)	Daily; 3:2 mix (YCT: *Pseudokirchneriella subcapitata*)	Lettuce (*ad libitum*)	None	None	Daily (Algae/YTC)	Infusoria (Rabbit pellet slurry)	1.5 mL slurried Tetra Min® flake fish food daily	Two times daily (Brine shrimp)

Table 4. Overview of the chronic test conditions with cobalt chloride for three different fish test species tested at

Parametrix/Oregon State University and Ghent University.

	Fathead minnow (*Pimephales promelas*)	**Rainbow trout (*Oncorhynchus mykiss*)**	**Zebrafish (*Danio rerio*)**
Origin	In-house culture, PERL (Albany, Oregon, USA)	Eggs and sperm Trout Lodge (Sumner, Washington, USA). Fertilized in-house	Aquatica (Plant City, Florida, USA)
Test protocol	ASTM [2002b], OECD [1992]	ASTM [2002b], OECD [1992]	ASTM [2002b],
Test duration	34-days	81-days	33-days

Culture and Test medium	Lab Blended H_2O (Well water blended with reverse osmosis H_2O); Hardness ~100 mg/L as CaCO3	Lab Blended H_2O (Well water blended with reverse osmosis H_2O); Hardness 115 mg/L as CaCO3	Lab Blended H_2O (Well water blended with reverse osmosis H_2O); Hardness 103 mg/L as CaCO3
Endpoints	Survival and Growth	Survival and Growth	Survival and Growth
Tested life stage	Fertilized eggs	Fertilized eggs	Fertilized eggs (< 48-h)
Tested concentrations (nominal)	0 – 3 mg/L Co	0 – 4 mg/L Co	0- 8 mg/L Co
Renewal frequency	Flow-Through, 25 mL/min	Flow-Through, 22.5 mL/min	Flow-Through, 7.5 mL/min
# Replicates/conc.	4	4	4
# Individuals/replicate	25	50 (start), thinned to 20	25
Test conditions	1-L chamber; 400-600 mL/replicate; 27°C; 16L:8D	1-L chamber; 600-800 mL/replicate; 12°C; 16L:8D	1-L chamber; 400-600 mL/replicate; 28°C; 16L:8D
Feeding regime	Two times daily (Brine shrimp)	Two times daily (Trout Chow Slurry)	Daily (Infusoria and encapsulated shrimp diet)

Table 5. Summary of acute toxicity test conditions and results for aquatic organisms exposed to cobalt chloride (µg Co/L dissolved).

Test species	**Common name**	**Life stage**	**Hardness (mg/L as $CaCO_3$)**	**Alkalinity (mg/L as $CaCO_3$)**	**pH (SU)**	**DOC (mg/L)**	**E(L)C_{50} (95% CI)**
Lemna minor[1]	Duckweed		55	6	6.5	0.3[2]	90.1 (69.9 - 116.1)
Pseudokirchneriella subcapitata[1]	Algae		27	29	7.7	0.3[2]	144 (118 - 176)
Oncorhynchus mykiss	Rainbow trout	Juvenile	60	60	7.2	0.5	1,512 (1,343 - 1,704)
Ceriodaphnia dubia	Water flea	Neonates	100	100	8.1	<0.5	2,154 (1,566 -

							2964)
Pimephales promelas	Fathead minnow	Larval	100	72	8.1	<0.05	3,090 (2,720 - 3,520)
Pimephales promelas	Fathead minnow	Juvenile	118	112	7.7	0.6	54,100 (45,500 - 64,300)
Hyalella azteca	Amphipod	Juveniles	125	NR	7.9	NR	3,290 (2,920 - 3,710)
Daphnia magna	Water flea	Juveniles	250	NR	7.8	NR	5,890 (5,680 - 6,100)
Danio rerio	Zebrafish	Larval	100	108	7.7	0.5	15,980 (13,630 - 18,730)
Danio rerio	Zebrafish	Juvenile	120	92	7.8	0.6	85,290 (72,300 - 100,700)
Aeolosoma sp.	Worm	Neonates	48	80	7.7	<0.05	42,700 (39,680 - 45,960)
Lymnaea stagnalis	Snail	1 month old	140	NR	7.2 - 7.7	NR	61,600 (44,100 - 86,100)
Chironomus tentans	Midge	2nd instar larvae	84	108	7.6	0.5	429,000 (359,000 – 512,700)

[1] Not included in calculation of an USEPA AWQC Final Acute Value

[2] Estimate of “initial” DOC of the dilution medium (based on earlier measurements of laboratory DI water); does not account for DOC development / excretions during tests.

NR: Not reported

Table 6. Water quality results from chronic toxicity tests

Parameters	*P. subcapitata*	*L. minor*	*C. dubia*	*D. magna*	*H. azteca*	*C. tentans*	*Aeolosoma* sp.	*B. calycifl orus*	*L. stagnalis*	*P. promelas*	*D. rerio*	*O. mykiss*
Average Hardne	27.4	54.9	46.7	250.3	125.2	34.3	50.1	52	140.1	98.4	98.4	115.8
Average Alkalini	28.6	5.8	76	36.3	46.8	34	42	72	116.2	120	117	130
Average pH in chamb	7.7	6.5	8.3	7.5	7.5	8.2	7.6	7.4	7.8	8.0	7.8	7.8
DOC	0.3	0.3	0.5	0.3	0.3	2.2	0.5	0.4	0.3	0.5	0.	1.1
Calciu	4.91	9.8	8.2	80.1	40.	8.8	12.8	11.5	40.1	22.3	22	26.8
Magnesium	3.7	7.4	6.4	12.2	6.08	3.0	4.41	5.32	9.73	10.4	104	11.9
Sodiu	15	33.	23.	17.7	23.	15	16.1	14.2	55.1	31.3	31	42.2
Potassium	0.46	3.74	2.2	3.04	1.96	1	1.6	0.382	11.7	1.14	1.14	0.8
Chlorid	26.7	17.	13.	144.	72.	1	1.4	12.9	99.3	21.1	43	47.8
Sulfate	5.9	29.	61.	48.1	24.	15	46.0	0.6	14.4	0.3	0.	0.6

Table 7. Summary of chronic toxicity data for aquatic organisms exposed to cobalt chloride (µg Co/L dissolved).

Test species	**Common name**	**Most Sensitive Endpoint**	**EC_{10} (95% CI)**	**EC_{20} (95% CI)**
Lemna minor	Duckweed	7d Growth rate	4.9 (2.7 – 8.7)	NC[1]
Hyalella azteca	Amphipod	Growth rate (28d dry	7.55 (4.00 – 14.27)	17.58 (11.92 – 14.27)

		weight)		
Ceriodaphnia dubia	Water flea	7d Reproduction	7.89 (0.72 – 86.37)	11.08 (1.88 – 65.29)
Lymnaea stagnalis	Snail	28d Growth rate	9.61 (3.65 – 25.24)	23.07 (12.03 – 44.22)
Pseudokirchneriella subcapitata	Algae	72h Growth rate	23.0 (14.1 – 37.5)	NC[1]
Daphnia magna	Water flea	21d net reproduction (# young/female)	32.4 (21.8 – 48.0)	45.7 (34.5 – 60.4)
Aeolosoma sp.	Worm	14d Growth	154.6 (124.9 – 191.5)	185.4 (154.3 – 222.7)
Chironomus tentans	Midge	20d Survival	167.1 (104.8 – 266.4)	225.2 (150.1 – 337.9)
Pimephales promelas	Fathead minnow	34d Survival	351.4 (210.6 – 586.5)	409.0 (268.1 – 623.9)
Brachionus calyciflorus	Rotifer	Population - # of individuals	442.2 (221 – 887)	784.9 (478 - 1289)
Danio rerio	Zebrafish	33d Biomass	1,085 (569 – 2,068)	1,593 (946 – 2,682)
Oncorhynchus mykiss	Rainbow trout	81d Biomass	2,171 (1,658 – 2,842)	2,495 (1,995 – 3,120)

[1] Endpoint not calculated.