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An Overview of Sensory Effects on Juvenile Salmonids Exposed to Dissolved Copper:

Applying a Benchmark Concentration Approach to Evaluate Sublethal Neurobehavioral Toxicity

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Applying a Benchmark Concentration Approach to Evaluate Sublethal Neurobehavioral Toxicity

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Table of Contents

List of Figures v
List of Tables vii
Executive Summary ix
Acknowledgments xi
Introduction 1
Previous Studies on the Effects of Copper 3
Application of the Benchmark Concentration Analysis 9
Results of the Benchmark Concentration Analysis 11
Discussion of Site Specific Considerations for Sensory System Effects 14
  Impairment from Short-term Increases of dCu 14
  Calculating an Acute Criterion Maximum Concentration 14
  Salmonids Are Typically Exposed to Multiple Stressors 15
  Bioavailability of dCu 15
  Olfactory Toxicity in Saltwater 16
  Avoiding Short-term Increases in dCu 17
  Coho Salmon–derived BMCs Should Apply to Other Salmonids 17
Conclusions 18
Glossary 19
References 21
Appendix A: Other Salmonid Sensory Effects of dCu 27
  Migratory Disruption 27
  Laboratory Avoidance Studies 28
  Other Adverse Effects 31
Appendix B: Supplementary Bibliography 33
  Copper Sources 33
  Benchmark Concentration/Dose 33
  Copper and Neurobiology 33
  Reviews 35
  Studies with Other Metals 36
  Selected Behavioral Studies 37
  Recovery of Olfactory Epithelium after Damage 38
  Additional Endpoints 38

EPA-6363-000006273

EPA-6363-000006274

List of Figures

Figure 1. Recording methods and features of the salmon peripheral olfactory system. 6

Figure 2. Copper-induced reductions in juvenile salmonid olfactory response and behavior are significantly correlated..... 8

Figure 3. Using a benchmark concentration approach to estimate a threshold for dCu toxicity in the salmonid olfactory system. 12

Figure A-1. Reduction in downstream migration of yearling coho salmon following 6-days exposure to copper at various concentrations..... 29

EPA-6363-000006275

EPA-6363-000006276

List of Tables

Table 1. Selected examples of adverse effects with copper to salmonids or their prey. 4

Table 2. Benchmark responses and benchmark concentrations for juvenile salmon exposed to dCu for 3 hours. 13

EPA-6363-000006277

EPA-6363-000006278

Executive Summary

Dissolved copper (dCu) is a ubiquitous surface water pollutant that causes a range of adverse effects in fish as well as in aquatic invertebrates and algae. This technical memorandum is a summary and targeted synthesis regarding sensory effects to juvenile salmonids from low-level exposures to dCu. As such, the material presented here serves to summarize scientific research on dCu and its impacts on salmonid sensory systems. In addition, this document provides a benchmark analysis of empirical data generated in recent National Marine Fisheries Service investigations that have focused on salmon olfactory function. The review section, Appendix A, discusses peer reviewed and gray literature on the effects of dCu on salmonid sensory systems, associated sensory-mediated behaviors, and physiology. It is intended to facilitate understanding of the effects of dCu on sensory system–mediated behaviors that are important to survival, reproduction, and distribution of salmonids. The review does not address the effects of dCu on salmonid habitats, although copper is also highly toxic at low µg/L concentrations to aquatic primary producers and invertebrates (i.e., the aquatic food web). Undoubtedly, new information will become available that enhances our current understanding of copper's effect on threatened and endangered salmonids and their supporting habitats.

A large body of scientific literature has shown that fish behaviors can be disrupted at concentrations of dCu that are at or slightly above ambient concentrations (i.e., background). In this document, background is operationally defined as surface waters with less than 3 µg/L dCu, as experimental water had background dCu concentrations as high as 3 µg/L dCu. Sensory system effects are generally among the more sensitive fish responses and underlie important behaviors involved in growth, reproduction, and (ultimately) survival (i.e., predator avoidance). Recent experiments on the sensory systems and corresponding behavior of juvenile salmonids contribute to more than four decades of research and show that dCu is a neurotoxicant that directly damages the sensory capabilities of salmonids at low concentrations. These effects can manifest over a period of minutes to hours and can persist for weeks.

To estimate toxicological effect thresholds for dCu in surface waters, benchmark concentrations (BMCs) were calculated using a U.S. Environmental Protection Agency methodology. This paper presents examples of BMCs for juvenile salmonid olfactory function based on recent data. BMCs ranged 0.18–2.1 µg/L, corresponding to reductions in predator avoidance behavior of approximately 8–57%. The BMC examples represent the dCu concentration (above background) expected to affect the ability of juvenile salmonids to avoid predators in freshwater. These concentration thresholds for juvenile salmonid sensory and behavioral responses fall within the range of other sublethal endpoints affected by dCu such as behavior, growth, and primary production, which is 0.75–2.5 µg/L.

The paper also discusses the influence of water chemistry on the bioavailability and toxicity of copper to fish sensory systems. Studies exploring behavioral avoidance as well as representative studies of other effects to salmonids are also summarized. Salmon may be able to

EPA-6363-000006279

avoid dCu in environmental situations where distinct gradients occur. However, avoidance of dCu originating from nonpoint sources appears unlikely. Given the large body of literature on copper and responses of aquatic ecosystems, we focused on a subset of fish sensory system studies relevant to anadromous salmonids.

Point and nonpoint source discharges from anthropogenic activities frequently exceed these thresholds by one, two, and sometimes three orders of magnitude, and can occur for hours to days. The U.S. Geological Survey ambient monitoring results for dCu representing 811 sites across the United States detected concentrations ranging 1–51 µg/L, with a median of 1.2 µg/L. Additionally, typical dCu concentrations originating from road runoff from a California study were 3.4–64.5 µg/L, with a mean of 15.8 µg/L. Taken together, the information reviewed and presented herein indicates that impairment of sensory functions important to survival of juvenile salmonids is likely to be widespread in many freshwater aquatic habitats. Impairment of these essential behaviors may manifest within minutes and continue for hours to days depending on concentration and exposure duration. Therefore, dCu has the potential to limit the productivity and intrinsic growth potential of wild salmon populations by reducing the survival and lifetime reproductive success of individual salmonids.

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EPA-6363-000006281

EPA-6363-000006282

Introduction

Copper, a naturally occurring element, is an essential micronutrient for plants and animals. However, copper is also recognized as a priority pollutant under the U.S. Clean Water Act. Historical and current anthropogenic activities have mobilized significant quantities of copper. Vehicle emissions and brake pad dust (Drapper et al. 2000), pesticides (USEPA 2005), industrial processes, municipal discharges, mining, and rooftops (Good 1993, Thomas and Greene 1993) are a few of the sources of copper in the environment. These various human activities may lead to the unintended and, in some circumstances, intended introduction of copper into aquatic ecosytems (Sansalone and Buchberger 1997, Wheeler et al. 2005). Once in the aquatic environment, copper is detected in multiple forms. It can be dissolved, or bound to organic and inorganic materials either in suspension or in sediment. This so called speciation of copper is dependent on site specific abiotic and biotic factors. As an element, copper will persist and cycle through ecosystems. Copper in its dissolved state is worthy of particular scrutiny as it is highly toxic to a broad range of aquatic species including algae, macrophytes, aquatic invertebrates, and fishes. The latter include anadromous salmon and steelhead within the *Oncorhynchus* and *Salmo* genera that are, in part, managed by the National Marine Fisheries Service.

Currently, anadromous salmonid populations inhabit waters of Alaska, Oregon, Washington, California, Idaho (*Oncorhynchus* spp.), and Maine (Atlantic salmon [*Salmo salar*]). Dissolved copper (referred to as dCu herein) is consistently detected in salmonid habitats including areas important for rearing, migrating, and spawning (Alpers et al. 2000, Soller et al. 2005). Dissolved copper is known to affect a variety of biological endpoints in fish (e.g., survival, growth, behavior, osmoregulation, sensory function, and others, as reviewed in Eisler 1998). More than three decades of experimental results have shown that the sensory systems of salmonids are particularly vulnerable to the neurotoxic effects of dCu. Recent experimental evidence showed that juvenile sensory system–mediated behaviors are also affected by short-term exposures to dCu.

Given the ecological significance of these behaviors to salmonids, it is important to characterize the potential effects from dCu. The growing body of scientific literature indicates that dCu is a potent neurotoxicant that directly damages the sensory capabilities of salmonids at low concentrations (see the Previous Studies on the Effects of Copper section). These concentrations may stem from anthropogenic inputs of dCu to salmonid habitats. Salmonid sensory systems mediate ecologically important behaviors involved in predator avoidance, migration, and reproduction. Impairment of these behaviors can limit an individual salmonid's potential to complete its life cycle and thus may have adverse consequences at the scale of wild populations.

The purpose of this paper is to: (1) summarize information on the effects of dCu to the sensory systems of juvenile salmonids in freshwater (also see Appendix A), (2) conduct a

benchmark concentration analysis to generate examples of dCu effect thresholds, and (3) to discuss site-specific considerations for sensory system effects. As such, it focuses on a single contaminant (dCu), two relevant sensory system endpoints (olfaction and alarm response behavior), and a single salmonid life stage (juvenile, <10 months old).

EPA-6363-000006284

Previous Studies on the Effects of Copper

Examples of copper's effects on a suite of selected biological endpoints from laboratory and field exposures are presented in Table 1. Additionally, Appendix A contains a targeted review and summary of some of the previous studies showing copper's effect on salmonid behavior, including avoidance and migratory disruptions. Appendix B is a supplementary bibliography that provides further information sources on salmonid sensory systems. The following analysis of sensory effects on juvenile salmonids primarily emphasizes recent and ongoing research conducted at the National Marine Fisheries Service's Northwest Fisheries Science Center. However, the phenomenon that copper and some other trace metals can interfere with chemoreception, alter behaviors, and influence the movements of fish was first described at least 40 years ago, and a large body of knowledge on the adverse effects of dCu has subsequently developed (Table 1).

The salmonid olfactory sensory system relies on olfactory receptor neurons (ciliated ORNs) to detect and respond to cues in the aquatic environment. The receptors are in direct contact with the aqueous environment. Olfactory receptors detect chemical cues that are important in finding food, avoiding predators, navigating migratory routes, recognizing kin, reproducing, and avoiding pollution. The architecture of the salmon olfactory system consists of a pair of olfactory rosettes, each positioned within an olfactory chamber near the midline of the fish's rostrum (Figure 1A). Each rosette contains ORNs that respond to dissolved odorants as water passes through the olfactory chamber (Figure 1B) and over the surface of the rosette in which the receptor neurons are embedded (Figure 1C). These chemical cues convey important information about the surrounding aquatic environment.

Direct exposure to dCu can impair and destroy olfactory sensory neurons, although the precise mechanism by which dCu interferes with the normal function of ORNs remains unknown (Hansen et al. 1999b, Baldwin et al. 2003, Sandahl et al. 2006, Sandahl et al. 2007). Impairment of olfaction (i.e., smell) can be measured by an electrophysiological technique called the electro-olfactogram (EOG) (Figure 1) (Scott and Scott-Johnson 2002, Baldwin and Scholz 2005, Sandahl et al. 2006). The EOG measures olfactory response of a population of receptor neurons in fish. Reductions in the EOG amplitude of copper-exposed fish compared to unexposed fish reflect functional losses in sensory capacity. Dissolved copper's toxic effect to olfactory sensory neurons is observable as a reduction in or elimination of the EOG amplitude to a recognizable odor (Figure 1D).

Several recent studies highlight some important aspects of copper olfactory toxicity (Baldwin et al. 2003, Sandahl et al. 2004, 2007). Baldwin et al. (2003) found that the neurotoxic effects of copper in coho salmon (*Oncorhynchus kisutch)* manifest over a timescale of minutes. At 10 minutes, EOG amplitude reductions were observed in juvenile coho exposed to 2, 5, 10, and 20 µg/L dCu above experimental background (3 µg/L). After 30 minutes at 2 µg/L dCu above experimental background, the EOG amplitude from juvenile coho to odors was reduced by approximately 25% compared to controls; in 20 µg/L dCu after 30 minutes by approximately

EPA-6363-000006285

80%. Sandahl et al. (2004) found similar effects following 7 days of exposure (both in EOG reductions and copper concentrations). This result indicated that the juvenile olfactory system does not appear to be able to adapt or otherwise compensate for continuous copper exposure for durations up to 7 days.

Table 1. Selected examples of adverse effects with copper to salmonids or their prey.[a]

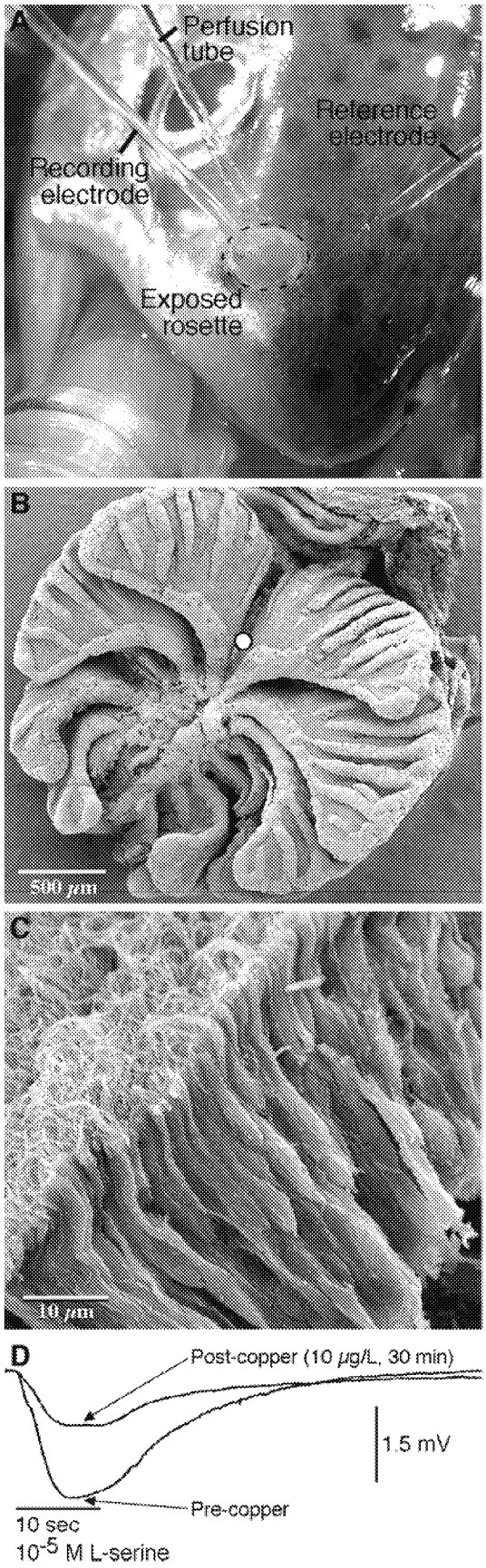
Species (lifestage)	Effect	Effect concentration (µg/L)[b]	Effect statistic	Hardness (mg/L)[c]	Exposure duration	Source
	Sensory and behavioral effects					
Coho salmon (juvenile)	Reduced olfaction and compromised alarm response	0.18–2.1	EC_{10} to EC_{50}	120	3 hours	Sandahl et al. 2007
Chinook salmon (*O. tshawytscha)* (juvenile)	Avoidance in laboratory exposures	0.75	LOEC	25	20 minutes	Hansen et al. 1999a
Rainbow trout (*O, mykiss)* (juvenile)	Avoidance in laboratory exposures	1.6	LOEC	25	20 minutes	Hansen et al. 1999a
Chinook salmon (juvenile)	Loss of avoidance ability	2	LOEC	25	21 days	Hansen et al. 1999a
Atlantic salmon (juvenile)	Avoidance in laboratory exposures	2.4	LOEC	20	20 minutes	Sprague et al. 1965
Atlantic salmon (adult)	Spawning migrations in the wild interrupted	20	LOEC	20	Indefinite	Sprague et al. 1965
Chinook salmon (adult)	Spawning migrations in the wild apparently interrupted	10–25	LOEC	40	Indefinite	Mebane 2000
Coho salmon	Delays and reduced downstream migration of dCu-exposed juveniles	5	LOEC	95	6 days	Lorz and McPherson 1976, 1977
Rainbow trout	Loss of homing ability	22	LOEC	63	40 weeks	Saucier et al. 1991
	Ecosystem effects					
NA[d]	Ecosystem function: Reduced photosynthesis	2.5	LOEC	49	≈ 1 year	Leland and Carter 1985
NA[d]	Ecosystem structure: loss of invertebrate taxa richness in a mountain stream	5	LOEC	49	≈ 1 year	Leland et al. 1989
	Other sublethal effects					
Chinook salmon	Reduced growth (as weight)	1.9	EC_{10}	25	120 days	Chapman 1982
Rainbow trout	Reduced growth (as weight)	2.8	EC_{10}	25	120 days	Marr et al. 1996

Table 1 continued. Selected examples of adverse effects with copper to salmonids or their prey.[a]

Species (lifestage)	Effect	Effect concentra-tion (µg/L)[b]	Effect statistic	Hardness (mg/L)[c]	Exposure duration	Source
	Other sublethal effects (cont.)					
Coho salmon	Reduced growth (as weight)	21–22	NOEC	24–32	60 days	Mudge et al. 1993
Steelhead (*O. mykiss*)	Reduced growth (as weight)	45 to >51	NOEC	24–32	60 days	Mudge et al. 1993
	Direct lethality[e]					
Chinook salmon (fry)	Death	19	LC_{50}	24	96 hours	Chapman 1978
Coho salmon (fry)	Death	28–38	LC_{50}	20–25	96 hours	Lorz and McPherson 1976
Steelhead/rain-bow trout (fry)	Death	9–17	LC_{50}	24–25	96 hours	Chapman 1978, Marr et al. 1999
Coho salmon (adult)	Death	46	LC_{50}	20	96 hours	Chapman and Stevens 1978
Steelhead (adult)	Death	57	LC_{50}	42	96 hours	Chapman and Stevens 1978
Coho salmon (juvenile)	Death	21–22	NOEC	24–32	60 days	Mudge et al. 1993
Steelhead (juvenile)	Death	24–28	NOEC	24–32	60 days	Mudge et al. 1993
Steelhead (egg-to-fry)	Death	11.9	EC_{10}	25	120 days	Chapman 1982

[a] Abbreviations: LOEC = Lowest observed adverse effect concentration (and most LOEC values given are not thresholds, but were simply the lowest concentration tested); NOEC = No observed adverse effect concentration; LC_{50} = the concentration that kills 50% of the test population; ECp = effective concentration adversely affecting (p) percent of the test population or percent of measured response, e.g., 10% for an EC10, etc.; and Indefinite = field exposures without defined starting and ending times. NA = not applicable.
[b] Effects and exposure durations stem from laboratory and field experiments, therefore in some experiments multiple routes of exposure may be present (i.e., aqueous and dietary) and water chemistry conditions will likely differ (see reference for details).
[c] Hardness is reported, as it can influence the toxicity of copper.
[d] This study examined ecosystems consisting of a number of species or unidentified species.
[e] Acute sensitivity of salmonids to copper probably varies by life stage, and the swim-up fry stage is probably more sensitive than older juvenile life stages such as parr and smolts or adults.

Figure 1. Recording methods and features of the salmon peripheral olfactory system. A) Photograph showing the rostrum of a coho salmon during the recording of electro-olfactograms (EOGs). The mouthpiece provides chilled, anaesthetized water to the gills, while the perfusion tube delivers odor-containing solutions to the olfactory chamber. The recording electrode in the olfactory chamber and reference electrode in the skin monitor the response of the olfactory system to an odor. B) Scanning electron micrograph showing a rosette, located within an olfactory chamber of a juvenile coho salmon. Each rosette consists of lamellae (lobes) covered by an epithelium containing regions of sensory neurons. The open circle denotes the location and approximate size of the tip of the recording microelectrode. C) Scanning electron micrograph showing a cross section from a region of sensory epithelium of a lamella. In the upper left is the apical surface containing the cilia and microvilli of the olfactory receptor neurons (ORNs). The dendrites and somata of the ORNs appear in the center within the epithelium, while the axons of the ORNs emerge from the basal surface at the lower right to produce the olfactory nerve. D) Typical odor-evoked EOGs obtained from a salmon before and after exposure to copper. A 10-second switch to a solution containing 10^{-5} M L-serine is shown with a horizontal bar. The EOG evoked by the odor pulse consists of a negative deflection in the voltage. A 30-minute exposure to copper reduced the amplitude of the EOG evoked in the same fish by 57%. (Photos courtesy of Carla Stehr. Figure adapted from Baldwin and Scholz 2005).



Recently, using EOG measurements in combination with a predator avoidance assay, Sandahl et al. (2007) presented the first evidence that impaired olfaction (smell) resulted in a direct suppression of predator avoidance behavior (alarm response) by juvenile coho salmon at environmentally relevant dCu exposures (≥2.0 µg/L; 3 hr exposure). Unexposed juveniles (control treatment) reduced their swimming speed on average by 74% (alarm response) in response to an alarm odor (conspecific skin extract). A reduction in swimming speed is a typical predator avoidance response for salmonids and many other fish. In unexposed fish, the alarm odor elicited a mean EOG response of 1.2 mV. Juvenile coho salmon exposed to 2-20 µg/L copper exhibited measurable reductions in both EOG (50–92%) and alarm response (47 to >100%) (derived from data in Figure 2 of Sandahl et al. 2007). Juvenile coho exhibited statistically significant decline in antipredator behavior at 5, 10, and 20 µg/L dCu (Figure 2).

Importantly, concentrations of dCu below 2 µg/L were not tested in Sandahl et al. (2007). This is notable because all concentrations tested (between 2 and 20 µg/L) significantly affected olfaction with reductions in EOG ranging ≈50–92%. Because individual juvenile coho were significantly affected at the lowest concentration tested (2 µg/L), uncertainty remains with respect to the precise threshold for olfactory impairment. The results of this last study provide evidence that juvenile salmon exposed to sublethal dCu concentrations at 2 µg/L (resulting in approximately 50% reductions in EOG), and likely even lower, might not recognize and respond to a predation threat, and therefore have an increased risk of being eaten by other fishes or birds (a form of ecological death, Kruzynski and Birtwell 1994).

Typically dCu concentrations in road runoff are well within the range affecting antipredator behavior, for example, 3.4–64.5 µg/L, with a mean of 15.8 µg/L (Soller et al. 2005). A 3 hour exposure is also likely to be environmentally relevant, as stormwater runoff durations from roads typically range from a few minutes to several hours (Sansalone and Buchberger 1997). Fish may regain their capacity to detect odors fairly quickly in some cases; physiological recovery of olfactory neuron function is dose-dependent and occurs within a few hours at low copper concentrations (i.e., <25 µg/L dCu, Baldwin et al. 2003). However, long-term damage to the sensory epithelia has also been documented. Where cell death occurs (i.e., ≥25 µg/l copper, Hansen et al. 1999a, 1999b) recovery is on the order of weeks (Moran et al. 1992) and in some cases months (Evans and Hara 1985).

Interestingly, another fish sensory system, the lateral line, is also a target for the neurotoxic effects of dCu. It is composed of mechanosensory neurons (hair cells) that respond to surface water vibrations, flow, and other types of mechanical cues in the aquatic environment. The lateral line system thereby mediates shoaling, pursuit of prey, predator avoidance, and rheotaxis (orientation to flow). In a recent study, dCu (i.e., ≥20 µg/L; 3 hour exposure) killed 20% of hair cells in zebrafish (*Danio rerio*) (Linbo et al. 2006). As mentioned earlier, juvenile salmon ORNs may also be killed at higher concentrations of dCu, highlighting the similar sensitivity of olfactory and lateral line receptors to this toxic metal. Consequently, dCu may damage or destroy either or both of these important sensory systems. Currently, we are not aware of any research on the effects of dCu to the lateral line of salmonids, although the comparable sensitivity of the olfactory system across species suggests that the salmon lateral line is likely to be vulnerable as well.

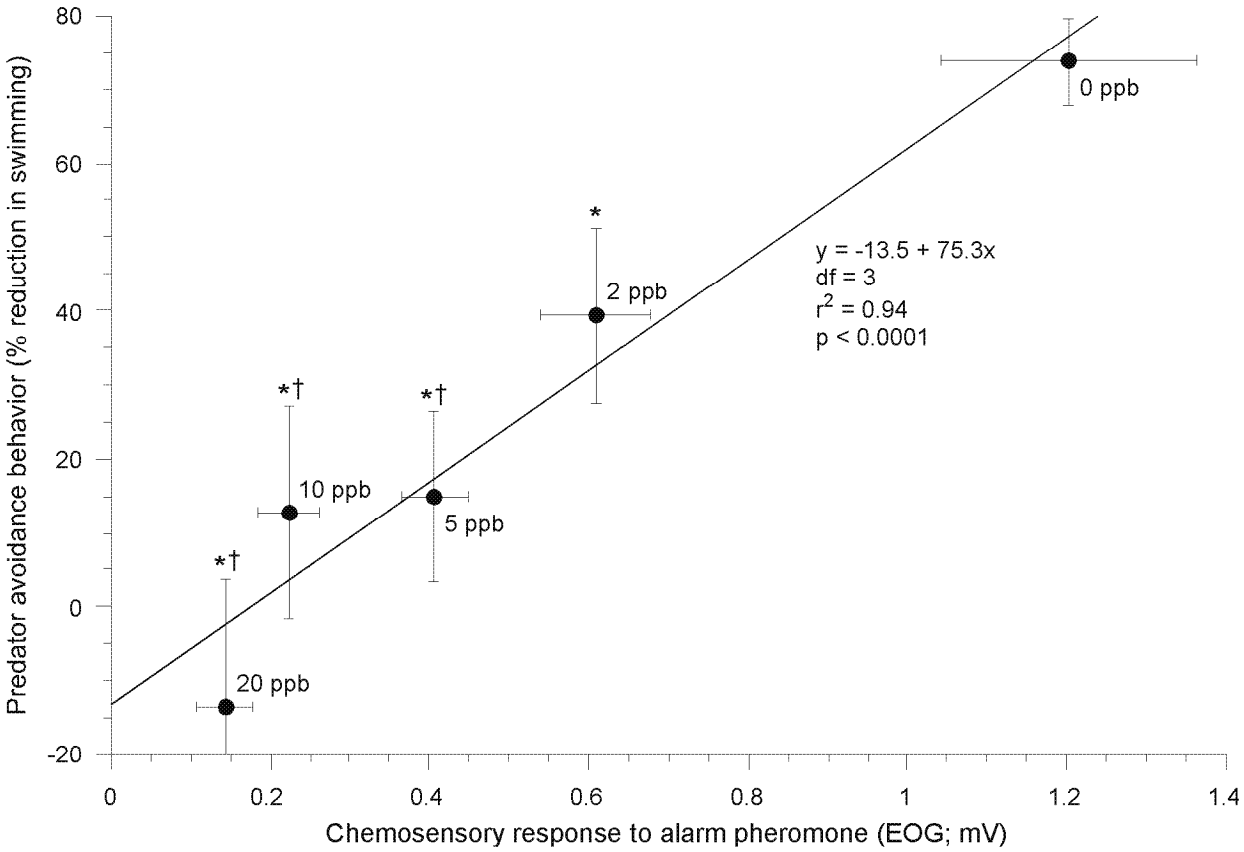


Figure 2. Copper-induced reductions in juvenile salmonid olfactory response and behavior are significantly correlated. Fish exposed to dCu (3 hours) showed reduced olfactory sensitivity and corresponding reduction in predator avoidance behavior. Values represent treatment means (with copper exposure concentration labeled to the right); error bars represent one standard error; n = 8–12 individual coho salmon; asterisk (*) represents a statistically significant difference in olfactory response (EOG data) compared to controls (one-way ANOVA with Dunnett post hoc test, $p < 0.05$); †represents statistically significant difference in behavioral response to skin extract (% reduction in swimming) compared to controls (one-way ANOVA with Dunnett post hoc test, $p < 0.05$). The line represents a statistically significant linear regression based on treatment means (n = 5; $p < 0.0001$; $r^2 = 0.94$). 1 ppb = 1 μg/l. (Adapted from Figure 2C in Sandahl et al. 2007.)

In this paper, a benchmark dose (concentration) analysis (USEPA 1995) is applied to recent data from dose-response experiments on juvenile salmonids exposed to dCu (Sandahl et al. 2007) to determine the exposure concentrations that may adversely affect salmonid sensory systems. In previous studies, benchmark concentrations (BMCs) were determined for olfactory responses, however, concomitant behavioral responses were not measured (Baldwin et al. 2003, Sandahl et al. 2004). The BMC analysis conducted herein determined concentrations of dCu that could be expected to affect juvenile salmonid olfaction and, by extension, alarm response behavior involved in predator avoidance.

Application of the Benchmark Concentration Analysis

The BMC, also referred to as a benchmark dose, is a method that has been used since 1995 by agencies such as the U.S. Environmental Protection Agency (EPA) to determine no observable adverse effect level (NOAEL) values. The method statistically fits dose-response data to determine NOAEL values (EPA 1995). This is in contrast to other methods (e.g., using an analysis of variance) that rely on finding a no observable effect concentration (NOEC) and lowest observable effect concentration (LOEC) to establish the NOAEL. Multiple difficulties arising from the traditional approach of selecting a NOAEL from dose-response data were previously identified by the EPA. Specific shortcomings associated with traditional methods included: 1) arbitrary selection of a NOAEL based on scientific judgments; 2) experiments involving fewer animals produced higher NOAELs; 3) dose-response slopes were largely ignored; and 4) the NOAEL was limited to the doses tested experimentally (EPA 1995). These as well as other concerns with selection of a NOAEL led to the development of an alternative approach, the BMC analysis. The BMC approach uses the complete dose-response data set to identify a NOAEL, thereby selecting an exposure concentration that may not have been tested experimentally.

The BMC is statistically defined as the lower confidence limit for a dose that produces a predetermined adverse effect relative to controls. This effect is referred to as the benchmark response (BMR) (EPA 1995). Unlike the traditional method of selecting the NOAEL (e.g., establishing a NOEC), the BMC takes into account the full range of dose-response data by fitting it with an appropriate regression equation. These can be linear, logarithmic, sigmoidal, etc. The BMR is generally set near the lower limit of responses (e.g., an effect concentration of 10%) that can be measured directly in exposed or affected animals.

In the present context, a BMC approach was used to estimate thresholds for dCu's sublethal effects on the chemosensory physiology and predator avoidance behaviors of juvenile coho salmon (Sandahl et al. 2007). An example of this approach is shown in Figure 3. This methodology has been used previously to determine toxicity thresholds in Pacific salmon (Sandahl and Jenkins 2002, Baldwin et al. 2003, Sandahl et al. 2004). The dose-response relationship for copper's effect on the EOG was described by fitting the data with a sigmoid logistic model:

$$y = m/[1+(x/k)^n]$$

where m is maximum EOG amplitude (fixed at the control mean of 1.2 mV), y is EOG amplitude, x is copper concentration, k is copper concentration at half-maximum EOG amplitude (EC_{50}), and n is slope.

EPA-6363-000006291

For this nonlinear regression, the average olfactory response of the control fish to a natural odor was used to constrain the maximum odor evoked EOG (m in the above equation). Consequently, the control fish were not used in the regression other than to set m. The regression incorporated the individual response of each exposed fish (n = 44 total) rather than the average values for each exposure group. As shown in Figure 3, the sigmoid logistic model was a very good fit for both the sensory and behavioral data ($r^2 = 0.94$, $p < 0.0001$). Benchmark concentrations were then determined based on the concentration at which the estimated curve intersected benchmark responses.

EPA-6363-000006292

Results of the Benchmark Concentration Analysis

Examples of benchmark concentrations and responses are presented in Figure 3 and Table 2. The EPA methodology recommends using the concentration that represents a 10% reduction in response compared to controls when limited biological effects data are available (EPA 1995). This is the BMC_{10} and is synonymous with the concentration producing an effect of 10% (EC_{10}), in this case a 10% reduction in the recorded amplitude of the salmon's chemosensory response (EOG). Since the predicted fish EOG response at the BMC_{10} falls well within the olfactory response of unexposed juveniles, that is, 95% CI (control fish, Figure 3), it is more than likely that this individual response (1.08 mV) at the BMC_{10} (0.18 µg/L) would not be detectable or biologically significant as an adverse response.

Other BMCs were derived using statistical criteria to determine benchmark responses. For example, Table 2 shows two BMCs that were determined using the statistical departure of the lower-bound confidence interval (CI) of the control mean (unexposed fish), 1.2 mV (either the 90 or 95% CI). The selection of different CIs results in different BMCs. The CI-derived BMCs represent a reasonable estimate of when an individual salmonid is likely to have a biologically significant reduction in olfaction and a concomitant reduction in predator avoidance behavior. The relative departures from controls in Table 2 are equivalent to effective concentrations for olfactory inhibition, that is, at the lower-bound 90% CI a BMC of 0.59 µg/L equates to a $BMC_{24.2}$. Put another way, the BMC analysis predicts a substantial 24.2 % reduction in olfaction (i.e., EOG amplitude) at 0.59 µg/L dCu. At the lower-bound 95% CI a 29.2% reduction in olfaction is predicted to occur at 0.79 µg/L.

The BMC_{50} is equivalent to the EC_{50} for olfactory responses (2.1 µg/L) and is very similar to the lowest observable effect concentration (LOEC) of 2 µg/L. Since the EC_{50} approximately equals the LOEC, it is almost certain that effects to juvenile salmonid olfaction will occur at lower concentrations than those measured. Therefore it is appropriate and useful to apply a BMC analysis to these data to predict effects occurring between 0 and 2 µg/L dCu. The predicted effect thresholds for sensory responses in juvenile coho salmon ranged 0.18–2.1 µg/L, which corresponded to reductions in predator avoidance behavior (i.e., reduced alarm response) of 8–57%. Comparatively, the other two studies that conducted a BMC approach with salmon olfaction data sets (e.g., EOG measures) estimated dCu BMCs of 3.6–10.7 µg/L (BMC_{20}–BMC_{50}) (Sandahl et al. 2004) and 2.3–3.0 µg/L (BMC_{25}) (Baldwin et al. 2003).

Together these three studies highlight that different experimental conditions including age of fish, exposure duration, and experimental background of dCu may influence BMCs. Importantly, of the three experiments that derived BMCs for olfactory impairment, the data set used in this technical memorandum from Sandahl et al. (2007) empirically linked impaired olfaction to an ecologically relevant behavior, that is, reduced alarm behavior (Figure 2).

EPA-6363-000006293

Therefore, we believe that the dCu BMC analysis herein is derived from the most ecologically relevant of the three studies.

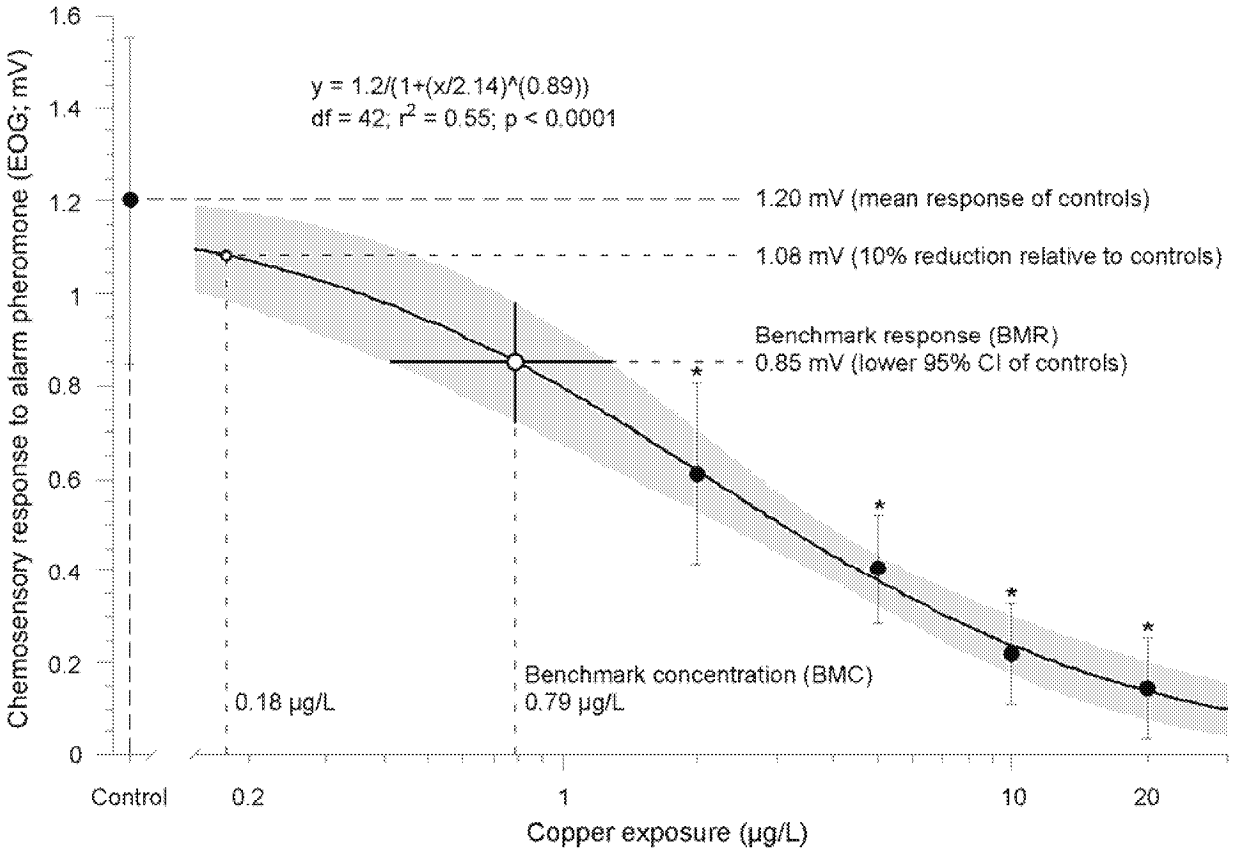


Figure 3. Using a benchmark concentration approach to estimate a threshold for dCu toxicity in the salmonid olfactory system. Filled circles represent treatment means; error bars represent the 95% confidence interval for each mean (n = 8–12 individual coho salmon). An asterisk (*) indicates a statistically significant difference in the size of the olfactory response (EOG data) compared to controls (one-way ANOVA with Dunnett post hoc test, $p < 0.05$). The line represents a statistically significant nonlinear regression based on individual fish ($n = 44$, $p < 0.0001$, $r^2 = 0.55$). The gray shading shows the 95% confidence band for the nonlinear regression. The regression used a standard sigmoid function with the maximum constrained to the control mean (1.2 mV, indicated by the upper horizontal dashed line). Therefore, the control fish were not included in the nonlinear regression. The lower bound of the 95% confidence interval of the control mean (0.85 mV) is indicated by the lower horizontal dashed line and is an example of a BMR. The large open circle shows where the regression line crosses the BMR and denotes the corresponding BMC, which in this case is a dCu concentration of 0.79 µg/L. Horizontal and vertical lines through the open circle highlight the 95% confidence intervals for the BMC based on the results of the nonlinear regression. The small open circle shows where the regression line crosses the BMR (1.08 mV) and denotes the corresponding BMC_{10} (0.18 µg/L) at which a 10% reduction in olfactory capacity is expected. (Data from Sandahl et al. 2007.)

Table 2. Benchmark responses and benchmark concentrations for juvenile salmon exposed to dCu for 3 hours. Benchmark response values represent a reduction in olfactory response to an alarm pheromone as measured via EOG recordings. Behavioral impairment indicates a predicted decrease in predator recognition and avoidance as indicated by a reduced alarm response. CI = confidence interval; NA = not applicable.

Benchmark responses[a]		**Benchmark concentrations**[b]		**Behavioral impairment (predicted)**[c]
Departure from mean of controls				Departure from mean of controls
Statistical[d] (CI of control mean)	Relative[e] (% reduction in olfactory response)	Value[f] (µg/l)	95% CI[g] (µg/l)	Relative[h] (% reduction in alarm response)
NA	10.0	0.18	0.06–0.52	8.3
Lower 90%	24.2	0.59	0.30–1.16	25.6
Lower 95%	29.2	0.79	0.44–1.42	31.8
NA	50.0	2.10	1.60–2.90	57.2

[a] The predetermined level of altered response or risk at which the benchmark dose (concentration) is calculated (EPA/630/R-94/007, 02/1995).
[b] The dose (concentration) producing a predetermined, altered response for an effect (EPA/630/R-94/007; 02/1995).
[c] Based on the linear regression shown in Figure 2; note behavioral responses were determined by inputting the Benchmark response value (EOG, mV) into the regression equation.
[d] Location of the value with respect to a confidence interval of the mean of the controls.
[e] Amount of reduction in the olfactory response represented by the value relative to the mean of the controls.
[f] Corresponding concentration; see Figure 3 and text for calculation method.
[g] Confidence interval for the value based on the nonlinear regression.
[h] Amount of reduction in alarm response represented by the value relative to the mean of the controls.

EPA-6363-000006295

Discussion of Site Specific Considerations for Sensory System Effects

Below we identify several issues to consider when using the BMCs to evaluate dCu concentrations under natural conditions.

Impairment from Short-term Increases of dCu

These BMCs reflect expected impairment of chemosensory systems from short-term increases of dCu above ambient concentrations (defined here as < 3 µg/L) (Baldwin et al. 2003, Sandahl et al. 2004, 2007) and are not expected to be alleviated by homeostatic mechanisms. Specifically, the BMCs are predicated on increases of dCu in salmon habitats that result from specific human activities. Effects to juvenile salmonid olfaction are expected following a few minutes of exposure. Salmonids are capable of regulating the amount of internal copper via uptake and elimination processes. These so called homeostatic mechanisms (such as metallothionein induction) can reduce copper's toxic effects and may result in acclimation. Consequently, fish may tolerate certain dCu exposures without showing overt toxicological responses; however, at higher levels these mechanisms could ultimately fail.

Initial evidence indicates that homeostatic mechanisms are not likely to reduce copper toxicity to the olfactory sensory system for pulsed or short-term exposures lasting less than a week (Hansen et al. 1999a) or for chronically exposed fish (McPherson et al. 2004). Moreover, lateral line neurons exposed continuously to dCu for 72 hours showed no signs of acclimation within this exposure interval (Linbo et al. 2006). For other measures of copper toxicity from long-term exposures, evidence suggests that olfactory acclimation may not occur (Table 1, Appendix A). Fish exposed to higher dCu concentrations for longer periods may lose much of their olfactory function. For example, field evidence suggests that wild fish living in heavy metal contaminated lakes where total copper concentrations ranged 9.7–15 µg/L showed reduced olfactory-mediated predator avoidance behavior; that is, homeostatic mechanisms appeared insufficient to alleviate metal toxicity, including copper (McPherson et al. 2004).

Calculating an Acute Criterion Maximum Concentration

The EPA sets acute water quality criteria by calculating an acute criterion maximum concentration (CMC) (Stephan et al. 1985). The CMC is an estimate of the highest concentration of a substance in surface water to which an aquatic community can be exposed briefly without resulting in an unacceptable effect (EPA 2002).We calculated an acute CMC using the Biotic Ligand Model (BLM) (EPA 2007). Interestingly, the estimated acute CMC based on the BLM using measured and estimated water quality parameters from Sandahl et. al. (2007) was 0.63 µg/L with a range from 0.34 to 3.2 µg/L, while the EPA hardness-based acute CMC (EPA 2002) was 6.7 µg/L. Because the BLM-based acute criterion is sensitive to pH and

EPA-6363-000006296

DOC, the range of measured test pH values (6.5–7.1) and the range of estimated DOC values (0.3–1.5 mg/L) produced this range of BLM-based acute criterion values. It is also interesting that the acute CMC range (0.34–3.2 µg/L) overlapped with the olfactory-based BMC range (0.18–2.1 µg/L).

Salmonids Are Typically Exposed to Multiple Stressors

These BMCs are specifically focused on the impact of dissolved copper alone on olfaction and predator avoidance behavior. Salmonids are rarely exposed to dCu only under natural conditions. In fact, exposure to complex environmental mixtures of other toxic compounds (e.g., metals, pesticides, PAHs, etc.) in conjunction with other stressors (e.g., elevated temperatures, low dissolved oxygen, etc.,) is the norm for many salmonid-bearing habitats. Equally important are exposure routes other than the water column, such as consumption of contaminated prey items (dietary) or direct contact with contaminated sediments. Threshold examples (BMCs) presented here are based solely on juvenile salmonids exposed to dCu. Presently, these thresholds do not take into account multiple routes of exposure or the potential impacts of complex mixtures of contaminants on olfaction. That said, several studies have shown a greater than expected toxicity (i.e., nonadditive) to other fish endpoints from mixtures of metals (Sprague et al. 1965, Norwood et al. 2003). For example, mixtures containing zinc and copper were found to have greater than additive toxicity to a wide variety of aquatic organisms including freshwater fish (Eisler 1998). Other metal mixtures also yielded greater than additive toxic effects at low dissolved concentrations (Playle 2004). The toxic effects of metals to salmonids may also be exacerbated by other types of contaminants such as pesticides (Forget et al. 1999). While interactions among multiple stressors, including contaminant mixtures, are beyond the scope of this document, they warrant careful consideration in site-specific assessments.

Bioavailability of dCu

These BMCs were derived from experiments using a single freshwater source (dechlorinated, soft municipal water). Hardness, alkalinity, and dissolved organic carbon (DOC) are known to alter the bioavailability of dissolved copper in surface waters to ligands in the fish gill. These water chemistry parameters can therefore influence the potential for dCu exposure in the field to cause an acute fish kill. Acute copper lethality mediated via the gill route of exposure is typically estimated using the Biotic Ligand Model (BLM; reviewed by Niyogi and Wood 2004). However, recent unpublished research by McIntyre et al. (in press) suggest that these parameters may have less of an influence on salmonid olfactory function across environmentally realistic ranges of hardness, alkalinity, and DOC.

To date, the U.S. Geological Survey (USGS) has monitored hardness, alkalinity, and DOC for more than 10 years in many West Coast river basins including the Willamette River basin, Puget Sound basin, Yakima River basin, and the Sacramento-San Joaquin River basin (USGS no date). Several at-risk species of anadromous salmonids inhabit these basins. The monitoring data indicate that surface waters within these basins typically have very low hardness and alkalinity and seasonally affected DOC concentrations. Hardness, alkalinity, and DOC levels found in most freshwater habitats occupied by Pacific salmonids would be unlikely to

EPA-6363-000006297

confer substantial protection against dCu olfactory toxicity (Winberg et al. 1992, Bjerselius et al. 1993, Baldwin et al. 2003, McIntyre et al. in press).

Recent experimental results suggest that significant amelioration of olfactory toxicity due to hardness is unlikely in typical Pacific salmonid freshwater habitats. The experiment showed that hardness at 20, 120, and 240 mg/L Ca (experimentally introduced as $CaCl_2$) did not significantly protect juvenile coho salmon from olfactory toxicity following 30 minute laboratory exposures to 10 µg dCu/L above an experimental background of 3 µg/L (Baldwin et al. 2003). In another experiment, a 20 µg dCu/L exposure (30 minutes) in water with low hardness and alkalinity and no DOC produced an 82% inhibition in juvenile coho olfactory function (McIntyre et al. in press). A hardness of ≥82 mg/L Ca was needed to reduce the level of olfactory inhibition to ≤50% at 20 µg/L dCu (McIntyre et al. in press). However, 82 mg/L was never exceeded in any of the surface water samples from USGS-sampled NAWQA basins (McIntyre et al. in press).

Typical alkalinity values from Pacific Northwest and California freshwater surface waters are also unlikely to protect salmonids from olfactory toxicity (USGS no date). Some reduction in dCu olfactory toxicity was observed in a recent study (McIntyre et al. in press). However, only 0.4% of stream samples contained alkalinity levels sufficient to reduce olfactory toxicity of dCu by half (McIntyre et al. in press). Bjerselius et al. (1993) and Winberg et al. (1992) also found that hardness and alkalinity provided limited amelioration of olfactory responses in juvenile Atlantic salmon exposed to dCu.

Increases in DOC showed greater protection to dCu compared to increases in alkalinity and hardness. Twenty-nine percent of USGS surface water samples from West Coast basins had a DOC concentration sufficient to limit olfactory impairment to 50% or less at 20 µg dCu /L (McIntyre et al. in press). Only a small fraction (6%) of all samples contained DOC levels (greater or equal to 6 mg/L) sufficient to completely protect the olfactory responses of juvenile coho salmon from the toxic effect of 20 µg dCu /L (McIntyre et al. in press). This information underscores the importance of evaluating site-specific DOC data to address the potential influence of this water quality parameter on olfactory toxicity.

Because the typical range of hardness, alkalinity, and DOC concentrations are unlikely to confer substantial protection against dCu toxicity, we expect that the BMC thresholds presented in this document will be applicable for most of the freshwater environments that provide migrating, spawning, and rearing habitats for salmonids.

Olfactory Toxicity in Saltwater

Dissolved copper's effect on salmonid olfaction in saltwater environments remains a recognized data gap and it is presently uncertain whether the BMC thresholds derived in this document apply to salt water environments. Estuarine and nearshore salt water environments, despite their higher salinity (in part due to increased cation concentrations) and hardness may or may not confer protection against dCu-induced olfactory toxicity. One source of this uncertainty is whether or not free copper (Cu^{2+}) is the sole species of copper responsible for olfactory toxicity. In freshwater, evidence suggests that Cu^{2+} is not the only toxic species that adversely affects olfaction in fish (McIntyre et al. in press) as well as more conventional endpoints such as

EPA-6363-000006298

mortality (Niyogi and Wood 2004). Other copper species (e.g., CuOH; Cu^{1+}) will also bind to the gill, thereby causing toxicity (Niyogi and Wood 2004). While the physiological basis for salmonid olfaction is well characterized, the transition to saltwater may involve important changes in olfactory receptor neuron function that ultimately influence the expression of the as yet unidentified ligands for dCu.

Avoiding Short-term Increases in dCu

Salmonids may or may not avoid short-term increases in dCu. Salmonids will actively avoid water containing dCu if they can detect it. As a consequence, fish may not use otherwise high quality rearing and spawning habitats. In addition, the presence of dCu may affect migratory routes of juveniles and adults. Smith and Bailey (1990) and Mebane (2000) derived regulatory "zones of passage" around wastewater discharges that were based on salmonid avoidance responses. However, in areas with diffuse, nonpoint source pollution, or multiple point source discharges, it may be difficult to apply "zones of passage", and in some cases available zones of passage may not exist. Despite a fish's preference to avoid dCu, circumstances may force migrating juveniles and adults to be exposed. For dCu contaminated, high quality rearing habitats, juveniles could either remain and be exposed or move to lower quality habitats. Juveniles could therefore suffer either reduced predator avoidance or reduced growth. For contaminated spawning habitats, adult salmon may either remain and be exposed as well as their offspring or move to lower quality habitats. Both of these scenarios result in potential reductions in reproductive success.

Coho Salmon–derived BMCs Should Apply to Other Salmonids

These BMCs were derived using data from juvenile coho salmon, but should apply to other fish species. The examples of BMC thresholds were derived from data based on juvenile coho salmon (4–5 month old, mean of 0.9 grams wet weight). However, we expect these BMC examples to be generally applicable to other species of salmon, trout, and steelhead in freshwater habitats. For example, 3 hour exposures of 4-month-old steelhead to a similar range of dCu produced comparable olfactory toxicity to that reported for 4-month-old coho salmon (Baldwin et al. in prep.). Studies on 10-month-old juvenile coho had similar reductions in olfaction compared to 4-month-old fish (Baldwin et al. 2003, Sandahl et al. 2004). Juvenile chum salmon (*O. keta)* (2–3 month old) also showed a dose dependent reduction in EOG amplitude following exposure to dCu (3–58 µg/L) (Sandahl et al. 2006). Taken together these findings suggest that the BMC threshold derived herein should be applicable to juvenile life stages of coho, Chinook, sockeye (*O. nerka)*, and pink salmon (*O. gorbuscha*) as well as steelhead, bull trout (*Salvelinus confluentus*), and other members of the family Salmonidae. As noted earlier, the toxicity of dCu to other life stages (particularly marine phases of life) remains to be determined.

EPA-6363-000006299

Conclusions

Dissolved copper (dCu) is a ubiquitous, bioavailable pollutant that can directly interfere with fish sensory systems and by extension important behaviors that underlie predator avoidance, juvenile growth, and migratory success (see Appendix A). Recent research shows that dCu not only impairs sensory neurons in a salmonid's nose, but also impairs juvenile salmonids' ability to detect and respond to predation cues. A juvenile salmonid with disrupted predator avoidance behaviors stands a greater risk of mortality and by extension a reduction in the likelihood of surviving to reproduce. The degree to which effects on individual behavior and survival impact a given population will depend in part on the number of the individuals affected and the status of the population (numbers, distribution, growth rate, etc.).

In this report, BMCs were calculated using an EPA methodology to provide examples of effect thresholds of dCu's impacts on salmonid sensory biology and behavior. The BMC examples represent increases in the dCu concentration above background or ambient levels (where background is less than or equal to 3 μg/L) expected to affect juvenile salmonid ability to avoid predators in fresh water. Benchmark concentrations ranged 0.18–2.1 μg/L, corresponding to reductions in predator avoidance behavior (alarm reaction) that ranged approximately 8–57%. Taking into account the olfactory responses of unexposed fish, a more biologically relevant range of BMCs is 0.59–2.1 μg/L (Table 2). This second range of BMC thresholds is similar to or slightly less than documented effects to other copper-affected sublethal endpoints such as behavior and growth that range 0.75–2.5 μg/L (see Table 1).

The primary objective of this report was to present examples of threshold concentrations for effects of dCu on a critical aspect of salmonid biology: olfaction. A secondary objective of this paper was to summarize a selection of recent and historical information related to the effects of dCu on salmonid sensory systems. This document is based on the current state of the science. Importantly, this overview is not a comprehensive summary of the myriad effects of copper to anadromous salmonids. As such, new information will undoubtedly become available that enhances our understanding of copper's effect on salmonid populations and their supporting habitats. The information reviewed and presented herein indicates that significant impairment of sensory functions important to survival of threatened and endangered juvenile salmonids is likely to be widespread in many freshwater aquatic habitats. Impairment of these essential behaviors may occur following 10 minutes of exposure and continue for hours to weeks depending on concentration and duration.

EPA-6363-000006300

Glossary

Acute exposure. Short-term continuous exposure usually lasting 96 hours or less.

BLM. Biotic Ligand Model

Chronic exposure. Longer-term continuous or pulsed exposures generally lasting greater than 96 hours.

Confidence interval (CI). A random interval constructed from data in such a way that the probability that the interval contains the true value can be specified before the data are collected.

dCu. dissolved copper.

DOC. dissolved organic carbon.

EC_p. Effective concentration adversely affecting (p) percent of the test population or percent of measured response, for example, 10% for an EC_{10} and so forth.

EOG. electro-olfactogram.

LC_{50}. The aqueous concentration of a substance that kills 50% of the test population.

Lower-bound 90% confidence interval. The lower half of the 90% confidence interval of the mean.

Lower-bound 95% confidence interval. The lower half of the 95% confidence interval of the mean.

LOEC. lowest observable effect concentration.

Mean. The average of the response values in a treatment population. Numerically the mean represents the sum of the individual response values divided by the number of individuals in a treatment.

mV. millivolts.

NOAEL. no observable adverse effect level.

NOEC. no observable effect concentration.

ORN. olfactory receptor neuron.

ppb. part(s) per billion, equivalent to µg/L.

EPA-6363-000006301

Relative departure from control response. A user selected level of response compared to control response; for example, a 10% reduction from the control response (unexposed individuals).

Statistical departure from control response. Uses statistical methods to select a response based on the distribution of responses seen in unexposed individuals. For example, the 95% lower bound confidence interval of the mean response from controls (unexposed individuals).

EPA-6363-000006302

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Appendix A:
Other Salmonid Sensory Effects of dCu

In this appendix, results are highlighted from several studies that we thought were particularly relevant, including comparing the concentrations that have caused sensory effects to concentrations causing lethality or growth reductions in field and laboratory experiments. As such, the following review is not an exhaustive summary of copper's adverse effects to anadromous salmonids. We emphasize studies that were conducted in waters with low alkalinity and hardness (<50 mg/L as calcium carbonate), and if reported, low concentrations of dissolved organic material. These conditions were emphasized since we believe these are the most relevant water quality conditions for an area of particular concern to us—freshwater habitats used by juvenile salmonids in the Pacific Northwest and California.

Migratory Disruption

Laboratory and field experiments with salmonids have shown avoidance of low concentrations of copper, disruption of downstream migration by juvenile salmonids, loss of homing ability, and loss of avoidance response to even acutely lethal concentrations of copper following long-term habituation to low level copper exposure. Saucier et al. (1991) examined the impact of a long-term sublethal copper exposure (22 µg/L, 37–41 weeks in duration) on the olfactory discrimination performance in rainbow trout (*Oncorhynchus mykiss*). When controls were given a choice between their own rearing water or other waters, they significantly preferred their own rearing water, whereas both copper-exposed groups showed no preference. They concluded that their results demonstrate that a long-term sublethal exposure to copper, as it commonly occurs under "natural" conditions, may result in olfactory dysfunction with potential impacts on fish survival and reproduction.

Field studies have reported that copper impairs both upstream spawning migration of salmonids and downstream outmigration of juveniles. Avoidance of copper in the wild has been demonstrated to delay upstream passage of Atlantic salmon (*Salmo salar*) moving past copper-contaminated reaches of the river to their upstream spawning grounds, cause unnatural downstream movement by adults away from the spawning grounds, and increase straying from their contaminated home stream into uncontaminated tributaries. Avoidance thresholds in the wild of 0.35 to 0.43 toxic units were about seven times higher than laboratory avoidance thresholds (0.05 toxic units), perhaps because the laboratory tests used juvenile fish rather than more motivated spawning adults. For this study 1.0 toxic unit was defined as an incipient lethal level (ILL, essentially a time independent LC_{50}), of 48 µg/L in soft water (Sprague et al. 1965, Saunders and Sprague 1967). Studies of home water selection with returning adult salmon showed that addition of 44 µg/L copper to their home water reduced the selection of their home stream by 90% (Sutterlin and Gray 1973). Releases of about 20 µg/L from a mine drainage into a salmon spawning river resulted in 10–22% repulsion of ascending salmon during four consecutive years compared to 1–2% prior to mining (Sutterlin and Gray 1973). The upstream

EPA-6363-000006309

spawning migration of Chinook salmon (*O. tshawytscha*) in Panther Creek, Idaho, may have been interrupted during the 1980s and early 1990s when the fish encountered dCu concentrations of 10–25 µg/L. In Panther Creek, the majority of spawning habitat and historical locations of Chinook salmon spawning were high in the watershed, upstream of copper discharges. However, Chinook salmon were only observed spawning below the first major diluting tributary, a point above which copper concentrations averaged about 10–25 µg/L during the times of the spawning observations (Mebane 1994, 2000).

Sublethal copper exposure has been shown to interfere with the downstream migration to the ocean of yearling coho salmon (*O. kisutch*). Lorz and McPherson (1976, 1977) and Lorz et al. (1978) evaluated the effects of copper exposure on salmon smolts' downstream migration success in a series of 14 field experiments. Lorz and McPherson (1976, 1977) exposed yearling coho salmon for six to 165 days to nominal copper concentrations varying from 0–30 µg/L. They then marked and released the fish during the normal coho salmon migration period and monitored downstream migration success. The fish were released simultaneously, allowing for evaluation of both copper exposure concentrations and exposure duration on migration success. All dCu exposures resulted in reduction of migration compared with unexposed control fish. Migration success decreased with both increasing copper concentrations and increased exposure time for each respective concentration. Exposure to 30 µg/L dCu for as little as 72 hours caused a considerable reduction in migration (≈60%) compared to control fish. The reductions in migration following short-term exposures to dCu are illustrated in Figure A-1. Following exposure to 30 µg/L dCu, 80% of coho did not reach the migratory point in 49 days. These concentrations (5-20 µg/L) were one-tenth to one-third the 96-hour LC_{50} for the same stock of juvenile coho salmon in the same water. Lorz et al. (1978) further tested downstream migration with yearling coho salmon previously exposed to copper, cadmium, copper-cadmium mixtures, zinc, and copper-zinc mixtures. Copper concentrations in all tests were held at 10 µg/L. In all cases, the copper exposed fish again had poorer migratory success than did controls. The other metals did not show the dose-dependent result found for copper. These studies suggest that exposure to copper concentrations at levels found in streams subject to nonpoint copper pollution may impair downstream migration, a result of direct and indirect effects to salmon smolts, including reproductive success.

Laboratory Avoidance Studies

Studies have shown that salmonids can detect and avoid copper at low concentrations when tested in troughs or streams that allow them to choose between concentration gradients. To our knowledge, the lowest copper concentration reported to cause avoidance in laboratory conditions was 0.1 µg/L (Folmar 1976). However, these results may have low applicability to ambient conditions because copper exposure concentrations were not analytically verified. Avoidance thresholds of 2 µg/L copper have been reported for Atlantic salmon (*Salmo salar*), concentrations that are less than one-tenth of acute LC_{50} values (Saunders and Sprague 1967). Giattina et al. (1982) reported that rainbow trout appeared to detect copper concentrations down to 1.4–2.7 µg/L, because declines in residence time started to occur at these lower concentrations. However, the responses were only statistically significant at 4.4 to 6.4 µg/L depending on whether fish were exposed to a gradually increasing or abruptly increasing concentration gradient respectively. At exposure to extremely high dCu levels, for example,

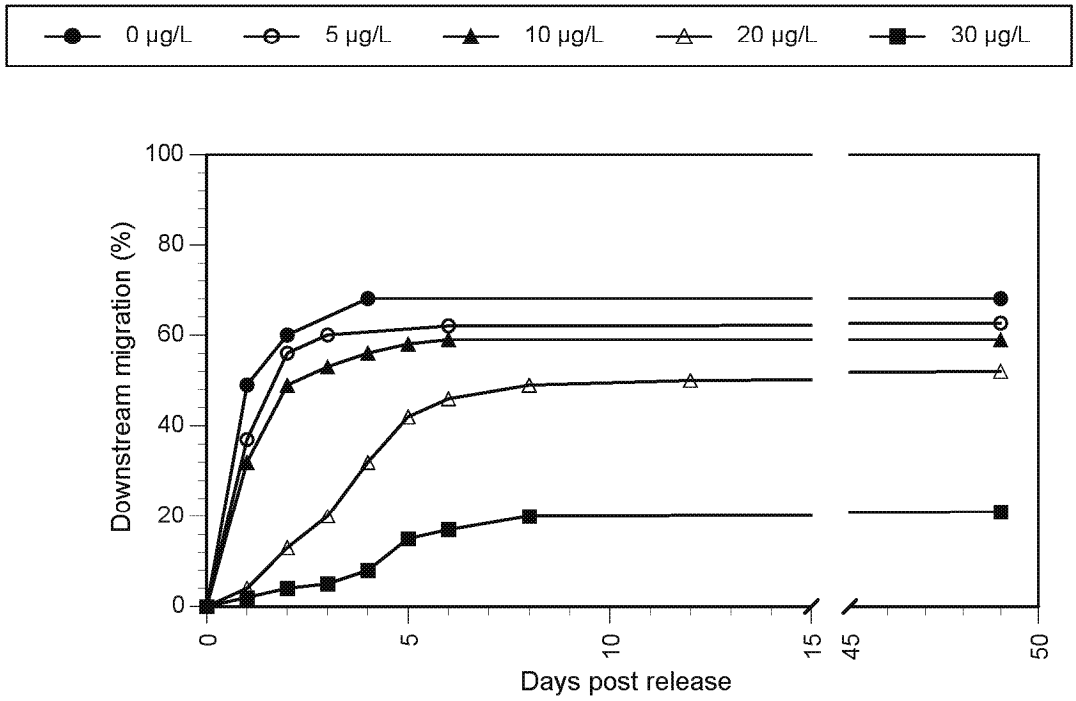

Figure A-1. Reduction in downstream migration of yearling coho salmon following 6 days of exposure to copper at various concentrations. (Redrawn from Lorz and McPherson 1977, their Figure 19.)

330–390 µg/L, trout showed diminished avoidance and sometimes attraction to acutely lethal concentrations (Giattina et al. 1982, Hansen et al. 1999a, Chapman unpubl. data).

Chapman (unpubl. data) reported that long-term sublethal copper exposures had impaired the avoidance performance of salmonids. Steelhead (*O. mykiss*), acclimated to low copper levels by surviving about 3 months early life stage toxicity testing, subsequently failed to avoid much higher, acutely lethal concentrations. Following about 3 month continuous exposure to 9 µg/L copper (from fertilization to about 1 month after swim up) the copper-acclimated fish and control fish with no previous copper exposure were exposed to a range of copper concentrations from 10 to 80 µg/L in avoidance-preference testing. The tests used the same counter flow avoidance-preference test chambers described by Giattina et al. (1982). The acclimated steelhead failed to avoid even the highest copper concentrations while most of the unexposed fish avoided all concentrations.

Hansen et al. (1999a) and Marr et al. (1995) conducted a variety of behavioral and other toxicity studies with Chinook salmon and rainbow trout exposed to copper. In these studies they used well water that was diluted with deionized water and spiked with copper to obtain a hardness, alkalinity, and pH that simulated those in Panther Creek, a mine-affected stream in Idaho. The avoidance response of the Chinook salmon was statistically significant for 0.8 and 2.8–22.5 µg/L copper but was not significant for a 1.6 µg/L copper treatment. Since the avoidance responses (percent time spent in test water) were similar between the 0.8, 1.6, and 3 µg/L treatments, but the 1.6 µg/L treatment had fewer replicates than the other treatments (10 vs. 20), the lack of statistical significance for the 1.6 µg/L treatment was probably an artifact of the

different sample sizes rather than a true lack of response. Rainbow trout consistently avoided copper at concentrations of 1.6 µg/L and above. To simulate avoidance responses that might result on exposing fish to background levels of copper, Hansen et al. (1999a) acclimated both Chinook salmon and rainbow trout to 2 µg/L copper for 25 days, and repeated the avoidance experiments. They observed that the avoidance response of Chinook salmon was greatly dampened such that no copper treatments resulted in statistically significant responses. In contrast, the avoidance response of rainbow trout was unaffected by the acclimation. This dramatic difference between Chinook salmon and rainbow trout avoidance was so unexpected that Hansen et al. (1999a) ran a second set of experiments that yielded the same results. Background dCu concentrations (<4 µg/L) are commonly observed in natural waterways, yet Chinook salmon failed to avoid any higher dCu concentrations following an acclimation to a nominal 2 µg dCu/L. Importantly, if Chinook salmon will not avoid any dCu concentrations following acclimation to low dCu concentrations, the behavioral defense against chronic and acute exposures to dCu is lost, and high mortality or chronic physiological effects are probable if subsequent higher levels of dCu exposure occur. Unlike Chinook salmon, dCu-acclimated rainbow trout preferred clean water and avoided higher dCu concentrations. Other differences between Chinook salmon and rainbow trout avoidance responses to copper were that addition of 4 and 8 mg/L dissolved organic carbon (DOC) did not appreciably affect the avoidance response of Chinook salmon to copper, nor did altering pH across a range of 6.5 to 8.5. In contrast, the addition of DOC (4 and 8 mg/L) did reduce the avoidance response of rainbow trout to copper. Although variable, avoidance responses of rainbow trout were slightly stronger at pH 7.5 and 8.5 than at 6.5 (Marr et al. 1995).

A further repeated finding from these laboratory avoidance tests was that although rainbow trout, steelhead, and Chinook salmon avoided low concentrations of dCu, they were apparently intoxicated and sometimes attracted to very high concentrations (Giattina et al. 1982, Hansen et al. 1999a, Chapman unpubl. data). The direct relevance of laboratory avoidance studies to the behaviors of fish in the wild is debatable since in natural waters fish likely select and move among habitats based on myriad reasons such as access to prey, shelter from predators, shade, velocity, temperature, and interactions with other fish. In contrast, laboratory preference/avoidance tests are commonly conducted under simple, highly artificial conditions to eliminate or minimize confounding variables other than the water characteristic of interest. Laboratory tests may overestimate the actual protection this behavior provides fish in heterogeneous, natural environments (Hartwell et al. 1987, Korver and Sprague 1989, Scherer and McNoil 1998).

However, at least one study suggested that experimental avoidance responses observed with salmonids are relevant to fish behaviors in the wild. From 1980 to 1982, sublethal levels of a contaminant (fluoride) from an aluminum mill at the John Day Dam on the Columbia River were associated with a significant delay in salmon passage and decreased survival (Damkaer and Dey 1989). Salmon took an average of 36 hours to pass up the fish ladder at the Bonneville and McNary dams compared to 157 hours delay at the John Day Dam. Greater than 50% mortality occurred between the Bonneville and McNary dams (above and below the John Day dam), compared to about 2% mortality associated with the other dams. Damkaer and Dey (1989) introduced similar levels of the contaminant in streamside test flumes alongside a salmon spawning stream (Big Beef Creek, Washington). Significant numbers of adult Chinook salmon failed to move out of their holding area and continue upstream; those that did move upstream

chose the noncontaminated side of the flume. By adjusting the dose, Damkaer and Dey (1989) predicted a threshold detection limit for avoidance by salmon. The mill subsequently reduced its release of the contaminant to below these experimental threshold levels, which did not show a response in the streamside tests. Afterwards, fish passage delays and salmon mortality between the dams decreased to 28 hours and <5%, respectively (Damkaer and Dey 1989). This study suggested that the delay due to avoidance of a chemical affected the spawning success of migrating adult salmonids. These results are also consistent with the field studies of salmon migration in copper-contaminated streams and from laboratory avoidance/preference testing. Experimental avoidance/preference testing thus appears to be relevant to fish behavior in nature.

Other Adverse Effects

The focus of this literature synthesis is sensory effects of copper on juvenile salmonids. However, other adverse effects of copper to salmonids reported in the literature include weakened immune function and disease resistance, increased susceptibility to stress, liver damage, reduced growth, impaired swimming performance, weakened eggshells, and direct mortality (McKim and Benoit 1971, Stevens 1977, Schreck and Lorz 1978, Waiwood and Beamish 1978a, 1978b, Chapman 1982, Farag et al. 1994, Marr et al. 1996, Farag et al. 2003). While a comprehensive review of other adverse effects of copper on fish is beyond the scope of this synthesis, we discuss several studies of interest below.

Stevens (1977) reported that preexposure to sublethal levels of dCu interfered with the immune response and reduced the disease resistance in yearling coho salmon. Juvenile coho salmon were vaccinated with the bacterial pathogen *Vibrio anguillarum* prior to copper exposure to investigate the effects of copper upon the immune response and survival. Following copper exposure (9.6–40 µg/L), surviving juveniles were challenged under natural conditions to *V. anguillarum*, the causative agent of vibriosis in fish. Vibriosis is a disease commonly found in wild and captive fish from marine environments and has caused deaths of coho and Chinook salmon. Coho salmon were exposed to constant concentrations of dCu for about one month at levels that covered the range from no effect to causing 100% mortality, 9.6–40 µg/L. The antibody titer level against *V. anguillarum* was significantly reduced in fish exposed to 13.9 µg/L of dCu when compared to that developed in control fish. The survivors of the dCu bioassays were then exposed in saltwater holding ponds for an additional 24 days to the *V. anguillarum* pathogen. The unvaccinated, non-dCu exposed control fish had 100% mortality and the vaccinated, non-dCu exposed fish had the lowest mortality. The vaccinated, dCu-exposed fish had increasing mortality corresponding to the lower antibody titer levels which in turn corresponded to the increasing dCu exposure levels. Therefore, dCu exposure can significantly reduce a fish's immune function and disease resistance at concentrations as low as 13.9 µg/L following 30 days of exposure (Stevens 1977).

Schreck and Lorz (1978) studied the effects of copper exposure to stress resistance in yearling coho salmon. Fish that were exposed for 7 days to 15 µg/L dCu and unexposed control fish were subjected to severe handling and confinement stress. Copper-exposed fish survived this additional stress for a median of 12–15 hours while control fish experienced no mortality at 36 hours. Schreck and Lorz (1978) concluded that exposure to copper placed a sublethal stress on the fish which made them more vulnerable to handling and saltwater adaptation. Further,

EPA-6363-000006313

they hypothesized that dCu exposure may make salmonids more vulnerable to secondary stresses such as disease and pursuit by predators.

Exposure of brook trout (*Salvelinus fontinalis*) eggs to 17.4 µg dCu/L for 90 days resulted in weakened chorions (eggshells) and embryo deformities. After hatching, poor yolk utilization and reduced growth were demonstrated. These overall weakened conditions may reduce survival chances in the wild (McKim and Benoit 1971, McKim 1985). Copper accumulation in the liver of rainbow trout caused degeneration of liver hepatocytes, which resulted in reduced ability to metabolize food, reduced growth, or eventual death (Leland and Carter 1985, Farag et al. 1994, Meyer 2005). Waiwood and Beamish (1978a), Chapman (1982), Seim et al. (1984), McKim and Benoit (1971), and Marr (1996) have also observed reduced growth of salmonids in response to chronic copper exposures as low as 1.9 µg/L. Waiwood and Beamish (1978b) reported that rainbow trout exposed to copper levels had reduced swimming performance (10, 15, 20, 30 µg/L dCu) and reduced oxygen consumption (25, 40 µg/L dCu) apparently due to gill damage and decreased efficiency of gas exchange.

In sum, there is a large body of literature showing that behavior of salmonids and other fishes can be disrupted at concentrations of dCu that are only slightly elevated above background concentrations. Further, dCu stress has been shown to increase the cost of maintenance to fish and to limit oxygen consumption and food metabolism. Reduced growth may result in increased susceptibility to predation, and impaired swimming ability may result in reduced escape reaction and prey hunting, with a possible consequence of reduced survival at the population level. We summarize selected examples of effect concentrations reported with copper for several different types of effects in Table 1 of this technical memorandum. In general, typical copper exposures probably do not kill juvenile salmonids directly until concentrations greater than about 10 times that of sensory thresholds, and then only if the concentrations are sustained for at least several hours. In selecting these examples, we sought to list representative effects and concentrations rather than extreme values that could be gleaned from the literature. However, the selected examples do not constitute an exhaustive review of the effects of copper to fish; more general reviews of effects of copper to fish and other aquatic organisms are available elsewhere (Leland and Carter 1985, Sorensen 1991, Eisler 1998, USEPA 2007).

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