PROTECTIVENESS OF COPPER AQUATIC LIFE CRITERIA/GUIDELINES AGAINST OLFACTORY IMPAIRMENT IN FISH: AN INTERNATIONAL COMPARISON

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Introduction

The olfactory system (sense of smell) is crucial to the ability of fish to avoid predators, recognize kin, and synchronize reproduction (Baldwin et al. 2003). Copper (Cu) has been shown to impair olfaction in juvenile freshwater life stages of salmonids (including Pacific salmon and trout) in some laboratory experiments; and in the United States, public concerns have been expressed that current regulatory criteria for Cu in fresh water are not adequately protective of olfactory function in salmonids (e.g., OSU 2007; Pearson 2007). These concerns have largely been raised because some Cu concentrations that have caused olfactory impairment in salmonids in the laboratory are less than Cu concentrations that have been shown to reduce salmonid survival, growth, or reproduction (the effects endpoints that are typically considered in deriving ambient water quality criteria). However, two important and interrelated points that must be considered: (1) freshwater Cu criteria are "driven" by the sensitivities of several invertebrate species that are more sensitive than the olfactory impairment threshold in salmonids when (2) the influence of water chemistry on Cu bioavailability is properly considered in deriving Cu criteria. Bioavailability refers to the ability of various chemical forms of Cu to interact with receptors on the surface(s) of organisms and thus directly cause toxicity or uptake by the organisms.

The toxicity of Cu and other metals to aquatic organisms is a function of water chemistry. For example, acute Cu toxicity in fresh water generally decreases as pH, water hardness (Ca^{2+} and Mg^{2+}), alkalinity, and dissolved organic carbon (DOC) concentrations increase (Meyer et al. 2007). The U.S. Environmental Protection Agency (USEPA) has recommended hardness-based freshwater aquatic life criteria for Cu and other metals since the 1980s (e.g., USEPA 1985); and hardness-based metals criteria decrease (i.e., become more restrictive) as water hardness decreases. More recently, the USEPA has recommended freshwater aquatic life criteria for Cu based on the biotic ligand model (BLM; USEPA 2007), which accounts for the influence of multiple water chemistry parameters, including DOC, pH, alkalinity, Ca^{2+}, Mg^{2+}, and several other cations and anions. The BLM-based Cu criteria generally decrease as DOC concentration, pH, alkalinity, and water hardness decrease. Because DOC has a stronger influence on Cu bioavailability than water hardness, hardness-based and BLM-based Cu criteria can differ considerably. For example, in water that has low DOC and high hardness, the hardness-based Cu criterion would be relatively high (because it is driven by the high hardness) but the BLM-based Cu criterion would be relatively low (because it is driven by the low DOC). Thus, any evaluation of the protectiveness of Cu criteria against a given effect, such as olfactory impairment, must properly account for the chemistry of the exposure water.

Meyer et al. (2013) compiled data on Cu-induced olfactory impairment and olfactory-induced behavioral effects from tests in which BLM parameters were measured. They demonstrated that the USEPA's BLM-based freshwater life criteria for Cu are protective against olfactory impairment, because it adequately accounts for water chemistry (whereas the USEPA's hardness-based Cu criteria are not consistently protective). Olfactory impairment by Cu is now receiving increased attention in Canada and Europe (e.g., Wall 2013; Stang 2013). Accordingly, in this paper we have expanded the analysis of Meyer et al. (2013) to consider copper guidelines from various other countries and whether these guidelines are protective against olfactory impairment.

Methods

Data related to the olfactory effects of Cu were compiled from published scientific literature. The studies used in the Meyer et al. (2013) evaluation were augmented with recently published studies. Because DOC is a crucial parameter that influences Cu bioavailability, measured DOC data were required in the studies evaluated. A typical approach for measuring olfactory impairment is the use of an electro-olfactogram (EOG), which is a measurement of the transepithelial electrical potential across the surface of an olfactory rosette (located in the nostril of the fish) in response to an odorant (e.g., an amino acid or a predator alarm substance) when Cu-contaminated water is perfused across the rosette. Another approach for measuring olfactory impairment is the use of an electro-encephalogram (EEG), which is a measurement of the electrical activity recorded in the olfactory bulb of the fish's brain in response to an odorant when Cu-contaminated water is perfused across the olfactory rosette (see Fig. 1 in Sandahl et al. [2004] for a diagram of EOG and EEG measurements). In addition, data were compiled from behavioral studies in which fish were exposed to an odorant in the presence and absence of Cu-contaminated water. Where possible, IC20 values were calculated for each test (i.e., 20% inhibition concentrations, such as a 20% inhibition in the EOG response). The corresponding test water chemistries were also compiled, including DOC, pH, and hardness.

Water quality guidelines for Cu were compiled for Canada (federal: CCME 2007; provincial: BCMOE 1987, Alberta Environment 1999), the United Kingdom (UKTAG 2008), and Australia/New Zealand (ANZECC 2000). These international Cu guidelines are adjusted as a function of water hardness, either as defined values for ranges of water hardness or using an equation (Table 1).

Ratios of olfactory-related IC20 values to each of these various guidelines were then calculated to determine whether the guidelines are protective against olfactory impairment. If the ratio was greater than or equal to 1, the guideline was considered protective; but if the ratio was less than 1, the guideline was considered not protective.

Results and Discussion

Seven freshwater olfactory or behavioral studies that were conducted with Cu-contaminated laboratory or field water had adequate water chemistry for calculation of various international guidelines:

- Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*) avoidance (pH = 7.5-7.7, alkalinity = 28 mg/L as $CaCO_3$, hardness = 25 mg/L as $CaCO_3$, DOC = 0.1 mg/L; Hansen et al. 1999a, and Meyer and Adams 2010);
- Chinook salmon (*O. tshawytscha*) and rainbow trout (*O. mykiss*) EEG responses (pH = 7.5-7.7, alkalinity = 28 mg/L as CaCO3, hardness = 25 mg/L as $CaCO_3$, DOC = 0.1 mg/L; Hansen et al. 1999b, and Meyer and Adams 2010);
- Coho salmon (*O. kisutch*) EOG responses (pH 7.1-8.5, alkalinity = 9-160 mg/L as CaCO3, hardness = 27-190 mg/L as $CaCO_3$, DOC = 0.1-6.0 mg/L; McIntyre et al. 2008a,b);
- Fathead minnow (*Pimephales promelas*) EOG responses (pH = 6.8, alkalinity = 23 mg/L as $CaCO_3$, hardness = 23 mg/L as $CaCO_3$, DOC = 1 mg/L; Green et al. 2010);
- Fathead minnow (*P. promelas*) EOG responses (pH = 7.5, alkalinity = 50.4 mg/L as CaCO3, hardness = 48 mg/L as CaCO3, DOC = 1.6 mg/L; Dew et al. 2012);

Table 1. International Copper Guidelines

	Values or Equations (µg Cu/L)	
Country or Jurisdiction	**Acute**	**Chronic**
Australia/New Zealand (ANZECC 2000)	none	$1.4(\text{hardness}/30)^{0.85}$
Canada (CCME 2007)	none	2 (0-120 mg/L hardness) 3 (120-180 mg/L hardness) 4 (>180 mg/L hardness)
British Columbia, Canada (BCMOE 1987)	0.094(hardness)+2	2 (≤50 mg/L hardness) 0.04 × hardness (>50 mg/L hardness)
Alberta, Canada (Alberta Environment 1999)	exp(0.979123× [ln(hardness)]-8.64497)	7 (>50 mg/L hardness)
United Kingdom (UKTAG 2008)	none	1 (0-50 mg/L hardness) 6 (50-100 mg/L hardness) 10 (100-250 mg/L hardness) 28 (>250 mg/L hardness)

- Chinook salmon (*O. tshawytscha*) avoidance (pH = 7, hardness = 6.1 mg/L as $CaCO_3$, DOC = 0.5-20 mg/L; Kennedy et al. 2012); and
- Yellow perch (*Perca flavescens*) EOG responses (field exposures, pH = 6.9-7.2, alkalinity = 26-38 mg/L as $CaCO_3$, hardness = 40-64 mg/L as $CaCO_3$, DOC = 3.9-4.3 mg/L; Azizishirazi et al. 2013).

With the exception of the yellow perch data from Azizishirazi et al. (2013), all Cu exposures were conducted with naive (no Cu-exposure history) fish in laboratory-prepared waters. In Azizishirazi et al. (2013), EOG responses were measured on wild fish collected from a reference lake (mean Cu ± standard error = 1.6 ± 0.3 µg/L) and two metal-enriched Canadian lakes with Cu concentrations of 8.6 ± 1.5 and 13.7 ± 2.3 µg/L. Mean nickel (Ni) and zinc (Zn) concentrations in the metal-enriched lakes ranged from 32.5 to 48.2 and 1.7 to 4.2 µg/L, respectively, and were assumed to have a negligible influence on the EOG responses in the wild fish. EOG responses were measured on perch that were exposed in their native waters and in reference site fish that were exposed to each of the metal-enriched waters.

Overall, 223 different olfactory threshold-to-guideline ratios were derived from the compiled data (Table 2). The ratio was <1 in 26% of these cases, meaning that the hardness-based guideline would not have been protective of the olfactory-based Cu threshold for those particular tests. Approximately 80% of the ratios <1 were associated with DOC concentrations <1 mg/L (Figure 1). This reflects a limitation of hardness-based criteria, in that they tend to be under-protective when DOC concentration is low. In other words, the decreased capacity of low DOC concentrations to bind Cu and render it non-bioavailable to aquatic life is more important than the ability of increasing water hardness concentrations to decrease Cu bioavailability.

At least based on the international Cu guidelines evaluated here, hardness-based Cu guidelines are sometimes under-protective of olfactory thresholds for Cu (mainly in waters with low DOC). This is consistent with the evaluation of Meyer et al. (2013), who found that the USEPA's hardness-based Cu criteria may be under-protective in some waters. However, Meyer et al. (2013) found that the USEPA's BLM-based Cu criteria were consistently protective across a wide range of water chemistries. Because USEPA and European BLMs for Cu were derived by different researchers using different methodologies, and because the approach for deriving the chronic "guideline" ("criterion" using USEPA nomenclature and "environmental quality guideline [EQG]" using EU nomenclature) differs between the two jurisdictions, including the types of toxicity data considered and the statistical procedure for determining the final criterion or EQG, it is currently uncertain whether European BLM-based Cu EQS values will be similarly protective against olfactory impairment.

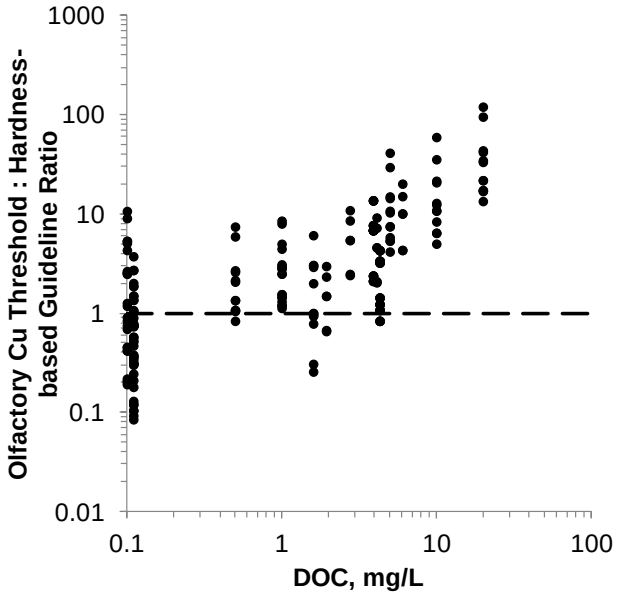

Figure 1. Relationship between ratio of olfactory Cu threshold to corresponding hardness-based Cu threshold and dissolved organic carbon (DOC) concentration in the exposure water.

Conclusions

A previous evaluation (Meyer et al. 2013) concluded that the USEPA's BLM-based freshwater aquatic life criteria for Cu are protective against olfactory impairment in salmonids and other fish species, whereas the USEPA's hardness-based criteria are not always protective. Because concerns related to Cu-induced olfactory impairment have started to receive increasing attention in Canada and Europe, the evaluation of Meyer et al. (2013) was expanded to evaluate whether Cu guidelines applied in other countries and jurisdictions are protective against olfactory impairment. We found that most countries and jurisdictions still apply hardness-based Cu criteria, and that, like the USEPA's hardness-based Cu criteria, international hardness-based Cu guidelines were not always protective of thresholds for olfactory impairment (particularly at DOC concentrations <1 mg/L). Additional evaluations on the protectiveness of European BLM-based guidelines, including EQS values derived by individual European Union member states, are ongoing.

Acknowledgments

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Table 2. Ratios of Cu thresholds for olfactory impairment or olfactory-mediated behavior (ratios <1 indicate Cu guidelines that may not be protective of olfactory impairment).

Study	Species[1]	Test description	Measure	Cu IC20 (µg/L)	Australia/ New Zealand Chronic	Canada Chronic	British Columbia, Canada Acute	British Columbia, Canada Chronic	Alberta, Canada Acute	Alberta, Canada Chronic	UK Chronic
McIntyre et al.	Coho salmon	Low-ion	EOG	1.50	1.1	***0.8***	***0.3***	***0.8***	***0.3***	-	1.5
2008		Calcium-1	EOG	0.73	***0.3***	***0.4***	***0.1***	***0.3***	***0.1***	***0.1***	***0.1***
		Calcium-2	EOG	1.47	***0.4***	***0.7***	***0.1***	***0.4***	***0.1***	***0.2***	***0.2***
		Calcium-3	EOG	3.60	***0.5***	***0.9***	***0.2***	***0.5***	***0.1***	***0.5***	***0.4***
		Alkalinity-1	EOG	1.49	1.1	***0.7***	***0.3***	***0.7***	***0.3***	-	1.5
		Alkalinity-2	EOG	2.72	2.0	1.4	***0.6***	1.4	***0.6***	-	2.7
		Alkalinity-3	EOG	3.74	2.7	1.9	***0.8***	1.9	***0.8***	-	3.7
		Fulvic acid-1	EOG	2.98	2.3	1.5	***0.7***	1.5	***0.7***	-	3.0
		Fulvic acid-2	EOG	10.9	8.6	5.5	2.4	5.5	2.5	-	10.9
		Fulvic acid-3	EOG	20.2	15.1	10.1	4.3	10.1	4.3	-	20.2
		Natural organic matter	EOG	9.2	7.3	4.6	2.0	4.6	2.1	-	9.2
Hansen et al.	Rainbow trout	-	Avoidance	0.84	***0.7***	***0.4***	***0.2***	***0.4***	***0.2***	-	***0.8***
1999a	Chinook salmon	-	Avoidance	0.91	***0.8***	***0.5***	***0.2***	***0.5***	***0.2***	-	***0.9***
Hansen et al.	Rainbow trout	-	EEG	5.1	4.3	2.6	1.2	2.6	1.3	-	5.1
1999b	Chinook salmon	-	EEG	10.7	9.1	5.4	2.5	5.4	2.7	-	10.7
Green et al. 2010	Fathead minnow	-	EOG	5.0	4.5	2.5	1.2	2.5	1.3	-	5.0
Dew et al. 2012	Fathead minnow	24 h	EOG	2.0	1.0	1.0	***0.3***	1.0	***0.3***	-	2.0
		96 h	EOG	6.1	2.9	3.1	***0.9***	3.1	***0.8***	-	6.1
Kennedy et al.	Chinook salmon	24 h, 0 mg DOC/L	Avoidance	2.2[a]	5.9	1.1	***0.8***	1.1	2.1	-	2.2
2012		24 h, 1 mg DOC/L	Avoidance	2.9[a]	8.0	1.5	1.1	1.5	2.8	-	2.9
		24 h, 5 mg DOC/L	Avoidance	15.0[a]	41.4	7.5	5.8	7.5	14.5	-	15.0
		24 h, 10 mg DOC/L	Avoidance	21.6[a]	59.6	10.8	8.4	10.8	20.8	-	21.6
		24 h, 20 mg DOC/L	Avoidance	43.8[a]	120.7	21.9	17.0	21.9	42.2	-	43.8
		96 h, 0 mg DOC/L	Avoidance	2.7[a]	7.4	1.4	1.0	1.4	2.6	-	2.7
		96 h, 1 mg DOC/L	Avoidance	3.1[a]	8.6	1.6	1.2	1.6	3.0	-	3.1
		96 h, 5 mg DOC/L	Avoidance	10.8[a]	29.7	5.4	4.2	5.4	10.4	-	10.8
		96 h, 10 mg DOC/L	Avoidance	12.9[a]	35.6	6.5	5.0	6.5	12.4	-	12.9
		96 h, 20 mg DOC/L	Avoidance	34.7[a]	95.7	17.4	13.5	17.4	33.5	-	34.7
Azizishirazi et al.	Yellow perch	Native fish, med. site, L-alanine	EOG	8.6[b]	3.2	4.3	1.1	3.4	***0.8***	1.2	1.4
2013		Native fish, med. site, TCA	EOG	8.6[b]	3.2	4.3	1.1	3.4	***0.8***	1.2	1.4
		Native fish, high site, L-alanine	EOG	13.7[b]	7.7	6.9	2.4	6.9	2.1	-	13.7
		Native fish, high site, TCA	EOG	13.7[b]	7.7	6.9	2.4	6.9	2.1	-	13.7
		Ref. fish, med. site, L-alanine	EOG	8.6[b]	3.2	4.3	1.1	3.4	***0.8***	1.2	1.4
		Ref. fish, med. site, TCA	EOG	8.6[b]	3.2	4.3	1.1	3.4	***0.8***	1.2	1.4
		Ref. fish, high site, L-alanine	EOG	13.7[b]	7.7	6.9	2.4	6.9	2.1	-	13.7
		Ref. fish, high site, TCA	EOG	13.7[b]	7.7	6.9	2.4	6.9	2.1	-	13.7

[1] Laboratory Cu exposures except for yellow perch (Azizishirazi et al. 2013), which were exposed to Cu in the field (Ni = 32.5-48.2 µg/L; Zn = 1.7-4.2 µg/L).
[a] IC50 values divided by 2 to approximate an IC20; [b] Cu concentrations are associated with significant reduction ($p \leq 0.05$) in mean EOG response relative to reference site.
UK = United Kingdom; IC20 & IC50 = 20% & 50% impairment concentrations; EOG = electro-olfactogram; DOC = dissolved organic carbon; TCA = taurocholic acid.