Sub-lethal metal toxicity concerns for Unuk watershed salmonids from Seabridge Gold's proposed KSM mine

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Executive summary

Heavy metals are common pollutants of aquatic systems, often associated with human activities. Hard-rock mining in particular can mobilize significant quantities of heavy metals to aquatic ecosystems, sometimes with profound ecological harm. Mine tailings are often the most contaminated material at metal mines, and impacts to freshwater fish from heavy metals are thoroughly documented.

While metals naturally leach from rock when they are exposed to weathering processes, human activities can intensify metal leaching by excavating and exposing vast quantities of rock (such as during metal-mining). Metal leaching can be further accelerated by acidic drainage, which occurs when acid-generating rock is exposed to air and/or water. Acid mine drainage is typically produced in tailings ponds and waste rock dumps at metal- and coal-mining sites, and is characterized by acidic water and high metal concentrations. These acidic waters can then dissolve and mobilize more heavy metals as they flow across the landscape and contact other minerals and exposed rock. Acid mine drainage is a major source of water contamination in many mining districts on Earth.

Fish are particularly vulnerable to metals because of sensitive organs that are continuously in contact with the environment, and because metals are highly soluble in water. Most metals can disrupt the essential functions of the fish gill (responsible for gas and ion exchange) and the olfactory system (a fish's sense of smell). Even relatively low concentrations of heavy metals can cause harm to fish. The olfactory system specifically plays an essential role in the survival of fish. While there is a wealth of scientific information describing the concentrations of metals that cause death in freshwater fish, much less reported are the sub-lethal effects (i.e., negative impacts that do not cause direct death) on salmon and trout from low metal concentrations.

Herein is a brief examination and summary of the proposed KSM project's predicted concentrations for 7 heavy metals to be released during ore-extraction operations into the salmon-bearing Sulphurets Creek and Unuk River, including relevant examples of the effect concentrations known to adversely affect salmonids. These metals are: aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn). Of the 7 most thoroughly documented metals and associated impacts to salmon, the following assessment was made:

1. The predicted concentrations of Al in Sulphurets Creek and Unuk River during all phases of mine development, operation, closure, and post-closure will have a negative effect on salmonids if water acidity increases, which it may. Salmonids exposed to the predicted concentrations have shown reduced growth, increased mucous production, and impaired downstream migration.

2. The predicted concentrations (mean and maximum) of Cd at Sulphurets Creek and Unuk River (UR1) during all phases of the project will likely have a negative effect on salmonids. Salmonids exposed to these concentrations have shown disorientation, reduced efficiency at capturing prey, impaired predator avoidance, social disruption, reduced growth and development, impaired biological health, reduced reproductive

functioning, and death. Maximum concentrations predicted in the Unuk River (UR2) located further downstream from the mine sites are also of concern.

3. The predicted concentrations (mean and maximum) of Cu at all sites in Sulphurets Creek and Unuk River during all phases of the project will negatively affect salmonids. Salmonids exposed to these concentrations have shown habitat avoidance, impaired olfaction, migratory disruption, impaired anti-predator response, reduced growth and swim speed, increased stress, impaired reproduction, and death.

4. The predicted maximum concentrations of Pb and Ni in Unuk River (UR1) during all phases of the project may adversely affect salmonids. Salmonids exposed to these metals have shown avoidance behaviour and impaired development.

5. The predicted maximum concentrations of Ag and Zn at all sites in Sulphurets Creek and Unuk River during all phases of the project may have a negative effect on salmonids. Salmonids exposed to these metals have shown habitat avoidance, physiological stress, and impaired development.

6. The concentrations of mixed-metals predicted during all phases of mine development may cause habitat avoidance behaviour in salmonids residing at all sites in Sulphurets Creek and Unuk River, and may cause death in fish from Sulphurets Creek.

Despite such documentations of sub-lethal effects on salmonids, there remains a high level of uncertainty as to what effect concentration for each metal will cause undo harm to fish. Specifically, there are at least four limitations when applying the reported effect concentrations to real-life scenarios: i) the effect concentrations reported in this review are most often the lowest *detected effect*, not the actual *lowest effect concentration*, ii) scientific studies rarely reflect natural exposure conditions, iii) laboratory studies tend to examine metals in isolation, which may not be environmentally realistic or relevant for assessing actual impacts on fish, and iv) dietary metal concentrations are not considered despite the likely simultaneous occurrence of both waterborne and dietary routes of metal toxicity.

Importantly, there remains a paucity of information for 23 other metal and non-metal contaminants to be released from the proposed project, and the associated concentrations that might adversely affect salmonids. Such inadequate information makes it impossible to reasonably assess the full potential adverse impacts on salmonids. Further research is thus required not only to predict the potential downstream flow of metal and non-metal contaminants into the Unuk River, but to adequately assess harm.

This review assumes that all predicted metal values reported by the proponent are accurate, and are the concentrations that will occur as a result of the proposed project. However, the accuracy of such predictions will be assessed in a follow-up document.

Introduction

Heavy metals are widely occurring pollutants commonly associated with human activities. Hard-rock mining in particular has mobilized significant quantities of heavy metals to aquatic ecosystems (Boyd 2010; Tierney et al. 2010), sometimes with profound ecological harm (Downs and Stocks 1977; Balistrieri et al. 2002). Different types of mine-waste have vastly different contaminant concentrations. Mine tailings are often the most contaminated material at metal mines. As ore is separated by milling and flotation, commonly 90% of it is discarded as tailings, and some of the world's largest toxicant deposits, and primary contaminant sources associated with metal extraction, occur in tailings ponds (Moore and Luoma 1990). Impacts to freshwater fish from heavy metals are thoroughly documented (Woody et al. 2010; Dennis and Clair 2012).

Heavy metals enter aquatic systems through natural weathering and leaching processes, which can be greatly accelerated by humans. Metals naturally leach from rock when they are exposed to air and/or water, and the resulting chemical reactions mobilize them into biologically available forms (Wilkin 2007). However, human activities often intensify this process through the excavation of vast quantities of rock from mineral deposits (such as during metal- and coal-mining), and the subsequent exposure of that material to weathering processes (Kelly 1988; Moore and Luoma 1990; Hogsden and Harding 2012). The leaching process can be further accelerated by acidic drainage, which occurs when sulphide minerals previously "locked" in rock are exposed to air and water and naturally oxidize without the presence of sufficient quantities of neutralizing minerals (Wilkin 2007). Acid mine drainage is typically produced in tailings ponds and waste rock dumps at mining sites, and is characterized by acidic water (low pH) and high concentration of dissolved metals. Bacteria contribute to metal leaching by catalyzing the reactions and speeding-up the rate in which water becomes acidified. These acidic waters can then dissolve and mobilize more heavy metals as they flow across the landscape and contact other minerals and exposed rock. Acid mine drainage is a major source of water contamination in many mining districts on Earth (Kelly 1988; Hogsden and Harding 2012).

Fish are extremely vulnerable to metal toxicants because of sensitive organs that are continuously in contact with the environment, and because metals are highly soluble in water. For example, the fish gill is a sophisticated, yet delicate, organ with multiple physiological functions that range from gas exchange to excretion of nitrogenous waste (Hogstrand and Wood 1998). High concentrations of most metals can disrupt these functions, damage the gill structurally, and cause suffocation and death (Mallat 1985). Even relatively low concentrations of heavy metals can fatally impair physiological functions (such as the regulation of ions; ionoregulation) of the gill (Wood 1992). The olfactory organ, and its associated nerve cells, is also directly exposed to the environment and thus highly susceptible to damage by metal toxicants in water. Heavy metals can interfere with a fish's sense of smell (chemoreception) by blocking the effects stimulated by natural odorants or by directly damaging the receptor sites (Hara et al. 1983; Klaprat et al. 1988). Olfaction plays an essential role in the survival of fish, initiating behaviours such as food gathering, predator avoidance, schooling, defense, navigation between ocean and freshwater habitats, and reproduction, and low concentrations of heavy metals can alter such behaviours and reduce survival (Sandahl et al. 2007; Tierney et al. 2010; McIntyre et al. 2012).

Seabridge Gold has proposed to build a metal mine and associated tailings impoundment in northwest British Columbia. The metal-mine sites, specifically, are predicted to release numerous heavy metals, non-metals, and other contaminants into the Unuk River, an important salmon-bearing system. The following is a state-of-knowledge report for 7 heavy metals predicted to be elevated in Sulphurets Creek and Unuk River as a result of ore extraction. Included are relevant examples of the concentrations known to adversely affect salmonids for each metal examined. These metals were selected because they are the most commonly described with regards to impacts on salmonids. Notably, 23 other metals and non-metals that are proposed to be discharged from the mine site into receiving streams are not examined in this review because of a paucity of data.

All predicted concentrations are derived from Seabridge Gold's application for an environmental assessment certificate (SGA 2013). Specifically, all reported predicted values are from Chapter 14, Tables 14.7-27 through to 14.7-38 (Scenario 4: upper case) for the following receiving sites: Sulphurets Creek (SC3), and Unuk River (UR1 and UR2; Figure 1). These predicted concentrations can be compared to background levels recorded at multiple sites within the Sulphurets Creek, and Unuk River during 2007-2012 (Table 1). Background surface water quality data are from Chapter 14, Table 14.1-1.

While this review assumes that all predicted metal values reported by the proponent are accurate (and are the concentrations that will occur as a result of the proposed project), several fundamental weaknesses with the data incorporated into the predictive simulations challenge the validity of the predicted results. Such weaknesses will be described in a follow-up document.

Table 1. Background mean, and 95th percentiles (in brackets), dissolved heavy metal concentrations recorded within the Sulphurets Creek (4 sites), and Unuk River (9 sites; see Figure 1) during May to October, 2007-2012. Metals include: aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn); concentrations are in μg/L.

Location	Al	Cd	Cu	Pb	Ni	Ag	Zn
Sulphurets	50.2 (79.6)	0.65 (2.5)	7.54 (10.7)	0.037 (0.098)	1.03 (2.52)	0.005 (0.005)	30.1 (125.0)
Unuk	57.0 (130.0)	0.191 (0.463)	2.64 (4.49)	0.073 (0.2)	1.95 (1.33)	9.62 (0.005)	4.9 (19.1)

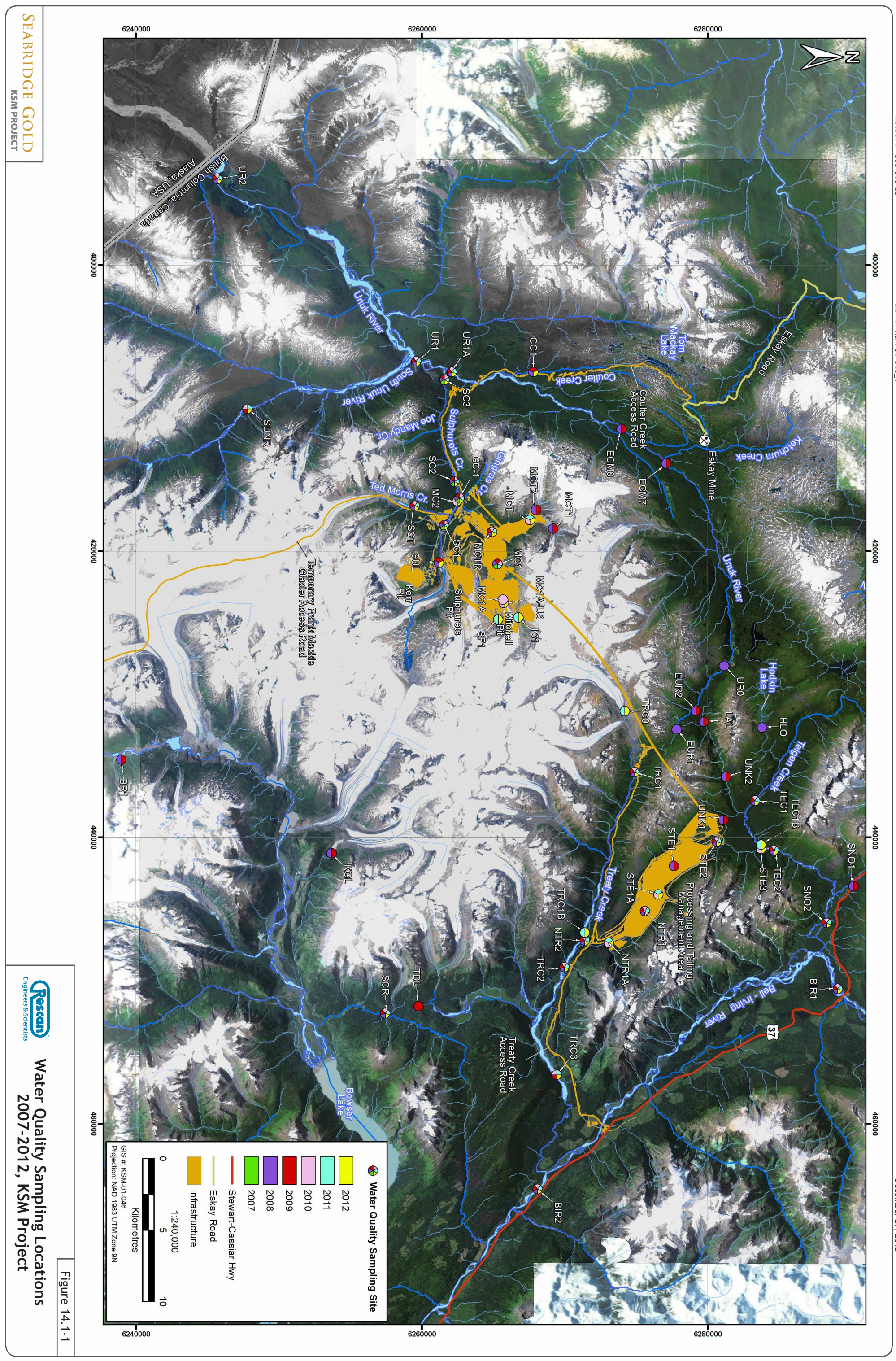

Figure 1. Water quality sampling locations for the proposed KSM project recorded during 2007-2012. Map from Rescan for Seabridge Gold's 2013 application for an environmental assessment certificate.

Metals and predicted concentrations

Aluminum (Al)

The predicted concentrations (mean and maximum) of Al in Sulphurets Creek (SC3), and Unuk River (UR1 and UR2; see Figure 1) during all phases of mine contruction, operation, closure, and post-closure have been shown to adversely affect salmonids sub-lethally (Table 2). However, the reported effects in the literature are all dependent on low pH (i.e., pH ~5; Kroglund and Finstad 2003; Kroglund et al. 2008). Unfortunately, there appears to be an ommission of predictions for the surface water pH of Sulphurets Creek, and Unuk River during and after mine development. Given that there will be a large volume of acid generating material exposed during mine development and operation, (and unless the pH of surface water quality predictions are made for Sulphurets Creek and Unuk River that suggest otherwise), the predicted Al concentrations have the potential to affect salmonids in Sulphurets Creek and Unuk River during the life of the project.

Relevant examples include:

1. Sub-lethal levels of Al can affect the feeding behavior, growth, and swim speed of salmonids. For example, reduced growth rates have been observed in juvenile brown trout exposed to total Al concentrations greater than 27 μg/L in waters with pH below 5.5 (Sandler and Lynam 1987). Juvenile rainbow trout exposed to 30.0 μg/L total Al in waters of 5.2 pH showed a 30% reduction in the maximum sustainable swimming speed within 7 days, and these effects were roughly two times greater than for fish exposed only to low pH (i.e., 5.2; Wilson and Wood 1992). In a separate study, juvenile rainbow trout pre-exposed to 38 μg/L total Al at 5.2-5.4 pH for 36 days suffered impaired swim speed, and the maximum swim speed remained depressed even when fish were subsequently placed in waters with pH of 6.5 and 0 μg/L total Al (Wilson et al. 1994).

2. Aluminum can accumulate rapidly on the gill lamellae surface of juvenile rainbow trout, and may gradually penetrate within the gill cells themselves over time (Wilson and Wood 1992). Juvenile rainbow trout exposed to 38 μg/L Al at pH 5.2 for 5 days showed a five-fold increase in the number of mucous cells present in the filamental epithelium compared to fish exposed to 0 μg/L Al in waters with pH 5.2 and 6.5 (Wilson et al. 1994). After 34 days exposure to 38 μg/L Al at pH 5.2, juvenile rainbow trout showed a four-fold increase in mucous cells compared to unexposed fish (Wilson et al. 1994), suggesting that fish do not acclimate to Al toxicity. Gill hyperplasia, which is an abnormal increase in cell numbers that can lead to respiratory impairment, may result from Al toxicity. At minimum, low Al concentrations, especially in waters with pH between 5.0 and 5.6, will cause fitness degradation, and reduce the ability of salmonids to adequately deal with other stressors, such as smoltification (Dennis and Clair 2012).

3. Exposure to low levels to Al during long-term and episodic (single or re-occurring episodes lasting several days) events may disrupt the downstream migration of juvenile salmonids and reduce survival in seawater. Several studies have reported that non-lethal Al concentrations can compromise the ability of juvenile Atlantic salmon to balance body fluids (osmoregulation) during smoltification (Staurnes et al. 1995; Magee et al. 2001, 2003; Kroglund et al. 2007). Juvenile Atlantic salmon exposed for three months to 6 (+/-

2) μg/L Al showed a 20-30% reduction in survival compared to control fish (Kroglund and Finstad 2003). Juvenile Atlantic salmon exposed to 28-64 μg/L inorganic Al for 2 to 5 days in acidic water (pH 5.4-6.3) also showed reduced seawater tolerance compared to control fish (Monette et al. 2008). Concentrations of inorganic Al of 5-10 μg/L is predicted to cause a 25%–50% reduction in the survival of Atlantic salmon when smolts are exposed for 3 days during seaward migration (Kroglund et al. 2008).

4. Aluminum may cause physical alteration in the olfactory epithelium of salmonids and influence the electrical properties of olfactory sensory neurons. For example, juvenile rainbow trout exposed to 9.5 μg/L Al in acidic water (pH 4.7) for 2 weeks resulted in loss of receptor cell cilia, anatomically altered olfactory knobs, and clumped microvilli compared to control fish, and showed reduced olfactory nerve responses compared to fish only exposed to acidic water (Klaprat et al. 1988).

Table 2. The proponent's "Scenario 4: upper case" predicted mean, and maximum (in brackets), total metal concentrations for Sulphurets Creek (SC3) and Unuk River (UR1 and UR2; Figure 1) during the life of the project. Bold font represents concentrations known to adversely affect salmonids. Metals include: aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), silver (Ag), and zinc (Zn); concentrations are in μg/L.

Location	Al	Cd	Cu	Pb	Ni	Ag	Zn
SC3[a]	**1,710 (8,030)**	**0.410 (1.27)**	**30.0 (130.0)**	2.0 (7.0)	3.0 (8.0)	0.05 **(0.226)**	**34.0 (102.0)**
SC3[b]	**1,690 (7,060)**	**0.411 (1.22)**	**31.5 (120.7)**	1.7 (6.23)	3.0 (8.0)	0.05 **(0.2)**	**33.8 (97.2)**
SC3[c]	**1,890 (7,840)**	**0.439 (1.34)**	**35.2 (134.5)**	1.88 (6.89)	3.0 (8.0)	0.054 **(0.221)**	**36.7 (107.6)**
SC3[d]	**1,730 (7,840)**	**0.416 (1.34)**	**32.1 (134.6)**	1.73 (6.89)	3.0 (8.0)	0.05 **(0.221)**	**34.2 (107.7)**
UR1[a]	**1,810 (12,710)**	0.0 **(1.0)**	**20.0 (80.0)**	1.62 **(8.71)**	3.0 **(18.0)**	0.043 **(0.290)**	**20.0 (60.0)**
UR1[b]	**1,790 (11,880)**	**0.231 (0.725)**	**21.7 (83.6)**	1.6 **(8.14)**	3.0 **(17.0)**	0.043 **(0.272)**	**22.3 (60.6)**
UR1[c]	**1,890 (12,560)**	**0.234 (0.736)**	**22.7 (85.9)**	1.68 **(8.59)**	3.0 **(18.0)**	0.045 **(0.287)**	**23.0 (63.9)**
UR1[d]	**1,820 (12,560)**	**0.234 (0.736)**	**22.0 (85.8)**	1.62 **(8.6)**	3.0 **(18.0)**	0.043 **(0.287)**	**22.6 (64.0)**
UR2[a]	**1,440 (6,580)**	0.061 **(0.231)**	**7.6 (31.3)**	1.23 (5.41)	2.0 (7.9)	0.025 **(0.108)**	**9.0 (28.0)**
UR2[b]	**1,440 (6,330)**	0.06 **(0.23)**	**7.6 (31.0)**	1.23 (5.2)	2.0 (7.7)	0.024 **(0.104)**	**9.0 (28.0)**
UR2[c]	**1,480 (6,530)**	0.059 **(0.231)**	**7.8 (31.5)**	1.26 (5.36)	2.0 (7.9)	0.025 **(0.107)**	**9.0 (28.0)**
UR2[d]	**1,460 (6,530)**	0.061 **(0.230)**	**7.7 (31.5)**	1.25 (5.36)	2.0 (7.9)	0.025 **(0.107)**	**9.0 (28.0)**

[a]Construction phase
[b]Operational phase
[c]Closure phase
[d]Post-closure phase

Cadmium (Cd)

The predicted concentrations (mean and maximum) of Cd in Sulphurets Creek (SC3) and Unuk River (UR1 and UR2) during all phases of the project will likely have a negative effect on salmonids. Cadmium concentrations are predicted to average ~ 0.41 µg/L at Sulphurets Creek, with maximum concentrations estimated to be ~ 1.2 µg/L, and will average ~ 0.23 µg/L at Unuk River (UR1), with maximum concentrations estimated to be ~ 0.73 µg/L (Table 2). Salmonids exposed to these concentrations have shown disorientation, reduced efficiency at capturing prey, impaired predator avoidance, social disruption, reduced growth and development, impaired biological health, reduced reproductive functioning, and death. Maximum concentrations (~ 0.23 µg/L) predicted in the Unuk River (UR2) located further downstream from the mine sites are also of concern.

Relevant examples include:

1. Salmonids can detect and respond to Cd. Lake whitefish demonstrated disoriented behaviour of both attraction and avoidance to the pollutant when exposed to 0.2 µg/L Cd (McNicol and Scherer 1991).

2. Foraging behaviour can be a sensitive indicator of metal toxicant stress on fish (Atchison et al. 1987; Little et al. 1990; Scherer et al. 1992). Several authors have reported a significant relationship between chronic sub-lethal Cd toxicity and reduced predation success in salmonids. For example, adult lake trout showed a significant reduction in the number of captured and consumed prey when exposed to 0.5 µg/L Cd for 106-112 days (Kislalioglu et al. 1996). A similar study reported that adult lake trout exposed to 0.5 µg/L Cd for 9 months showed reduced predation success compared to control fish when presented with unexposed rainbow trout prey, and foraging success decreased with increasing Cd concentration (Scherer et al. 1997). Adult lake trout that were not exposed to Cd showed the highest predation success when presented with juvenile rainbow trout that had been previously exposed to 0.5 µg/L Cd for 9 months, though the results were not significantly different from controls. Finally, research by Riddell et al. (2005a) showed that exposure of juvenile brook trout to 0.5 µg/L Cd for 30 days reduced their capture efficiency of prey by 20%, yet the activity of Cd-exposed fish increased by 25% compared to unexposed fish.

3. The social behaviour of individual fish, and dominance hierarchies within populations, can be altered by low levels of Cd. Juvenile rainbow trout showed a decreased tendency to become dominant within a social hierarchy when exposed to 0.8 µg/L Cd for 24 hours (Sloman et al. 2003).

4. Cadmium exposure can reduce the growth and development of salmonids. For example, Atlantic salmon alevins showed a significant reduction in growth when exposed to 0.47 µg/L Cd, and the results further indicated that these fish had a lower growth response threshold around 0.13 µg/L Cd (meaning that growth reduction likely begins to occur when fish are exposed to as little as 0.13 µg/L Cd; Rombough and Garside 1982). Additionally, rainbow trout alevins showed impaired growth when exposed to 0.25 µg/L Cd for 56 days (Lizardo-Daudt and Kennedy 2008). Finally, juvenile bull trout showed a

28% reduction in weight change when exposed to 0.79 µg/L Cd for 56 days (Hansen et al. 2002a).

5. Cadmium can reduce the biological performance of salmonids. Juvenile brown trout exposed to 0.87 µg/L Cd, and Atlantic salmon alevins exposed to 1.0 µg/L Cd, showed a 20% and 38% reduction in biomass of the test population, respectively (Rombough and Garside 1982; Brinkman and Hansen 2007). Juvenile brook trout showed significantly poorer biological health, as measured by condition factor, when exposed to 0.5 µg/L Cd for 30 days, and their condition declined by 12-18% over the 30-day period, whereas the condition of unexposed fish increased by 34% (Riddell et al. 2005a).

6. Cadmium exposure can negatively affect the reproductive functioning in salmonids. Rainbow trout eggs exposed to 0.05 µg/L Cd have been shown to hatch prematurely compared to unexposed eggs (Lizardo-Daudt and Kennedy 2008).

7. Cadmium can elevate stress in salmonids. Juvenile rainbow trout exposed to 1 µg/L Cd for 2 days showed elevated plasma cortisol levels compared to control fish, and a similar response was observed after 30 days (Brodeur et al. 1998; Ricard et al. 1998). Cortisol production is a general adaptation response of fish to stress (Brodeur et al. 1998).

8. The lowest concentration found to cause death in 50% of fish (LC50) for bull trout ranged from 0.83 to 0.88 µg/L Cd (Hansen et al. 2002b).

Copper (Cu)
The predicted concentrations (mean and maximum) of Cu in Sulphurets Creek (SC3) and Unuk River (UR1 and UR2) during all phases of the project will negatively affect salmonids. Copper concentrations are predicted to average > 30 µg/L at Sulphurets Creek, with maximum concentrations estimated to be > 120 µg/L (Table 2). At Unuk River, average Cu concentrations will be > 20 µg/L and > 7 µg/L at UR1 and UR2, respectively; maximum concentrations will be > 80 µg/L and > 31 µg/L at UR1 and UR2, respectively. Salmonids exposed to these concentrations have shown habitat avoidance, impaired olfaction, migratory disruption, impaired anti-predator response, reduced growth and swim speed, increased stress, impaired reproduction, and death.

Relevant examples include:
1. Where distinct Cu gradients are present (e.g., near a point-source discharge), salmonids may use their sense of smell to detect and avoid contaminated waters. Several studies have reported that juvenile salmonids rearing in freshwater avoid Cu concentrations ranging from 0.7 µg/L to 7.3 µg/L (Sprague et al. 1965; Giattina et al. 1982; Hansen et al. 1999a; Svecevicius 2007), with Chinook salmon, rainbow trout, and Atlantic salmon, all displaying avoidance behavior in waters with Cu concentrations ≤ 2.4 µg/L. A recent study estimated that Cu concentrations as low as 0.84 µg/L for rainbow trout and 0.91 µg/L for Chinook salmon produced an avoidance response in 20% of the population (Meyer and Adams 2010). An avoidance response to Cu-contaminated water may ensure that fish select favorable habitat conditions for survival, but also indicates that fish habitat is lost when contaminated (Saucier et al. 1991; Baldwin et al. 2003).

2. Long-term sub-lethal Cu exposure may also impair a fish's avoidance response to higher Cu concentrations. For example, juvenile Chinook salmon exposed to 2 μg/L Cu for 25 to 30 days showed no preference for clean water versus contaminated water, and failed to avoid waters with Cu concentrations higher than 2 μg/L, including a failure to avoid Cu-contaminated water of 21 μg/L (Hansen et al. 1999a). Prior to acclimation to 2 μg/L Cu, Chinook salmon consistently avoided waters up to 21 μg/L Cu (Hansen et al. 1999a). The failure to avoid higher Cu concentrations suggests that the sensory mechanism responsible for avoidance responses was impaired by the long-term sub-lethal concentration of 2 μg/L Cu, which could result in further impairment of sensory-dependent behaviors essential for survival, or result in mortality if fish are later exposed to higher concentrations.

3. Copper exposure may delay the upstream migration of salmonids to spawning habitat, and induce downstream movement by adults away from spawning grounds. For example, the upstream spawning migration of Atlantic salmon can be interrupted by Cu concentrations of 20 μg/L (Sprague et al. 1965; Sutterlin and Gray 1973), with reverse downstream migrations occurring whenever Cu concentrations exceeded 16.8 μg/L to 20.6 μg/L (Sprague et al. 1965; Saunders and Sprague 1967; Hecht et al. 2007). There is also observational evidence that the spawning migration of Chinook salmon may be interrupted at Cu concentrations between 10 μg/L and 25 μg/L (Hecht et al. 2007).

4. Copper exposure can disrupt the downstream migration of juvenile salmonids and reduce survival in seawater. For example, coho salmon smolts exposed to ≥5 μg/L Cu exhibited delayed downstream migration to the ocean, and reduced seawater survival, compared to unexposed control fish (Lorz and McPherson 1976). Migration success for juveniles decreased more with higher Cu concentrations and increasing exposure time. A 76% reduction in downstream migration success over a distance of 6.4 km was observed for juvenile coho exposed to 20 μg/L Cu for 144 hours followed by transfer to seawater, compared to control fish (Lorz and McPherson 1976). Finally, juvenile coho exposed to 15 μg/L Cu for 7 days in freshwater followed by transfer to seawater resulted in 40% mortality compared to 100% survival of unexposed fish (Schreck and Lorz 1978).

5. Copper exposure can cause a loss in sensory capacity for salmonids, and interfere with a fish's ability to detect and respond to chemical signals. Juvenile salmon in natural environments typically alter their behaviour when alerted by the smell of predators to avoid being captured; studies show that low levels of Cu can disrupt this anti-predator response. For example, exposure of 5 μg/L Cu impaired the normal response of juvenile coho to odorants within minutes (Baldwin et al. 2003). Similar impairment of a fish's sense of smell has been reported for juvenile steelhead trout exposed to 5 μg/L Cu for 3 hours (Baldwin et al. 2011), juvenile chum salmon exposed to 3 μg/L Cu for 4 hours (Sandahl et al. 2006), and juvenile coho exposed to 3.6 μg/L for 7 days (Sandahl et al. 2004). When the chemical odor is conspecific skin extract (i.e., a predator), unexposed fish reduce their swimming speed on average by 75% as an anti-predator response. However, juvenile coho exposed to 2.0 μg/L Cu for 3 hours and then presented with a predator's scent showed significant impairment of the predator avoidance behaviour;

fewer fish became motionless compared to pre-exposure (Sandahl et al. 2007). In a separate study, upstream predator cues presented to juvenile coho previously exposed to 5.0 µg/L Cu for 3 hours did not elicit an alarm response in contrast to control fish (McIntyre et al. 2012). Importantly, Cu-exposed juvenile coho were more vulnerable to predation by cutthroat trout, as measured by attack latency, survival time, and capture success rate; and, pre-exposing predators to similar Cu concentrations did not improve the evasion success of coho prey (McIntyre et al. 2012).

6. Copper exposure can alter swimming and feeding behaviour. Rainbow trout fry exposed to 9.0 µg/L Cu showed a 10% reduction in critical swim speed, and the same effect was observed with juvenile rainbow trout exposed to 5.0 µg/L Cu in low pH water (Waiwood and Beamish 1978a, b). Juvenile brook trout exposed to 6 µg/L Cu showed a three-fold increase in swimming activity within minutes compared to pre-exposure activity levels (Drummond et al. 1973). However, the increase in activity did not equate to an increase in feeding behaviour, as these same fish showed a 40% reduction in foraging after 2 hours of exposure to 6 µg/L Cu (Drummond et al. 1973). The concomitant decrease in feeding behaviour with increased activity may best be explained by the need of fish to increase water flow across the gills for oxygen diffusion due to suffocation from gill damage and/or clogging of the lamellae with mucus, which is a direct effect of Cu toxicity (Scarfe et al. 1982).

7. Several authors have reported reduced growth rates in Cu-exposed fish. For example, juvenile brook trout exposed to 3.4 µg/L Cu for 1-23 weeks showed a reduction in growth by 15-25% compared to control fish (McKim and Benoit 1971). Rainbow trout fry exposed to 4.6 µg/L Cu for 20 days experienced significantly reduced growth during the same period, and a 40-day exposure to 9.0 µg/L Cu resulted in a 45% reduction in mean body mass relative to control fish (Marr et al. 1996). A reduction in growth by 20% relative to control fish occurred in juvenile rainbow trout exposed to 4.0 µg/L Cu, and the same effect was observed in fish exposed to 2.0 µg/L Cu in low pH water (Waiwood and Beamish 1978a).

8. Copper exposure can induce stress in fish, suppress resistance to pathogens, and increase susceptibility to secondary stressors. Brook trout fry exposed to 6 µg/L Cu for 5-20 hours showed increased cough frequencies, which is indicative of stress (Drummond et al. 1973). Juvenile coho salmon exposed to 18.2 µg/L for 30 days showed significantly reduced immune response to *Vibrio anguillarum*, the etiological agent of the fish disease known as vibriosis (Stevens 1977). Finally, coho fry exposed to 13.8 µg/L Cu for 7 days showed reduced survival after handling and confinement (Schreck and Lorz 1978), an indication that Cu exposure may increase the vulnerability of salmonids to secondary stressors such as disease and predator pursuits.

9. Copper exposure can disrupt the reproductive performance and spawning behaviour of exposed fish. For example, adult brown trout exposed to 10 µg/L Cu for 4 days and then presented with female pheromones produced significantly less milt than control fish, and control fish demonstrated more pre-spawning behaviours than exposed fish (Jaensson and Olsen 2010).

10. Acute lethality in salmonids can occur at Cu concentrations that range 9-17 µg/L for juvenile rainbow trout (Chapman 1978a; Marr et al. 1999) to 103-240 µg/L for juvenile sockeye salmon (Davis and Shand 1978).

Lead (Pb)
The predicted maximum concentrations of Pb in Unuk River (UR1) during all phases of the project may adversely affect salmonids. Maximum Pb concentrations are predicted to be > 8 µg/L at this location (Table 2), and salmonids exposed to these concentrations have shown impaired development.

Relevant examples include:
1. Salmonids exposed to lead can develop physical abnormalities. For example, juvenile rainbow trout exposed for 6 weeks during the eyed-egg stage to 7.6 µg/L Pb in soft water developed blacktail abnormalities (Davies et al. 1976).

Nickel (Ni)
The predicted maximum concentrations of Ni in Unuk River (UR1) during all phases of the project may have a negative effect on salmonids. Maximum Ni concentrations are predicted to be > 17 µg/L at this location (Table 2), and salmonids exposed to these concentrations have shown avoidance behaviour.

Specific relevant examples include:
1. Salmonids respond to Ni in different ways at sub-lethal concentrations. At 6 µg/L total Ni (unknown dissolved concentration), juvenile rainbow trout showed a 40% increase in time spent in the area of the experimental tank with toxicant water compared to control fish; yet these same fish detected and avoided the toxicant water when total Ni concentrations reached 10-19 µg/L (Giattina et al. 1982). The concentration that caused a 50% reduction in the amount of time fish spent in an area relative to control times was estimated at 23.9 µg/L total Ni (unknown dissolved concentration; Giattina et al. 1982).

Silver (Ag)
The predicted maximum concentrations of Ag in Sulphurets Creek (SC3) and Unuk River (UR1 and UR2) during all phases of the project may have a negative effect on salmonids. Maximum Ag concentrations are predicted to be > 0.2 µg/L at Sulphurets Creek, and > 0.27 µg/L and > 0.1 at Unuk River sites UR1 and UR2, respectively (Table 2), and salmonids exposed to these concentrations have shown impaired development.

Specific relevant examples include:
1. Silver exposure can alter feeding behaviour, growth, and swim speed. For example, juvenile rainbow trout exposed to 0.1 µg/L and 0.17 µg/L Ag were significantly smaller (in mean length and weight) than unexposed fish after 60 days (Davies et al. 1978; Nebeker et al. 1983). The maximum acceptable toxicant concentration based on the lowest significant effect level for these fish was estimated to be < 0.1 µg/L Ag (Nebeker et al. 1983).

2. Rainbow trout eggs exposed to 0.17 µg/L Ag hatched prematurely, and the resulting

sac-fry showed delayed development (Davies et al. 1978).

Zinc (Zn)

The predicted concentrations (mean and maximum) of Zn in Sulphurets Creek (SC3) and Unuk River (UR1 and UR2) during all phases of the project will likely adversely affect salmonids. Mean Zn concentrations are predicted to average > 34 µg/L at Sulphurets Creek, with maximum concentrations predicted to be > 97 µg/L; mean Zn concentrations are predicted to be > 20 µg/L and 9 µg/L at Unuk River sites UR1 and UR2, respectively, with maximum concentrations of > 60 µg/L and 28 µg/L at sites UR1 and UR2. Salmonids exposed to these concentrations have shown habitat avoidance and physiological stress.

Specific relevant examples include:

1. Zinc exposure may induce avoidance of rearing habitat for salmonids. Estimates of threshold concentrations for avoidance (i.e., the lowest concentration that causes at least 50% of fish to show significant avoidance) of juvenile rainbow trout to Zn are reported to be 8.6 µg/L (95% confidence limits range 7.3-10.3 µg/L; Sprague 1968). Importantly, a decrease in water temperature raised the avoidance threshold for fish. For example, the threshold avoidance for juvenile rainbow trout exposed to 17°C water was 7.3 µg/L, whereas exposure to 9.5°C resulted in an estimated threshold avoidance of 8.4 µg/L; though the differences were not statistically significant (Sprague 1968). Juvenile Atlantic salmon exposed under similar laboratory conditions also showed avoidance to Zn, with an estimated threshold concentration of 53 µg/L (range = 27-104 µg/L; Sprague 1964). The difference in avoidance thresholds between the two species is thought to be the result of differences in behaviour characteristics (i.e., while Atlantic salmon tend to be less mobile, rainbow trout are active swimmers that may become more aware of toxicant gradients), rather than a difference in sensory perception (Sprague 1968).

2. Sub-lethal exposure of salmonids to waterborne Zn can induce physiological stress and reduce immune responses. For example, juvenile rainbow trout exposed to 81 µg/L Zn for 1 day showed significantly higher plasma glucose levels compared to control fish, and the rise in glucose was attributed to, and a sign of, stress (Wagner and McKeown 1982). In a separate study of juvenile rainbow trout, fish exposed to 10 µg/L Zn for 30 days showed significantly inhibited immune response compared to control fish (Sanchez-Dardon et al. 1999).

Metal Mixtures

One complicating factor in an assessment of the toxicity potential for any particular heavy metal (or non-metal for that matter) is that, unlike laboratory studies that often examine metals in isolation, multiple contaminants typically occur and interact in aquatic systems (Boyd 2010). Toxicological studies that focus on the effects of single metals may not be environmentally realistic or relevant for assessing actual impacts on fish. Combinations of heavy metals may behave in three ways: additively (one metal acts independently from another, and the toxic effect of each metal in combination is the same as the effect of the individual metals), synergistically (different metals interact, and the toxic effect of the combined metals is greater than the additive effects of the individual metals), or antagonistically (different metals interact, but the toxic effect of the combined metals is less than the additive effects of the individual metals; Boyd 2010). The synergistic behaviour of metals in combination is of most concern for the health of salmonids, and there are three specific examples where fish in receiving systems will likely be affected by the predicted mixed-metal concentrations.

Relevant examples include:
1. The lowest concentration found to cause death in 50% of fish (LC50) for bull trout ranged from 0.83 to 0.88 µg/L Cd when exposed only to Cd, whereas the LC50 in a mixture with Zn was 0.51 µg/L Cd (Hansen et al. 2002b). Considering that Cd and Zn are predicted to occur together in receiving environments (particularly at sites SC3 and UR1), fish will likely be more impacted than if exposed to Cd alone.

2. A study that examined a mixture of Cu and Zn reported that the combination of the two metals reduced the avoidance threshold of fish by an order of magnitude below that for each metal tested individually. In combination, 0.4 µg/L Cu and 6.1 µg/L Zn produced an avoidance reaction in juvenile Atlantic salmon, whereas the individual thresholds were 2.3 µg/L Cu, and 53 µg/L Zn (Sprague 1964). Thus, fish at all sites in Sulphurets Creek and Unuk River will likely exhibit avoidance behaviour given the predicted concentrations.

3. The threshold of avoidance for juvenile rainbow trout exposed to metal mixtures has been estimated at 1.2 µg/L Cu, 0.11 µg/L Cd, 0.32 µg/L Pb, and 5 µg/L Zn (Hansen et al. 1999b), which is less than the single-metal avoidance concentrations for Cu and Zn, and may indicate that fish at all sites in Sulphurets Creek and Unuk River will be affected given the predicted concentrations.

Discussion

The extent of impact on salmon populations from sub-lethal metal toxicity is impossible to quantify due to the deficiency in data for most metals and non-metals that will be released by the proposed project into salmon-bearing streams. Of that which we know (i.e., of the most thoroughly researched metals), metals such as Cu will cause loss of olfaction and secondary death at the average concentrations predicted at all sites in Sulphurets Creek and Unuk River. Fish may not directly die when exposed to these concentrations (although there is evidence to suggest that juveniles may die of acute toxicity (Chapman 1978a; Davis and Shand 1978; Marr et al. 1999), but rather will eventually die as a result of increased susceptibility to predation. What cannot be assessed is whether such secondary death will directly equate to population-level declines. However, combined with all of the stressors currently facing salmonids in compromised habitats, such effects will undoubtedly reduce the abundance of returning adults. Add these known toxicity effects onto the numerous unknown but potential effects from metal-mixtures (and other metal and non-metal contaminants proposed to be generated), and the health of salmonids will undoubtedly be compromised. Furthermore, these potential effects must be placed within the context of a warming planet, increasing stream temperatures, reduced ocean productivity, degraded spawning and rearing habitat, and the cumulative effects of all human activities.

Effect concentrations

Despite the numerous documentations of sub-lethal effects on salmonids from the 7 reported metals, there remains a high level of uncertainty as to what exactly the lowest effect concentration is for each metal that will cause undo harm to fish. Specifically, there are at least four limitations when applying the reported effect concentrations for the 7 heavy metals to real-life scenarios. First, the effect concentrations reported are most often the lowest *detected effect*, not the actual *lowest effect concentration*. While the lowest *detected effect* describes the lowest concentration of a metal that was tested and found to cause an effect on fish, the *lowest effect concentration* is the actual lowest concentration of a metal that can cause a detectable effect on fish. For example, Sandahl et al. (2007) showed that juvenile coho exposed to > 2 µg/L Cu for 3 hours exhibited a suppression in predator avoidance behaviour (a lowest *detected effect*); yet, concentrations below 2 µg/L were not tested. Thus, uncertainty remains as to the precise threshold for olfactory impairment. This is also true for the olfactory impairment of juvenile rainbow trout (Baldwin et al. 2011), and juvenile chum salmon (Sandahl et al. 2006). No such studies have been performed for Chinook or sockeye salmon. Further research is needed to determine threshold concentrations for all metals on salmonids.

Second, scientific studies rarely reflect natural exposure conditions. Most of the studies reported in this review were performed in a laboratory, where conditions for fish are near optimal. Parameters such as water flow, temperature, and food all tend to be favorable for fish and constant throughout the experimental period (Pyle and Merza 2007). However, fish in natural settings are typically forced to cope with sub-optimal conditions, and are frequently exposed to multiple stressors (Hecht et al. 2007); these added stressors may or may not alter the toxic effects of heavy metals. Not only can the chemical properties of water influence the availability of toxicants (Newman and Unger 2003), but the nutritional status of fish may influence the uptake of toxicants from the environment (Holmstrup et al. 2010). Thus, the measured toxicity of a particular metal at a given concentration in the laboratory may be less than for fish in contaminated waters.

Third, laboratory studies tend to examine metals in isolation, which may not be environmentally realistic or relevant for assessing actual impacts on fish. This is because fish are more often exposed to an assortment of metals, as well as organic chemical pollutants, in contaminated aquatic systems (Boyd 2010). Of the three ways that metals can behave (antagonistically, additively, or synergistically) when combined in a mixture, the greatest concern for fish is one of synergy. There are examples of mixtures of Cd/Zn, Cd/Pb, Cd/Cu/Pb/Zn, Cu/Al, Cu/Fe, and Cu/Zn with resulting effects on bull trout, rainbow trout, brown trout, and Atlantic salmon that were more than additive (Sprague 1964; Sprague and Ramsay 1965; Sayer et al. 1991; Hansen et al. 1999c; Birceanu et al. 2008). Although a review of the relevant literature on mixed metals suggests that studies more often report synergistic effects than the other two behaviour types (an indication that laboratory studies may underestimate sub-lethal effects on salmonids), future research is needed.

Finally, dietary metal concentrations are not incorporated into Canada's water quality guidelines despite the likely simultaneous occurrence of both waterborne and dietary routes of metal toxicity. The results reported in this literature synthesis only describe waterborne effects of metals on fish; yet, the consumption of metal-contaminated prey is also a common route of toxicity for predatory animals such as salmonids. Dietary Cu may at times be more important than waterborne Cu at reducing survival of salmonids during early life stages (Woodward et al. 1994). Importantly, waterborne and dietary metal exposures occur simultaneously in aquatic environments, and sub-lethal toxic effects of waterborne metals in salmonids may be exacerbated by dietary uptake. For example, the switch in feeding preference from motile to non-motile (benthic) prey by juvenile brook trout as a result of Cd-exposure is hypothesized to exacerbate the effects of Cd by intensifying or prolonging exposure through a combination of trophic transfer and altered foraging behavior (Riddell et al. 2005b). Yet, the water quality guidelines for heavy metals assigned by the governments of British Columbia or Canada, and the toxicity tests performed in the proposed project's environmental assessment, do not factor the toxic effects of chronic dietary loading.

Thus, despite the numerous documentations of sub-lethal toxicity effects from the aforementioned 7 heavy metals on salmonids, the large degree of uncertainty makes a formal assessment of harm to salmon based on these metals tenuous at best.

Importantly, there are 23 other metals and non-metals proposed to be released into receiving waters that have generally not been described with regards to effects on salmonids. I have not touched on the potential effects of selenium (Se), which the proponent openly declares will become a management problem, and is an element of concern from a treatment and salmon conservation perspective. Research has shown that while Se is a required micronutrient for fish, it can be toxic when only slightly increased above essential requirements. Toxic effects of waterborne Se for fish can include decreased growth, immune suppression, reproductive failure, deformities, and mortality (Janz et al. 2010). While the inorganic forms of Se (e.g., selenate, selenite) can be toxic at concentrations in the 100 μg/L range via waterborne exposures, dietary exposure to organic Se may pose a greater hazard to fish (USEPA 1998; Janz et al. 2010). To my knowledge, no research has confirmed the lowest effect concentrations of Se that cause harm to salmonids. Combined with the other metals and non-metals, and their mixtures, to be released from the proposed tailings impoundment, there remains an enormous void in the scientific literature as to the extent of harm on salmonids.

I have not discussed the weaknesses of the GoldSim model used to predict the reported metal concentrations. Importantly, this review assumes that all predicted metal values reported by the proponent are accurate, and are the concentrations that will occur as a result of the proposed project. However, the accuracy of such predictions will be assessed in a follow-up document.

Downstream effects

River transport of toxicants from areas subject to long-term deposition of contaminants may be much more extensive than previously thought. Most studies of metal-contaminated riparian systems focus on only a few kilometres of river near the contaminant source (Moraiarity et al. 1982; Bradley and Cox 1987), and do not integrate contamination in the solid and aqueous phases with the biological system. Additionally, metal bioavailability in fish-bearing rivers is poorly understood, partly because the biological communities of such systems are complex and difficult to examine (Moore et al. 1991). However, there are a few studies that have shown long-distance transport of heavy metals from upstream sources. For example, metals from upstream sources in the Clark Fork Complex of Montana (an intensive metal-mine region) entered streams and rivers as solutes and particulates, and eventually contaminated river sediments at least 380 km downstream (with a predicted total river transport of more than 500 km; Moore and Luoma 1990). In the Blackfoot River, Montana, some solute and particulate contaminants from metal-mines extend downstream for 25 km from the primary source, with at least Cd and Zn remaining in a biologically available form over long stretches of river; Cd specifically has been observed in biota more than 75 km downstream (Moore et al. 1991).

Other metals and non-metals can biomagnify and persist in aquatic systems far from the point source of pollution. Similar to Cd, contaminants such as mercury (Hg), molybdenum (Mo), and Se all have the propensity to biomagnify within food-webs as concentrations increase progressively at each interacting trophic level. In many cases, top-level consumers can receive toxic concentrations from their diet even though concentrations in water may be small (Lemly and Smith 1987; Mason et al. 2000; Peterson et al. 2002; Jewett et al. 2003; Regoli et al. 2012). While there is insufficient information in the scientific literature to assess the biomagnification potential of all contaminants predicted to be generated by the proposed project, bioaccumulation factors should be used to consider secondary toxicity potential, and to more fully assess the risks that metals and non-metals will have to salmon health.

Riverine sediments are recognized as sensitive indicators for monitoring contaminants. River sediment is an important reservoir for heavy metals, and can be a secondary contamination source affecting the health of river ecosystems (Fan et al. 2002; Wang et al. 2010), including salmonids (Riddell 2005a). While the concentrations of heavy metals in a given river may be low, the contents in sediments can be exceptionally high (Ferreira et al. 1996). Once in the river, heavy metals can be removed from the water body and absorbed by sediment particles (Tam and Wong 2000), with metal concentrations tending to be greatest in fine, rather than coarse, sediments (Ujevic et al. 2010). Thus, heavy metals released into rivers or river reaches with fine sediments may be transported short distances (but will accumulate in high concentrations with long persistence times), whereas heavy metals in rivers with coarse sediments may be transported long distances. Unlike organic pollutants, heavy metals do not decay; they can persistence in sediments for long time periods, or cause increased exposure than is present in the environment alone through bioaccumulation and biomagnification among aquatic biota. Depending on the sediment composition of Sulphurets Creek, the Unuk River may become the most significant reservoir for heavy metals released by the proposed project. Further research is needed to determine the ultimate fate of the released metal and non-metal toxicants.

All of the metal concentrations predicted to occur in surface waters as a result of the proposed project are reported in *total* concentrations, whereas most of the relevant examples of toxic effects pertain to *dissolved* concentrations, except for Al. Dissolved concentrations are most important because they represent the mobile and biologically available amount of a given metal in water, whereas total metal concentrations are the total amount of a given metal in water whether in an available form or not. A dissolved metal concentration is a sub-set of the total metal concentration in water. It would be much more informative if the proponent could provide predictions for dissolved concentrations of each metal in surface waters as a result of the project. Otherwise, and to be conservative, it is fair to assume that the predicted concentrations represent the mobile and biologically available amount of a given metal in water.

To conclude, salmonids in receiving waters of metal-mine effluent from the proposed project will undoubtedly be subject to sub-lethal metal toxicity. In some circumstances, such as the loss of olfaction from Cu toxicity, the effect most probable is secondary death. Combined with all stressors currently facing salmonids in compromised habitats, such effects will undoubtedly reduce abundance. Importantly, the current state of knowledge regarding the specific metals and non-metals proposed to be released by project into salmon-bearing streams is insufficient to adequately assess the extent of impact to salmon and aquatic biota within the salmon food-web. Further research is thus required to not only predict the potential downstream flow of metal and non-metal contaminants into the Unuk River, but to adequately assess harm.

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