APPENDIX G

MEMO

Date: May 9, 2011 **HCP Ref No.:** CZN1682

From: John Wilcockson and Martin Davies

To: David Harpley, CZN

Subject: Prairie Creek Mine – Bioaccumulation of Mercury and Selenium in Fish (Memo 4)

1.0 INTRODUCTION

During the technical group meeting on April 12, when the assessed potential for aquatic effects related to planned effluent discharge from Canadian Zinc Corp. (CZN)'s Prairie Creek Mine was being considered, concern was expressed regarding predicted incremental increases of mercury in water downstream of the mine, and the potential for bioaccumulation of mercury in fish to levels of concern. It was felt that the increase in mercury, combined with the slight nutrient enrichment described could result in a significant increase in mercury concentrations in the tissues of sport fish. A particular target of concern was arctic grayling just upstream of the confluence of Prairie Creek with the Nahanni River, which are a potential target of Aboriginal harvest.

Selenium is another metalloid with potential for bioaccumulation in aquatic biota, and in some environments, high waterborne concentrations of selenium have lead to negative effects on fish populations. Selenium is taken up by fish primarily through their diet, and is an essential micronutrient; however, high concentrations of selenium in fish eggs can cause developmental abnormalities in eggs and larvae that may lead to reproductive impairment (Janz *et al.* 2010). Based on data from Se-impacted environments, aquatic invertebrate communities are relatively insensitive to elevated selenium concentrations (Stewart *et al.* 2010). Young *et al.* (2010) provide a thorough review of ecological effects of selenium in aquatic environments.

This memo provides further discussion and assessment of the potential for bioaccumulation of mercury and selenium in Prairie Creek fish.

1.1 MERCURY

1.1.1 Background

1.1.1.1 Factors Mediating Bioaccumulation

Mercury bioaccumulation can be a function of several biological and physico-chemical properties (Wyn *et al.* 2009, Gorski *et al.* 2001), including:

1. Age of fish assessed for mercury concentrations;
2. Structure/length of food chain;
3. Dietary preferences of fish;

4. Rate of release of mercury/bioavailability of mercury; and
5. Methylation rate of mercury in an aquatic system.

1.1.1.2 Factors Mediating Bioavailability of Mercury

Environmental impacts of mercury are highly dependent on the chemical form or species of mercury (Clarkson 1998 in Ullrich *et al.* 2001). The chemical form of mercury in aquatic systems, in turn, is strongly influenced by several biotic and abiotic factors. Of the chemical species, organic mercury compounds such as methylmercury and dimethylmercury have the greatest toxic potency and also have the greatest tendency to bioaccumulate in biota. An understanding of the factors that promote their formation is therefore important for the protection of human health (Risher 2003).

Mercury (Hg) in aquatic systems is generally bound to particulates, either in bed sediments or suspended in the water column (Ullrich *et al.* 2001). In sediments, methylmercury (MeHg) concentrations are only about 1 to 1.5% of total Hg. Anoxic conditions favour Hg and MeHg release from sediments, as do increasing temperature, nutrient additions and decreasing pH (Ullrich *et al.* 2001).

In freshwater systems, sediments constitute the main reservoir of Hg. "Settling of particulate matter is considered a major Hg delivery mechanism to the sediment/water interface, the main site for methylation ... "(Hurley *et al.* 1991, 1994 and Watras *et al.* 1994 in Ullrich *et al.* 2001). The most active methylation sites are in wetlands, lake sediments and anoxic bottom waters (Harris *et al.* 2007). However, in lake systems, the total rate of methylation in the water column may be greater than in sediments, because of the vastly larger volume of the water column.

In freshwater environments (lakes and rivers), up to 30% of total Hg can be MeHg. The proportion is higher in the water column than sediments (Ullrich *et al.* 2001).

Methylation is primarily influenced by bacterial activity, which in turn, is affected by pH, redox conditions, temperature and the presence of chemical species (Sultan 2002 and Ullrich *et al.* 2001). Biotic and abiotic factors are discussed below to assess the potential for enhanced biomagnification by fish if there is an increase of mercury and nutrient inputs once the mine becomes operational.

Microbiology: Anaerobic sulphate-reducing bacteria (SRB) are the principal methylators of inorganic Hg in both freshwater and estuarine environments. The same bacteria are responsible for mediating MeHg degradation. The rate of microbial MeHg production seems to be primarily dependent on the structure of the bacterial community, Hg availability, nutrients and the abundance of electron acceptors such as sulphate.

Temperature: Hg methylation often peaks in summer months and is thought to be linked to increases in bacterial activity associated with higher water temperatures. Optimum MeHg release from sediments occurs at 35ºC. Relative to this rate, the release of methyl mercury decreases to 70% at 20ºC and 50% at 4ºC. Temperature and anoxic conditions are determinants of MeHg concentration, though temperature only accounts for 30% of the influence. Demethylation has been found to be favoured by lower temperatures (Ullrich *et al.* 2001).

pH: The solubility and mobility of Hg and MeHg are influenced by pH. Low pH conditions generally facilitate the release of heavy metals from sediments; however, in the case of Hg, studies have been contradictory. Neutral and slightly alkaline conditions reduce MeHg concentrations and low pH waters may contain a higher relative amount of MeHg. "A pH decrease at the aerobic sediment-water interface resulted in a two to threefold increase in net methylation rates. In anaerobic sediments, on the other hand, net MeHg production was generally found to be decreased at low pH values. The acidification of surficial lake sediments always resulted in a significant decrease in Hg methylation rates" (Ullrich *et al.* 2001), but increase in release rates. A pH change from 7.0 to 5.0 doubles the release of MeHg from sediments (Ullrich *et al.* 2001).

Organic Material: The conversion of Hg to MeHg is generally much higher when sediments are rich in organic material or near sewage treatment plants. Increases in MeHg in water, sediment and fish tissues occur with increasing levels of organic carbon, however the exact relationship is unknown (Ullrich *et al.* 2001). Hg concentrations in sediments are strongly related to the amount of organic content; Hg is more strongly bound than MeHg, explaining why MeHg is more easily mobilized from sediments.

Redox Conditions: "Both methylation rates and the stability of methylmercury in sediments appear to be enhanced under anaerobic conditions, whereas methylation rates are low under aerobic conditions, probably because of the reduced activity of anaerobic sulphate-reducing bacteria" (Ullrich *et al.* 2001). Anoxic conditions favour Hg and MeHg enrichment. This may be due to the release of Hg when strong sediment binding sites (oxyhydroxides) dissolve under anoxic conditions. "The formation and dissolution of oxyhydroxides and organic complexes may influence methylation by controlling the availability of inorganic Hg" (Ullrich *et al.* 2001).

Sulphide: An inverse relationship between sulphide and MeHg production has been observed in sediments. It has been found that MeHg levels in sediments increase proportionally to sulphide concentrations up to 1.8 mg/g of sulphide, after which there was a sharp decline in MeHg production (Ullrich *et al.* 2001).

Based on these factors, methylation rates of mercury in Prairie Creek should be low, given well-oxygenated (aerobic), fast-flowing waters with low temperatures (especially through winter, when waters are expected to be near 0ºC), and with little sedimentation in the creek and very low organic matter (as suggested by low periphyton and benthic abundances).

1.1.2 Mercury Concentrations in Prairie Creek and Mine Effluent

Currently, all measurements of mercury in Prairie Creek water in the historical database have been non-detectable (i.e., <20 ng/L). The maximum predicted concentration in Prairie Creek downstream of the mine was 28 ng/L; the non-detectable Prairie Creek background concentration was the greatest contributor to this number.

We anticipate that the actual background concentration in upstream Prairie Creek is much lower than 20 ng/L; this will be assessed in ultra-trace mercury analyses that will be applied to Prairie Creek water samples planned for collection in mid-May 2011.

It is also noteworthy that the only measurement of mercury in water used in the prediction of downstream concentrations, where mercury was measurable, was in treated process water. Dissolved mercury makes up about 15% of total mercury measured in effluent, with the rest presumably associated with particulates. During operations, most of these particulates should be removed in the effluent treatment plant (clarification) and in the effluent holding pond. This would have the effect of reducing concentrations to closer to the dissolved mercury value observed in simulated effluent (280 ng/L in treated process water and 20 ng/L in treated mine water).

If upstream concentrations of mercury in Prairie Creek are found to be lower than the 20 ng/L background value currently used in the model, and the majority of mercury in mine effluent is removed in particulates before discharge, actual downstream mercury concentrations in Prairie Creek would be well below those predicted in water quality modeling.

1.1.3 Potential for Increased Bioaccumulation in Prairie Creek

Generally, the conditions found in Prairie Creek would not favour the formation of methylmercury. The creek is highly oligotrophic and fast-flowing; even with some small incremental increases in nitrogen and phosphorus, once the mine becomes operational, this will not result in conditions that facilitate significant methylmercury production. The water temperature is generally cold, ranging from 0 to 12ºC. There have been no fine sediments observed anywhere in the creek. The finest substrates observed have been pebbles. Thus the sediments will be well aerated and low in organic matter, thereby limiting potential for methylation. Furthermore, discharges of organic matter are predicted to be very small. Redox and pH is anticipated to remain very high. Thus, any free (dissolved) inorganic mercury present will tend to be bound strongly to oxyhydroxides of magnesium and iron.

Concentrations of mercury found in slimy sculpin taken from areas of Prairie Creek in 2006 by Spencer *et al.* (2008) exhibited mercury tissue burdens of 0.028 to 0.078 µg/g, well below Health Canada guidelines of 0.200 µg/g for subsistence consumers and 0.500 µg/g for general consumers. Although only slimy sculpin were sampled by Spencer *et al.*, additional sampling of metals in multiple fish species from Prairie Creek by Beak (1981) found higher mercury tissue burdens in slimy sculpins than in bull trout or mountain whitefish sampled from the creek, and that concentrations were typically non-detectable in mountain whitefish, a coregonid relative of arctic grayling. Given these lines of evidence, we anticipate that mercury concentrations in the muscle tissue of arctic grayling harvested at the mouth of Prairie Creek will not increase significantly and will not exceed the Health Canada guidelines once the mine becomes operational.

2.0 SELENIUM

2.1.1 Factors Mediating Bioavailability and Bioaccumulation

The process of bioaccumulation of selenium in aquatic organisms is very different from that of mercury. In contrast to mercury, greatest trophic transfer rates for selenium are from sediments/particulates to primary producers (i.e., from sediments to periphyton or aquatic plants), rather than from primary to secondary or tertiary consumers such as invertebrates and fish, and fish size does not typically correlate with selenium tissue burden (Stewart *et al.* 2010).

Key factors affecting bioaccumulation of selenium in aquatic systems include the following key factors (these and others are discussed in more detail in Chapman *et al.* 2010):

- **Lentic versus lotic environments:** Greatest bioaccumulation of selenium in the aquatic environment occurs in lentic (slow-flowing or standing-water) environments, particularly wetlands. This is because of long-term deposition of selenium in sediments and cycling of selenium between sediments and primary producers/bacteria in lentic environments (leading to increasing concentrations), and because the bulk of selenium in these often-low-oxygen environments is in the reduced selenite form, which is more bioavailable than the oxidized form of selenate (Stewart *et al.* 2010). In lotic (flowing) environments, with well-oxygenated conditions, little sediment accumulation, and typically less organic matter and algal growth than lentic environments, there is less potential for selenium accumulation.

- **Fish residency:** Toxicity to developing fish eggs and larvae occurs through maternal transfer of selenium to eggs (Young *et al.* 2010). As such, selenium burdens of importance in determining potential for adverse effect are those in ripe, female fish. For female fish to develop high levels of selenium in their tissues through diet, they must feed in high-selenium environments; fish that are resident in an area with high selenium concentrations will accumulate more than fish that migrate through such an area and only take a small part of their diet from that area.

2.1.2 Selenium Concentrations in Prairie Creek and Mine Effluent

The measured average concentration of selenium in Prairie Creek upstream of the mine site was 1.19 µg/L, with a measured, 90th-percentile concentration of 2.22. This 90th-percentile has been proposed as the downstream Site-Specific Water Quality Objective for selenium in Prairie Creek. Mixing-model predictions estimate downstream concentrations during mine operations to range seasonally between 1.15 and 1.81 µg/L.

2.1.3 Potential for Increased Bioaccumulation in Prairie Creek

Prairie Creek is a lotic, well oxygenated environment, with little potential for accumulation of selenium through sediment accumulation and cycling of reduced selenium between accumulating sediments and lower levels of the food chain. As such, one would expect selenium concentrations in biota to remain low if waterborne concentrations remain low, and to reach a steady state in biota following any long-term change in the background concentration of selenium in water.

Slimy sculpin is the only fish species that appears to reside in Prairie Creek year-round with potential for selenium accumulation in eggs over the egg-development phase (which in sculpin occurs over winter). Most bull trout and most or all mountain whitefish likely only migrate through the creek in summer/fall to spawn upstream of the mine (Beak 1981, Muchnacz 2001). As such, potential for substantial maternal accumulation and transfer of selenium to eggs is low for both bull trout and mountain whitefish, because these fish are not full-time residents of lower Prairie Creek, and greatest for slimy sculpin.

Although significant maternal accumulation of Se in bull trout is not expected in Prairie Creek for reasons mentioned above, it is worth noting that McDonald *et al.* (2010) calculated a Tissue Residue Threshold for Dolly Varden char (*Salvelinus malma*), a conspecific species with bull trout, of 54 mg/g Se in eggs, indicating that char are generally less sensitive to Se burdens than other fish species.

With regard to slimy sculpin, Spencer *et al.* (2009) found concentrations of selenium in Prairie Creek sculpin to average 1.25, 1.06 and 1.16 µg/g (dw) in sculpin muscle tissue in upstream, near-field and far-field areas of the creek, respectively. These values are very low relative to other slimy sculpin data: Carmichael and Chapman (2006) compiled baseline selenium data for slimy sculpin from 45 unimpacted sites throughout British Columbia and found that selenium burdens in sculpins from a majority of sites exceeded 4 µg/g (dw, whole weight); no site exhibited mean sculpin Se burdens as low as those observed by Spencer *et al.* (2009) for Prairie Creek.

Given the low baseline concentrations of Se in Prairie Creek sculpins, the small change in waterborne selenium expected during mine operations (i.e., concentrations remaining within the range of upstream variability), and characteristics of the Prairie Creek environment that make Se accumulation unlikely, the risk of adverse effects on the aquatic environment from selenium discharged with effluent from the Prairie Creek mine should be negligible.

3.0 REFERENCES

Beak Consultants Ltd. 1981. Prairie Creek Project. Fisheries and Invertebrate Studies, 1981. A report prepared for Cadillac Explorations Ltd., Calgary, AB, by Beak Consultants Ltd., Richmond, BC.

Carmichael, N.B. and P.M. Chapman. 2006. Baseline selenium in sculpins related to the northeast British Columbia coal zone. BC Ministry of Environment, Omineca Region, Prince George, BC. Accessed at: http://www.env.gov.bc.ca/epd/regions/omineca/water/reports

Chapman, P.M., W.J. Adams, M.L. Brooks, C.G. Delos, S.N. Luoma, W.A. Maher, H.M. Ohlendorf, T.S. Presser, and D.P. Shaw. 2010. Ecological Assessment of Selenium in the Aquatic Environment. SETAC Pellston Workshop on Ecological Assessment of Selenium in the Aquatic Environment. Pesacola, FL. SETAC Publications. CRC Press, New York.

Clarkson, T.W. 1983. Human toxicology of mercury. J. Trace Elem Exp. Med. 11(303).

Gorski, P.R., L.B. Clecknera, J.P. Hurleya, M.E. Sierszenc, and D.E. Armstrong. 2001. Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. Sci Tot Env, 304:327-348.

Harris, R.C., J.W.M. Rudd, M. Amyot, and L. Babiarz. 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. PNAS 104 (42): 165856-16591.

Health Canada. 2011. Mercury in Fish: Questions and Answers. Accessed on: May 2, 2011. Accessed at: http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/environ/mercur/merc_fish_qa-poisson_qr-eng.php#hr3

Hurley, J.P., C.J. Watras and N.S. Bloom. 1991. Mercury cycling in a northern Wisconsin seepage lake- The role of particulate matter in vertical transport. Water Air Soil Pollut., 56(53).

Hurley, J.P., D.P. Krabbenhoft, C.I. Babiarz, and A.W. Andren. 1994. Cycling of mercury across sediment-water interface in seepage lakes, Advances in Chemistry Series, 237(425).

Janz, D.M., D.K. DeForest, M.L. Brooks, P.M. Chapman, G. Gilron, D. Hoff, W.A. Hopkins, D.O. McIntyre, C.A. Mebane, V.P. Palace, J.P. Skorupa, and M. Wayland. 2010. Selenium toxicity to aquatic organisms. Pgs. 141-232 of Chapman *et al.*

McDonald, B.G., A.M.H. Debruyn, J.R.F. Elphick, M. Davies, D Bustard, and P.M. Chapman. 2010. Developmental Toxicity Of Selenium To Dolly Varden Char (*Salvelinus Malma*). Environ Tox Chem. 29:2800-2805.

Mueller, K.A. and A.C. Matz. Water quality, and metal and metalloid concentrations in water, sediment, and fish tissues from Innoko National Wildlife Refuge, Alaska, 1995–1997, Ecological Services, Fairbanks, Alaska, U.S. Fish and Wildlife Service. Technical Report NAES-TR-02-01; 2002. 155 pp.

Muchnacz, N.J. 2001. Interim Report: Fisheries Survey of Prairie Creek Watershed. Prepared for Parks Canada Agency Nahanni National Park Reserve and Department of Fisheries and Oceans Canada Fish Habitat Management Western Arctic Region. Natural Resources Institute, University of Manitoba.

Risher, D.F. 2003. Concise International Chemical Assessment Document 50. Elemental Mercury and Inorganic Mercury Compounds: Human Health Aspects. Published under joint sponsorship of the United Nations Environment Programme, the International Labour Organization, and the World Health Organization. Accessed April 19, 2011. Accessed at: http://www.who.int/ipcs/publications/cicad/en/cicad50.pdf

Stewart, R., M. Grosell, D. Buchwalter, N. Fisher, S. Luoma, T. Mathews, P. Orr, and W.X. Wang. 2010. Bioaccumulation and trophic transfer of selenium. Pgs. 93 to 140 in Chapman *et al.*

Sultan, K. 2006. Distribution of Arsenic and Heavy Metals in Soils and Surface Waters in Central Victoria (Ballarat, Creswick and Maldon). M.Sc. Thesis. University of Ballarat. 129 p + appendices.

Ullrich S.M., T.W. Tanton and S.A. Abdrashitova. 2001. Mercury in the aquatic environment: a review of factors affecting methylation. Crit Rev Environ Sci and Tech 31:241-293.

US EPA (United States Environmental Protection Agency). 1997. Mercury Study Report to Congress. Volume III: Fate and Transport of Mercury in the Environment. EPA-452/R-97-005. December 1997.

Watras, C.J. and N.S. Bloom. 1994. The vertical distribution of mercury species in Wisconsin lakes: accumulation in plankton layers. In: (Eds.) C.J. Watras and J.W. Huckabee. Mercury Pollution- Integration and Synthesis. Lewis Publishers, Boca Raton, Ch: I/12: 153-177.

Wyn, B., K.A. Kidd, N.M. Burgess, and R.A. Curry. 2009. Mercury biomagnifications in the food webs of acidic lkes in Kejimkujik National Park and National Historic Site, Nova Scotia. Can J of Fish and Aquat Sci 66:1532-1545.

Young, T.F., K. Finley, W. Adams, J. Besser, W.A. Hopkins, D. Jolley, E. McNaughton, T.S. Presser, D.P. Shaw, and J.Unrine. 2010. What You Need to Know About Selenium. Pgs. 7 to 46 in Chapman *et al.*