Subject Area 7.2: Evaluations of the impacts and interactions of chemicals in the laboratory or real environments, and their fate in biota, including animals and humans

Research Article

Toxicity of Cobalt-Complexed Cyanide to *Oncorhynchus mykiss*, *Daphnia magna*, and *Ceriodaphnia dubia*

Potentiation by ultraviolet radiation and attenuation by dissolved organic carbon and adaptive UV tolerance

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Abstract

Background. Cobalt cyanide complexes often result when ore is treated with cyanide solutions to extract gold and other metals. These have recently been discovered in low but significant concentrations in effluents from gold leach operations. This study was conducted to determine the potential toxicity of cobalt-cyanide complexes to freshwater organisms and the extent to which ultraviolet radiation (UV) potentiates this toxicity. Tests were also conducted to determine if humic acids or if adaptation to UV influenced sensitivity to the cyanide complexes.

Methods. Rainbow trout (*Oncorhynchus mykiss*), *Daphnia magna*, and *Ceriodaphnia dubia* were exposed to potassium hexacyanocobaltate in the presence and absence of UV radiation, in the presence and absence of humic acids. Cyano-cobalt exposures were also conducted with *C. dubia* from cultures adapted to elevated UV.

Results. With an LC50 concentration of 0.38 mg/L, cyanocobalt was over a 1000 times more toxic to rainbow trout in the presence of UV at a low, environmentally relevant irradiance level (4 $\mu W/cm^2$ as UVB) than exposure to this compound in the absence of UV with an LC50 of 112.9 mg/L. Toxicity was immediately apparent, with mortality occurring within an hour of the onset of exposure at the highest concentration. Fish were unaffected by exposure to UV alone. Weak-acid dissociable cyanide concentrations were observed in irradiated aqueous solutions of cyanocobaltate within hours of UV exposure and persisted in the presence of UV for at least 96 hours, whereas negligible concentrations were observed in the absence of UV. The presence of humic acids significantly diminished cyanocobalt toxicity to *D. magna* and reduced mortality from UV exposure. Humic acids did not significantly influence survival among *C. dubia*. *C. dubia* from UV-adapted populations were less sensitive to metallocyanide compounds than organisms from unadapted populations.

Conclusions. The results indicate that metallocyanide complexes may pose a hazard to aquatic life through photochemically induced processes. Factors that decrease UV exposure such as dissolved organic carbon or increased pigmentation would diminish toxicity.

Keywords: Aquatic organisms; *Ceriodaphnia dubia*; cyanocobalt; *Daphnia magna*; *Oncorhynchus mykiss*; organic carbon; radiation; rainbow trout; toxicity; ultraviolet radiation; UV tolerance

Introduction

Cyanide, the CN^- anion, binds chemically with a broad range of organic and inorganic substances (Flynn and Haslem 1995). Its strong affinity for metals, gold in particular, has made cyanide the reagent of choice for the extraction of precious metals from their ores (Smith and Mudder 1991). Young (2001) estimated that mining applications accounted for about 20% of worldwide cyanide production, or 0.6 million tons annually. Gold production at the world's mines has changed little since Young's estimate (George 2005), which suggests that the current mining usage of cyanide is similar.

The application of cyanide solutions to ores liberates gold as the $Au(CN)_2^-$ complex, and also produces cyanometallic complexes from the base- and ferro-metals that are commonly present. Cyanometallic complexes are grouped according to their tendency to dissociate on acid exposure to liberate free metal ions and free cyanide ($CN^- + HCN_{aq}$), the most toxic of the cyanide species (Eisler et al. 1999). More easily dissociated complexes (for example, $Zn(CN)_4^{2-}$, $Ag(CN)_2^-$, $Cu(CN)_3^{2-}$) can release environmentally significant amounts of free cyanide under some conditions (Doudoroff 1976). Consequently, where process solutions are discharged, cyanide regulatory compliance is commonly based on the weak acid dissociable (WAD) cyanide analytical procedure (e.g. State of Nevada, 0.2 mg/L WAD cyanide threshold), which reports both free cyanide and cyanide contained in easily dissociated cyanometallic complexes. In other locations, discharge compliance is based on the total cyanide analytical procedure (e.g. State of New Mexico, 0.2 mg/L total cyanide threshold) which reports cyanide contained in stronger cyanometallic complexes (for example, $Fe(CN)_6^{3-}$) as well as free and weakly complexed cyanide. The environmental risks posed by stronger complexes are less than the risks posed by free and weakly complexed cyanide (Smith and Mudder 1991). However, several CN species (e.g. cyanates, thiocyanates, etc.) are not detected by either WAD or Total determinations.

Recently, Johnson et al. (2001) reported effluent chemical analyses for several gold leach operations in the southwestern U.S. in which cobalt and cyanide concentrations were correlated at ratios consistent with the presence of cobalt

cyanocomplexes. The ores mined at these sites were not particularly rich in cobalt but, due to extensive evaporation during process solution recycling, solutes became concentrated and dissolved cobalt reached concentrations as high as 3 mg/L. This level of cobalt could carry as much as 7.5 mg cyanide/L as the $Co(CN)_6^{3-}$ complex. The presence of cobalt-complexed cyanide in gold leach effluents had not been suspected previously because this species reports neither in the WAD cyanide analytical procedure nor in the total cyanide procedure (Goulden et al. 1972, Grimes et al. 1999, Milosavljevic and Solujic 2001). Direct detection methods have been developed using ion chromatography methods (Haddad and Kalambaheti 1991, ASTM 2004), but these have not yet been proven for analysis of mineral processing effluents which are chemically complex and can be high in total dissolved solids (e.g. Johnson et al. 2000).

Toxicological data exist for most of the cyanide species that characterize mineral processing effluents (Burdick and Lipschuetz 1950, Doudoroff 1976, Eisler et al. 1999, Calfee and Little 2003a), but not for the cyano-cobalt complexes. Here we report experiments that were conducted to determine the toxicity of cobalt cyanocomplexes to fish and to two aquatic invertebrates. Testing was carried out with and without ultraviolet light (UV) to examine whether toxicity is influenced by photodissociation, a phenomenon that is known to affect cobalt cyanocomplexes (Flynn and Haslem 1995). Testing was also carried out to determine whether toxicity is influenced by adapted UV tolerance or by UV absorbance by dissolved organic carbon.

1 Materials and Methods

Exposures were conducted with potassium hexacyanocobaltate (III) ($K_3Co(CN)_6$) purchased from Alfa Aesar (Ward Hill, MA, USA). Rainbow trout (*O. mykiss*) were obtained as eggs from a hatchery and cultured at the Columbia Environmental Research Center. Fish were fed brine shrimp and trout food and were tested at approximately 30 days after yolk sac absorption. The freshwater crustaceans, *D. magna* and *C. dubia,* were obtained from laboratory cultures and were fed a mixture of algae (*Selenastrum* sp) and yeast (YCT) daily. *C dubia* produced from populations adapted to UV for a minimum of 6 generations (UV adapted) and *C. dubia* from populations cultured in the absence of UV (naïve) were used during exposures to cobalt-complexed cyanide.

1.1 Determination of laboratory LC50 concentrations

Laboratory exposures were conducted to determine the toxicity of cobalt-complexed cyanide over a 96-hour exposure by measuring the concentration toxic to 50% of the test organisms (LC50). The toxicity testing procedures were conducted in accordance with ASTM test guidelines (1998). Fish were exposed in 96-hour static acute toxicity tests to five dilutions of cyanocobalt and a well water control treatment (pH 7.8, hardness 286 milligrams/liter (mg/liter) as calcium carbonate, alkalinity 258 mg/liter as calcium carbonate).

Appropriate volumes of the stock solution prepared with well water were pipetted into the test vessels to obtain the desired exposure concentrations, then mixed thoroughly with a glass-stirring rod prior to placing test organisms in the exposure beakers. Nominal concentrations for toxicity tests were based on calculated values from dilutions. (Note: to convert µmoles CN/L to µg/L multiply by 26; to convert µmoles Co(CN)6/L to µg/L, multiply by 35.8).

Ten fish were exposed in 4-liter glass beakers (2 replicates) containing 3.5 liters of the chemical solution. Ten *D. magna* were exposed in 150 ml beakers (3 replicates) containing 100 ml of the test solution. Individual *C dubia* neonates (<12 hours old) were placed in a 30 ml beaker containing 25 ml test or reference water (1 neonate/beaker; 10 replicate beakers/treatment), and each concentration and light treatment (No UV and UV) was tested in a randomly-allocated block design (e.g. 5 beakers to a block for each concentration:light combination) to eliminate bias due to beaker location. Tests were conducted for a 96-hour period to determine survival. Mortalities and abnormal behavior (floating; hyperactivity; lethargy) were recorded daily.

Toxicity tests were conducted in a solar simulator with dimensions of approximately 1 m wide by 2 m long enclosed with reflective specular aluminum and suspended over a temperature-controlled water bath of similar dimensions (Little and Fabacher 1996). Test beakers containing the organisms were randomly positioned in the water bath. The simulator was equipped with cool white, UVB fluorescent lamps, UVA fluorescent lamps, and halogen flood lamps. The cool white, halogen, and UVA fluorescent lamps were controlled by a timer to operate for 16 hours daily. The UVB lamps were activated with a second timer to operate for 5 hours per day. The UVB photoperiod started five hours after the onset of the white light and UVA photoperiod. The simulator was checked daily for lamp function, water bath temperature, and photoperiod cycles. Exposure to each chemical treatment and control was performed under two light treatments; No UV (0 µW/cm²) and UV (4 µW/cm²) at 12–14°C. Artificial light tends to produce spikes of intensity at certain wavelengths and not all wavelengths of natural sunlight are generated. In terms of total UVB and total UVA the UV irradiance treatment applied during the toxicity tests was representative of the quality and intensity of natural sunlight measured in a variety of habitats in the western U.S. The light treatment was based on ultraviolet-b (UVB) radiation intensities since the UVB wavelengths (290–320 nm) are most injurious to aquatic organisms. Light intensity was measured at 1 nm intervals ranging from 280–700 nm with an Optronics OL-754 spectroradiometer (Optronics Laboratories, FL). The light treatments were achieved using various filters (i.e. polycarbonate, mylar, shade cloth) covering the exposure vessels.

Humic acids were generated by soaking 1 kg of white oak (*Quercus alba*) leaves in 5 L of water for 90 days. Aliquots of 5 ml dark amber-colored water overlying the oak leaves were diluted with 95 ml well water to yield an average 6 mg/L dissolved organic carbon. This dilution resulted in slight yellow-colored water, which was used in control exposures or dosed with the cyanocobalt. Water samples (0.5 liter) for measurement of dissolved organic carbon (DOC) were filtered with a 0.45 µm polycarbonate membrane filter (not a significant source of organic carbon), subjected to a persulfate/UV digestion technique, and analyzed on a Technicon Autoanalyzer II System (IRAMA Corp, Miilwaukii, Oregon). The detection limit was 0.05 mg/L carbon.

The test temperatures were set at 12.0°C for the *O. mykiss* exposures and 25°C for the *D. magna* and *C. dubia* exposures. Mortality, condition, and behavior abnormalities such as loss of equilibrium were recorded daily. In parallel exposures conducted in the absence of test organisms, water samples were collected for the analysis of cobalt-complexed cyanide and WAD cyanide at 0, 1, 8, 24, 48 and 96 hours of exposure in the presence and absence of UV.

1.2 Chemical analysis

Water samples were placed in 250-ml polypropylene bottles, preserved with NaOH, and analyzed within 6 days. The hexacyanocobaltate (III) complex was measured using a Dionex DX500 chromatograph system comprised of a GP50 gradient pump, AS16 and AG16 separation and guard columns, and AD20 absorbance detector following ASTM method D 6994-04 (ASTM 2004). The eluent was a 50:50 mix of 20 mM NaOH and 300 mM $NaClO_4$ solutions, flow was 1 mL $min^{-1}m_6$ L^{-1} passing through a 100 µL sample loop, and absorbance was measured at 215 nm. The detection limit was 0.2 mg $Co(CN)_6$ L^{-1}.

WAD cyanide was measured using the picric acid method (Fisher et al. 1952, with unpublished modifications by P. Emsbo). Reproducibility was ±10% or better; the detection limit was 0.02 mg CN L^{-1}. Inasmuch as the only source of cyanide in the analyzed samples was dissolved hexacyanobaltate (III), the WAD cyanide results are assumed to represent free cyanide (CN^- + HCN_{aq}) produced by dissociation. Mass balance calculations support this assumption.

1.3 Statistical analysis

Standard analysis of variance tests were conducted on mortality data to determine if toxicity resulted from the interaction of chemical concentration, UV light treatment, or duration of exposure. Probit analysis (Sokal and Rohlf 1987) was used to calculate LC50 values and 95% confidence intervals for each chemical based on nominal concentrations using TOXSTAT® v. 3.5 (Western Ecosystems Technology, Cheyenne, Wyoming). The criterion of non-overlapping 95% confidence intervals was used to determine significant differences ($p<0.05$) between LC50 values.

2 Results

Exposure to UV alone was not lethal to juvenile rainbow trout over the 96 hour exposure; however it was lethal to *D. magna* and *C. dubia*. In the absence of UV, the LC50 for cyanocobalt for *O. mykiss* was 112.9 mg/L, whereas in the presence of UV the LC50 was 0.38 mg/L (**Table 1**). Photo-enhanced toxicity was evident during exposures of *D. magna*. The 96 hour LC50 was 0.5 mg/L in the absence of UV, but below 0.25 mg/L in the presence of UV (see Table 1). Similar photo-enhanced toxicity of cyanocobalt was observed for *C. dubia*. In the absence of UV the LC50 was 2.29 mg/L for UV-naïve and 2.82 mg/L for UV-tolerant organisms, whereas in the presence of UV the LC50 was 0.12 mg/L for UV-naïve and 0.48 mg/L for UV-tolerant organisms (see Table 1).

The presence of humic acids reduced the toxicity of the cyanocobalt in the presence and absence of UV to *O. mykiss*. In the absence of UV at 113 mg/L mortality declined from 35% to 20% when humic acids were present. During UV exposure, a lower dose (0.38 mg/L) cyanocobalt was lethal to 30% of test organisms in the absence of humic acids, whereas there was no mortality among fish exposed to this concentration of cyanocobalt when humic acids were present (**Table 2**). Humic acids also significantly reduced the toxicity of cyanocobalt to *D. magna*. Mortality among UV exposed organisms declined from 100% to 40% among organisms exposed to 0.25 mg/L cyanocobalt and from 100% to 15% among controls. Humic acids did not significantly influence survival of *C. dubia* exposed to cyanocobalt alone or in the presence of UV (see Table 2). UV-adapted *C. dubia* were clearly less affected by cyanocobalt than non-adapted individuals, both in the presence and absence of UV (see Table 1).

Table 1: Acute toxicity (96 hr LC50 mg/L) of potassium hexacyanocobaltate to *Oncorhynchus mykiss*, *Daphnia magna*, and *Ceriodaphnia dubia* (naïve and UV adapted) exposed to a UV or No UV light treatment. Confidence intervals (95%) are in parentheses

Species	Replicates	No UV	UV
Oncorhynchus mykiss	**2**	112.9 (86.9–138.9)	0.3832 (0.3031–0.4634)
Daphnia magna	**3**	0.5019 (0.4021–0.6017)	< 0.25
Ceriodaphnia dubia (UV naïve)	**10**	2.2887 (0.471–4.11)	0.1250 (0.072–0.178)
Ceriodaphnia dubia (UV adapted)	**10**	2.8232 (2.13–3.52)	0.4790 (0.344–0.614)

Table 2: Percent mortality of rainbow trout exposed to potassium hexacyanocobaltate with or without the presence of dissolved organic carbon (DOC) under a UV and No UV light treatment. Standard deviations are in parentheses

Species	Conc. (mg/L)	No UV		Conc. (mg/L)	UV	
		No DOC	DOC		No DOC	DOC
Oncorhynchus mykiss	113	35 (2.2)	20 (1.4)	0.3832	30 (0.577)	0
	Control	0	0	Control	0	0
Ceriodaphnia dubia (UV naïve)	2.7	100	100	0.45	100	100
	Control	0	0	Control	100	100
Ceriodaphnia dubia (UV adapted)	2.7	0	0	0.45	20 (1.4)	30 (0.57)
	Control	0	0	Control	0	0

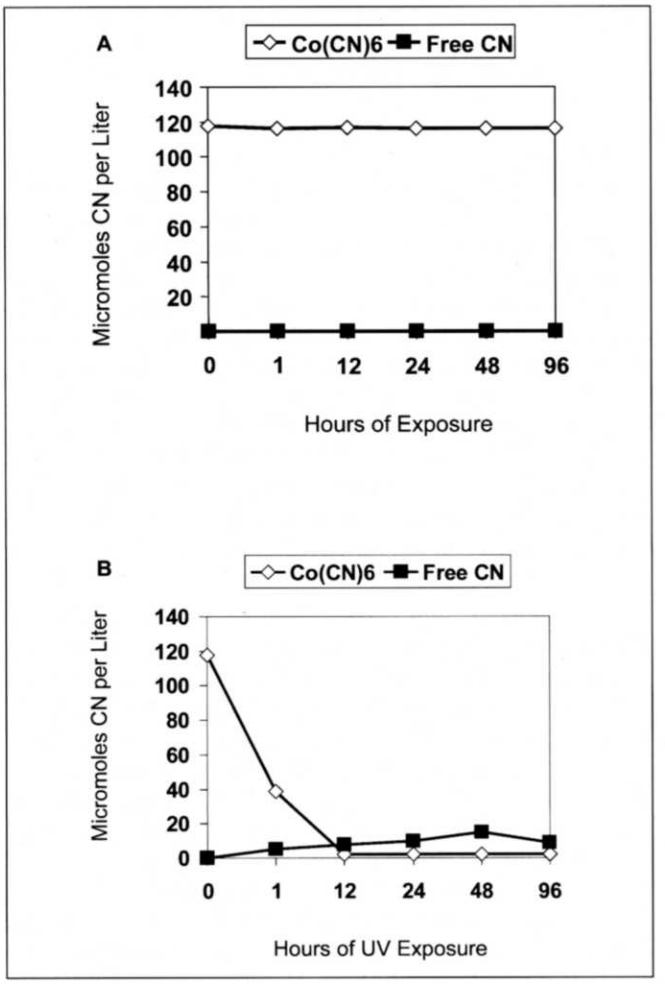

Fig. 1: Observed cyanocobalt and free cyanide concentrations observed (A) in the absence of UV and (B) in the presence of UV

Weak acid dissociable cyanide concentrations were below the limits of detection (0.2 mg/L) throughout the 96 hour exposure in the absence of UV. In contrast the concentration of potassium hexacyanocobaltate declined from 117 to 108 micromoles within the first 24 hours then remained stable at 108 micromoles for the duration of the 96 hour sampling period. In the presence of UV free cyanide was observed each sampling interval throughout the 96 hour period at concentrations ranging from 163 µg/L after a 1-hour UV exposure to a maximum of 343 µg/L following a 48-hour UV exposure. During this period, the concentrations of cyanohexacobaltate declined from 117 to 2 micromoles within the first 12 hours of UV exposure and remained at the limits of detection for the duration of the 96 hour exposure (**Fig. 1**).

3 Discussion

Cyanometallic complexes can result from mineral extraction and industrial processes and can be introduced into the environment as effluents from these activities, as well as from the use of cyanometallic compounds as pigments, anticaking supplements, corrosion inhibitors and water treatment flocculants (Eisler 1991). Whereas certain of the cyanometallic complexes may be decomposed by oxidation, hydrolysis, and catalysis, cobalt cyanocomplexes are resistant to attenuation by these pathways and to decomposition by acids. However, cobalt and iron cyanocomplexes can both photodissociate to release free cyanide (Flynn and Haslem 1995). In darkness, the cobalt cyanocomplex is relatively benign to fish and only moderately toxic to invertebrates, but in the presence of UV it becomes highly toxic. The onset of injury is apparent during the initial hour of exposure as abnormal behavioral activities and loss of equilibrium, a pattern that is also observed with ferrocyanide (Calfee and Little 2003a). During this time lethal concentrations of WAD cyanide are observed in the exposure water, as during ferrocyanide exposures. With respect to actual mineral processing effluents, free cyanide production from dissociation of strong cyanometallic complexes is generally considered to be environmentally insignificant (Smith and Mudder 1991). However, the results presented here support recommendations that have been made previously on the basis of photodissociation experiments (Broderius and Smith 1980) and chemical analyses of actual effluents (Johnson et al. 2002) that the environmental impact of photodissociation deserves further study.

Cyanide is a priority pollutant with a water quality criterion of 5 µg/L for freshwater and 200 µg/L for drinking water in the United States and Canada (EPA 1986). However, the criteria are vague as to the form of cyanide to be measured; the common interpretation is that it should be free cyanide. Free cyanide is highly reactive and is converted to other chemical and physical forms through reactions including volatilization, oxidation, sulfidation, complexation or precipitation by metals, hydrolysis to ammonia and carbonate, absorption to carbon, and biodegradation (Flynn and Haslem 1995). Derivative cyanide products can also be toxic, but less so than free cyanide (Boening and Chew 1999). Thiocyanates and cyanogens are highly reactive to ultraviolet radiation which can reconvert them to free cyanide. These species are not detected by the analytical methods that are commonly used for cyanide compliance. However in some jurisdictions, such as British Columbia, these may be measured incidentally during analysis of total cyanide or analysis of strong acid- dissociable cyanide plus thiocyanates (BCME 1986).

4 Conclusions

The humic and fulvic acids that form by decomposition of vegetation and give a tea color to water may play an important role in the toxicity of cyanometallic complexes. The reactive surfaces of the large humic acid carbon chain can absorb metals (McCarthy et al. 1990), effectively removing them from solution, and the same phenomenon may affect cyanide (Cao 1995). This type of response may explain the slight decrease in toxicity that occurred in the absence of UV when humic acids were present. Humic acids also absorb UV and thus limit irradiance of the water column, reducing the energy available for photochemical reactions (Scully and Lean 1990, Lean 1997). This effect was clearly demonstrated in tests with *O. mykiss* and *D. magna* in which a significant decrease in UV-induced mortality was observed when humic acids were added to the exposure water. Cyano-

cobalt LC50 concentrations for the UV studies were considerably lower than in the absence of UV, such that even in the presence of humic acids, the chemical was highly toxic. Studies with ferrocyanide have shown a similar decrease in UV toxicity enhancement when humic acids are present. In much the same manner, ferrocyanide was not toxic in stream studies conducted under heavily overcast conditions (Calfee and Little 2003b). Toxicity was also limited when turbidity was high (Little and Calfee 2002). Thus any environmental variable that limits UV will reduce the toxicity of both cobalt and iron cyanometallic complexes.

The specific physiological mechanism(s) responsible for the UV tolerance of UV-adapted *C. dubia* is not known, but there are several endogenous factors that might be proposed. The abundance and distribution of UV-absorbing compounds, such as melanin, would limit the dose of UV received by the organism (Little and Fabacher 2000). Alternatively, the adaptation may reflect selection of more effective detoxification mechanisms that could, for example, increase the deactivation of free radicals generated from UV or cyanide exposure, increase photo-repair of cellular injuries from UV or chemical exposure, or induce detoxification enzymes.

This study focused on cobalt cyanocomplexes because they appear to be present in effluents from gold leach operations, may be persistent in the environment, and may pose downstream toxicity risks as a consequence of photodissociation reactions in sunlight-exposed flow. Other cyanide-complexed metals, cyanogens, thiocyanates, and organically-bound cyanides might also be present in effluents of this type, such that the overall cyanide hazard would be most effectively monitored through whole effluent tests. However tests conducted in the absence of sunlight or UV irradiance may lead to an underestimation of toxicity.

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