Technical Memorandum 39

Toxic effects of cyanide on aquatic animals of the Alligator Rivers Region

G.D. Rippon, C.A. Le Gras,
R.V. Hyne and P.J. Cusbert

Supervising Scientist for
the Alligator Rivers Region

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ABSTRACT

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Due to the proposal to mine and process gold-bearing ore at Coronation Hill at the headwaters of the South Alligator River (SAR), the effect of cyanide in SAR water on local aquatic animals was studied using biological toxicity tests. The species tested were a water flea (*Moinodaphnia macleayi*), green hydra (*Hydra viridissima*), and the northern purple spotted gudgeon (*Mogurnda mogurnda*). The tests involved both lethal and sublethal endpoints, as well as chronic and acute exposures. Sodium cyanide dissolved in SAR water was used at concentrations of 2, 6.7, 20, 67 and 200 μg/L.

The number and size of water flea broods were significantly affected at 67 and 200 μg/L while no affect was seen for either hydra or the gudgeon embryos. Chemical analysis of the test waters showed, because of the high volatilisation of cyanide, that most of the cyanide was lost from test containers within 24 hours. The rate of loss was dependent on the surface area to volume ratio of the test container and solution; this complicates the interpretation of the results but does show the difficulty of trying to predict the fate, and therefore the toxicity, of any cyanide entry into the environment from such sources as a residue dam.

1 INTRODUCTION

A proposal to mine gold and associated metals at Coronation Hill in the Kakadu Conservation Zone was reviewed by the Resource Assessment Commission (1991). One of the issues assessed by the Commission was the potential risk to the environment posed by the use of cyanide in the gold extraction process. For instance, any water from mine residue dams associated with this process is likely to be contaminated with cyanide, and therefore pose a potential risk to the aquatic environment. This investigation was a preliminary assessment of the toxic effects of cyanide on aquatic animals of the Alligator Rivers Region which would allow risk assessment based on data for local animals and local water of the Region.

The general term 'cyanides' includes organic and inorganic compounds which contain the cyano (CN) group. It is the liberation of CN^- (and HCN thereafter) from these compounds that causes cyanide poisoning, of which the acute effect is well known. In the United States ambient water quality criteria to protect freshwater aquatic life from cyanides have been established in which the 4-day average concentration should not exceed 5.2 μg/L, nor the 1-hour average concentration exceed 22 μg/L, more than once every 3 years on average (USEPA 1985).

The toxicity of cyanide in aquatic systems can be greatly modified by local environmental conditions and can therefore be extremely complex. For example, some of the complex metal cyanides like the ferrocyanides and ferricyanides liberate HCN when exposed to sunlight. Nevertheless, the following can be said to give a general indication of the nature and fate of cyanides in the environment (USEPA 1978, 1985). Hydrogen cyanide and the alkali metal cyanides are very soluble in water while the alkaline earth metal cyanides are generally not. When pH is lower than 8 and temperature below 25°C, at least 94% of the free cyanide exists as HCN. If the pH is greater than 8 or the temperature above 25°C, more free cyanide will exist as CN^- (e.g. at pH 9 and 30°C, about 55% of the free cyanide will exist as HCN). The toxicity of cyanide to aquatic organisms is apparently due mainly to HCN, although CN^- is also toxic. There is significant loss of soluble free cyanide from the aquatic environment through volatilisation of HCN. The less soluble metal cyanides (e.g. CuCN) in acidic conditions may liberate some HCN which may therefore be lost through volatilisation. Microbial degradation and sorption onto particulates results in a significant loss of the less soluble metal cyanides from an aquatic environment. The biodegradation of complex metal cyanides (e.g. ferricyanides and ferrocyanides) is complex, with destabilising factors such as high temperature, sunlight, and extreme pH conditions interacting to cause breakdown. Without these factors complex metal cyanides can be expected to be persistent, and therefore transportable, in an aquatic environment. Simple metal cyanides and HCN do not bioaccumulate although the water soluble complex metal cyanides do, though to what extent is not known.

The possible routes for cyanide to reach the South Alligator River have been considered by Waite (1991) and Mackie (1990). These authors concluded that the seepage route is likely to give rise to negligible concentrations of cyanide in the river but that significant concentrations could result from overtopping of the residue dam or a road transport accident. Despite the complexities associated with cyanide toxicity described above, an assessment of the significance of these routes should be provided by measurement of the toxic effects induced by sodium cyanide dissolved in South Alligator River water.

The aim of this study was to determine the toxicity of cyanide (as sodium cyanide dissolved in SAR water) using the standard Biological Toxicity Tests (BTTs) of the Alligator Rivers Region Research Institute for three aquatic animals from the ARR (Holdway et al. 1991; McBride et al. 1991; Allison et al. 1991). The procedures use a freshwater fish (*Mogurnda mogurnda*), a water flea (*Moinodaphnia macleayi*) and a hydra (*Hydra viridissima*) as the test animals. These animals had been previously selected on the basis of

sensitivity and husbandry needs following an extensive screening program on nineteen aquatic species of the Region (Holdway et al. 1988). The time available for this project did not allow further screening of animals for sensitivity to cyanide nor for any alteration of the experimental design to enable the study of more complex interactions. The study did, however, incorporate measurements of the half-life of cyanide in solution for the various test procedures used.

2 MATERIALS AND METHODS

2.1 Biological toxicity testing

Three BTTs were used to assess the toxicity of cyanide, each using a different species and different endpoint(s). In this way it was assured that any chronic or acute effect of cyanide was likely to be assessed.

The purple-spotted gudgeon (*Mogurnda mogurnda*) was used in a 4 day embryo assay (Holdway et al. 1991). The test animals were less than 12h old at the start of the test and were observed each day for hatching and survival. There were ten embryos in each replicate with three replicates per concentration. Each replicate was held in a 9 cm diameter Petri dish with a test volume of 30 mL and a loosely-fitting lid. This is an acute test using both lethal and non-lethal endpoints.

Moinodaphnia macleayi, a water flea, was used in a 5 day reproduction assay (McBride et al. 1991). The test animals were less than 6h old at the start of the test, progeny of parthenogenic stock, and themselves also asexually reproducing through the test. There were ten individuals exposed to each concentration. Each individual was held in a 3.5 cm x 5.0 cm vial with a test volume of 35 mL and tightly-fitting cap; the lid had two ventilation holes. On each day of the test, brood numbers, including the number dead and living, and parent mortality was recorded. This is a chronic test with the water fleas exposed to the toxicant for most of their lifespan. The endpoint is reproduction based on brood size differences. Information was also gained on parent mortality but this is specifically tested under other experimental conditions (Hyne et al. 1991) and was not done for this study.

Green hydra (*Hydra viridissima*) were used in a 6 day population growth assay (Allison et al. 1991). The test animals were all in the process of budding (i.e. asexually reproducing) and their numbers were recorded each day. There were five hydra at the start of the test in each replicate with three replicates per concentration. Each replicate was held in a 9 cm diameter Petri dish with a test volume of 30 mL which was loosely sealed. This test is best described as an acute test using a combination of both lethal and non-lethal endpoints, i.e. population growth as the sum effect of mortality and reproduction.

All invertebrate stock were acclimatised to the SAR water one week prior to the commencement of the tests. The SAR water was filtered through two sheets of Whatman No. 1 filter paper prior to use in the cultures.

Sodium cyanide was the cyanide source for the test. A fresh 100 mg/L (in high purity water) stock solution was made each day of a test. This stock solution was diluted with the SAR water to give 6 L of a 200 μg/L test solution. Two litres of the 200 μg/L solution was needed for a dilution series to give the other test solutions; 67, 20, 6.7, and 2 μg/L. The remaining 4 L of 200 μg/L solution was used for chemical analysis of cyanide concentration using the isothermal diffusion method (see below). The control was the diluent alone, i.e. water of the South Alligator River. These solutions were stored in stoppered bottles with minimal head space until required. Minimal head space prevented any loss of cyanide by volatilisation of HCN from the test solutions. Individuals of each species were exposed to a nominal cyanide concentration of the above range. There was no attempt made to modify

the test containers to minimise volatilisation of HCN from the test solutions. The No-Observed-Effect-Concentration (NOEC) and the Lowest-Observed-Effect-Concentration (LOEC) values for sodium cyanide in SAR water were determined for each species/endpoint by statistical analyses of the results as specified in the respective BTTs.

Changes were made to the washing procedure of the BTTs to allow a more rapid turnover of test containers. The nitric acid rinse was omitted since it was only required for removal of metal residues. At the end of each test day, the test containers' contents were emptied into a waste container specifically designated for cyanide waste. This waste container was kept sealed when not in use. When full, for each litre of waste, 10 mL of 20% NaOH was added and mixed, and then any cyanide precipitated with 10 mL of a 1% $FeSO_4$ solution. After leaving to stand for 24 hours, it was emptied down a drain with copious volumes of water. The test containers were thoroughly rinsed with tap water before being briefly scrubbed with detergent, if necessary. The 24h immersion in detergent was not carried out according to BTT procedures. As long as the test containers were not allowed to become dry, a brief (but vigorous) scrub with detergent was enough since any cyanide residue should be minimal. The test containers were thoroughly rinsed again with tap water and then rinsed with distilled water and allowed to dry overnight. The test containers were thus ready for use the next test day.

The incubators were fitted with Sylvania *Gro Lux* fluorescent tubes which give a higher proportion of ultraviolet and near-ultraviolet light. The incubator had a 12 h photoperiod with the middle of the photophase coinciding with the solar midday. It had a temperature of 30°C $\pm$ 1°C.

2.2 Chemical analyses

Measurements of pH and alkalinity were made using a Metrohm model 682 titroprocessor. This apparatus was also used to determine the volume of 0.01M hydrochloric acid required to reduce the pH of SAR test water to 6.5. A Perkin-Elmer Lambda 2 spectrophotometer was used for absorbance measurements, with test water in the reference cell. The test water was filtered through Whatman 4.7 cm GF/C glass microfibre filters (nominal pore size about 1 μm) before use. Nuclepore 0.03 μm membrane filters were used to investigate the speciation of iron in the SAR water. Iron concentrations were determined using a Perkin-Elmer model 5000 atomic absorption spectrometer, with a model 500 graphite furnace. Determinations were performed on 1+19 dilutions using a 40 μg/L calibration standard and USEPA Trace Metal Sample No. 4 as reference. All chemicals used were of reagent grade and were dissolved in high purity (Millipore Super-Q) water.

Total free cyanide concentrations were determined spectrophotometrically with or without an isothermal diffusion step. Isothermal diffusion was based on the method of Broderius (1981). A 1 litre test sample or standard solution was placed in a 2 L Erlenmeyer flask which was sealed with a neoprene stopper. The pH of the solution had previously been adjusted to 6.5 by adding a predetermined volume of 0.01 M hydrochloric acid. A small petri dish was fused to a glass rod which was suspended from the stopper, and charged with 20 mL of 0.04 M sodium hydroxide solution using a pipette. The petri dish was suspended such that there was a clear space between it and the test solution.

Standard solutions of 0, 50, 100 and 200μg/L free cyanide were prepared in the SAR water by dilution of the stock solution (100 mg/L free cyanide in high purity water). The test solutions consisted of the 200 μg/L solution (i.e. the 4 L set aside for chemical analysis, as described above, in 1 L aliquots) and four 1 L aliquots of control (diluent) water. One litre of each test solution (with or without water flea food added at the rate of 1 μL of food for each mL of solution) was added to a 5 L cylindrical beaker. A control and treatment with and without food added were then taken for immediate analysis, while the others (i.e. control and treatment with and without food) were covered and left in the incubator for 24

hours and then analysed. These times corresponded to the start and end of each test day. The 1 L of solution in the 5 L beaker was meant to simulate the "worst-case" condition of cyanide volatilisation from the Petri dishes. It would also give some prior indication of the half-life of the cyanide solutions before a more detailed study was carried out in the following week.

Isothermal diffusion of HCN from test solutions at 0 and 24 hours and the standards was allowed to proceed for 4 hours, after which colour development and spectrophotometric measurement was performed on the sodium hydroxide absorbing solutions as described below. The proportion of HCN transferred from the test solutions to the absorbing solution was calculated to be about 7% under the conditions of the experiment, by comparison with standards determined directly. The detection limit for the method, defined as twice the standard deviation of the blank absorbance (n=15), was calculated to be 1 μg/L. The isothermal diffusion method has the advantages of eliminating interference from cyanide which is weakly complexed to metals, and providing a concentration factor which improves sensitivity (7% of the cyanide absorbed by 2% of the volume of the test solution). Nevertheless, it needs a large volume of test water, it is lengthy, tedious and labour intensive. Also, one litre of the test sample stored in a 5 L beaker did not accurately reproduce the conditions of the toxicity test.

Direct determination of cyanide in all test solution concentrations was, therefore, performed concurrently with the above isothermal diffusion method. These measurements were performed by adding the colour-forming reagents directly to an aliquot of the standard or sample solution. Because this aliquot was no greater than 20 mL, measurements could be performed on samples kept in containers identical to those used for the toxicity tests (i.e. 9 cm diameter Petri dishes and 5.0 cm x 3.5 cm vials). Determinations were performed at the start and end of each test day. Time and labour requirements were substantially reduced using the direct method, as was the volume of scarce test water used. In addition, very low blank absorbances were measured which demonstrated that interferences were absent. Also, the detection limit, as defined above was 2 μg/L (n=22), not much greater than for the isothermal diffusion method. Direct determination was used in addition to the former technique during the toxicity-testing experiments but was used exclusively for the experiments which measured the loss rate of HCN.

Experiments determining the rate of loss of HCN were performed on freshly prepared 200μg/L free cyanide solution only. Aliquots of this solution were dispensed into eight test containers of both types (i.e. Petri dish and vial). Each type of container was sampled at 2, 4, 8 and 24 hours (after preparation and dispensing) with duplicates for each time. Determinations were also performed in duplicate at 0 hours without regard to sample container. This protocol was performed on three successive days.

The spectrophotometric method used was modified from the standard method (American Public Health Association 1989). The reagents used were: 1% w/v chloramine-T solution in water; 15 g barbituric acid, 75 mL pyridine and 15 mL concentrated hydrochloric acid diluted to 250 mL with water; and 124.8 g sodium dihydrogen phosphate dihydrate and 40 mL concentrated phosphoric acid diluted to 1.0 L with water. 0.5 mL of the phosphate buffer, 0.5 mL of the chloramine-T solution and 1 mL of the barbituric acid reagent were added, in that order, to an aliquot of the solution to be determined and diluted to 25 mL in a volumetric flask. There was a 2 minute interval between addition of the chloramine-T solution and the barbituric acid reagent. The intensity of the magenta colour was measured after 15 minutes at 578 nm.

The aliquots of solutions used were as follows: for isothermal diffusion; 14mL for 0 μg/L and control water, 10 mL for 50 μg/L and 4 mL for 100 μg/L, 200 μg/L and test samples. For direct determinations: 1, 2, 5, 10 and 20 mL of 200 μg/L stock solution for 10, 20, 50, 100 and 200 μg/L calibration standards, and 20 mL for all test samples.

3 RESULTS

3.1 Biological toxicity testing

For the BTT using the purple spotted gudgeon, the 20 μg/L treatment group was excluded due to fungus. There was no significant effect of the toxicant on either of the endpoints (i.e. survival or hatchability) at any concentration used. Similarly, for the BTT using green hydra, no effect was observed. However, for the BTT using water flea, a marked effect was seen in reproduction as an end point (Table 1). The two highest concentrations were significantly different at the 0.05% level from the control. Therefore, 67 μg/L was deemed to be the LOEC while 20 μg/L was deemed to be the NOEC. Another effect was noted in the water flea, with the survival of the parental water flea being affected at 67 and 200 μg/L free cyanide (Table 1), although this can not be statistically analysed and confirmed. Death in the cyanide treated groups occurred mostly by Day 4 of the test.

Table 1. Effects of free cyanide on water flea reproduction

Concentration (μg/L nominal free cyanide)	0	2	6.7	20	67	200
Mean Brood No.§	47.4	42.2	42.5	43.5	27.2*	7.2*
Number of Surviving Parents	8ϕ	10	10	9	6	5

* significantly different from the control
ϕ 1 parent missing
§ n = number of surviving parents

3.2 Chemical analyses

The cyanide determinations performed concurrently with the toxicity tests gave two results. Firstly, they demonstrated that the concentrations of cyanide at t=0 in the toxicity-testing solutions compared satisfactorily with expected values. They were only about 10% lower than expected. Secondly, they demonstrated that most of the cyanide was lost from the test containers in a 24 hour period. The results are presented in Table 2.

Table 2. Mean measured concentrations of free cyanide (μg/L) for toxicity tests at t=0 hours and t=24 hours

Nominal Concentration	5L Beaker* (n=3)		Petri Dish* (n=2)		Screw-top Vial (n=4)	
	t=0h	t=24h	t=0h	t=24h	t=0h	t=24h
2.0					1.3	0.4
6.7					4.8	1.8
20					16	5.8
67					53	22
200	187	8.7	177	7.7	181	68
	(95% loss)		(96% loss)		(63% loss)ϕ	

* For the Petri dish experiments, the concentration of cyanide at t=24 hours was too low to measure for initial concentrations less than 200 μg/L. Concentration less than 200 μg/L were not prepared for the beaker experiment.

ϕ Determined by linear regression.

Experiments to determine the rate of loss of HCN from each of the containers used for toxicity tests were performed at 30°C. Cyanide determinations were carried out in duplicate at 0, 2, 4, 8 and 24 hours and the experiments were repeated twice on successive days. The results are presented in Table 3.

Table 3. Rate of loss of free cyanide (CN) from containers used in toxicity testing

Petri Dish			Screw-top Vial		
[CN](μg/L)	ln[CN]	t(h)	[CN](μg/L)	ln[CN]	t(h)
Experiment 1*					
203	5.31	0	203	5.31	0
125	4.83	2	153	5.03	2
82.5	4.41	4	152	5.02	4
35.5	3.57	8	105	4.65	8
4.1	1.41	24	51	3.93	24
Experiment 2					
191	5.25	0	191	5.25	0
139	4.93	2	173	5.15	2
98.5	4.59	4	153	5.03	4
54	3.99	8	124	4.82	8
4.6	1.52	24	595	4.09	24
Experiment 3					
197	5.28	0	197	5.28	0
132	4.88	2	185	5.22	2
90.5	4.51	4	169	5.13	4
44.5	3.80	8	134	4.89	8
4.7	1.55	24	63.5	4.15	24

* Screw-top vial results excluded from rate and half-life calculations

All plots of ln [free CN] versus time were linear with correlation coefficients > 0.99 except for experiment 1 with the screw-topped vial. The data for this experiment were not considered further. From the data in Table 3, a half-life ($t_{\frac{1}{2}}$) and an observed first-order rate constant (k_{obs}) can be calculated. These are presented in Table 4, together with data for the beakers used to prepare samples for isothermal diffusion. The rate of cyanide loss is not only dependent on surface area but also dependent on the volume of test solution. The limited available data suggest that the rate is inversely proportional to $V^{2/3}$. It must be stressed that each of the three vessels for which data are presented in Table 4 (and which were used in the toxicity tests) had a covering which, while impeding loss of HCN to the atmosphere, would not stop slow diffusion from the container. The use of different containers could give different results.

Table 4. Kinetic data for loss of cyanide from containers used in toxicity-testing

Container	Volume of Solution (mL)	Surface Area (cm^2)	k_{obs} (s^{-1}) $x10^{-5}$	$t^1/_2$ (h)
Beaker	1000	254	3.5	5.5
Petri Dish	30	63.6	4.3	4.5
Screw-top Vial	35	11.3	1.3	14.4

Iron is known to be present at significant concentrations in the South Alligator River. Trivalent iron forms a hexacyanoferrate ion with a stability constant of 10^{44} M^{-6}. This complex, having high thermodynamic stability, will form if any iron present exists as species which are kinetically labile. The concentration of iron was measured in four fractions based on filtration through different media. The results are presented in Table 5. It is evident that more than 97% of the iron is present in the >0.03μm fraction and therefore probably exists as hydrated iron oxides which are not kinetically labile.

Table 5. Speciation of iron in South Alligator River water based on filtration

Filtration Medium	Nominal Pore Size	[Fe] (μg/L)
Unfiltered	-	620
Whatman No. 1 Paper*	$\simeq$10 μm	550
Whatman GF/C Glass Microfibre$^{\phi}$	$\simeq$1 μm	380
Nuclepore Polycarbonate	0.03 μm	17

* Used to filter test water for acclimatising test species.
ϕ Used to filter test water before addition of reagents.

4 DISCUSSION

Only one BTT showed an effect of cyanide on the test organism, that of the water flea with reproduction as an end point. The results obtained were for the nominal concentrations of 67 μg/L for the LOEC and 20 μg/L free cyanide for the NOEC. However, the average cyanide concentrations would be much lower than nominal values because cyanide in the water flea test containers had a half-life of 14.4 hours. Our calculated average values compare favourably with the USEPA reported invertebrate chronic values (USEPA 1985). Although information gathered on the survival of parental water fleas can not be statistically validated, there would seem to be a pronounced effect of cyanide at the same concentrations as the chronic effects were seen. This is slightly lower than the literature results reported by the USEPA (1985) where another water flea, *Daphnia pulex*, had an LC_{50} for cyanide of 83 μg/L and 110 μg/L. Also, *Daphnia magna* was reported to have a 96h LC_{50} of 160 μg/L for cyanide as potassium cyanide (Dowden & Bennett 1965).

The other BTTs, using purple spotted gudgeon embryos and green hydra, showed no effect of cyanide on a variety of endpoints. This would appear to be most probably due to the very short half-life of cyanide in their test containers, only 4.4 hours. Nevertheless, warm water fish and fish embryos are generally less sensitive to cyanide (USEPA 1985).

Parent mortality, although not statistically substantiated because of the small parental numbers used in the test, did indicate an effect at 67 and 200 μg/L of free cyanide. Any

further study on the chronic effect of cyanide could more appropriately use the water flea survival test of Hyne et al. (1991). The survival test would be less lengthy and tedious.

The normal practice advocated by the Research Institute is to apply a safety factor of 10 to the results of laboratory tests to take into account statistical uncertainties in the experimental procedures and the variability of sensitivity of different species. Results from the water flea reproduction test therefore indicate a safe concentration of cyanide of about 3-6 μg/L, although it does not allow for the effect that volatilisation of cyanide had in lowering actual cyanide concentrations in the test. This preliminary figure is consistent with the recommendation of the United States Environmental Protection Agency national ambient water quality criterion of 5.2 micrograms per litre as a four day average, with the one hour average concentration not to exceed 22 micrograms per litre more than once every three years on average, for the protection of the aquatic environment.

When cyanide concentrations were determined for the test solutions, the measured values were 10% lower than expected. This effect seemed to be reproducible but the origin of the effect is not known. It may be related to degradation, complexation or loss of cyanide between the time when the 200 μg/L solution was prepared (about 8 a.m.) and the time when the toxicity experiments were begun (about 2p.m.). This is despite all solutions being stored in the interim in sealed bottles with minimal headspace.

The observed loss of cyanide, and therefore the effective concentration of cyanide to which the test organisms were exposed, was dependent on the container in which the test was performed. It is evident from the data for the petri dish and screw-topped vial that the rate of cyanide loss is directly proportional to the surface area of the vessel. This demonstrates that the primary mechanism for cyanide loss is very likely to be volatilisation of HCN rather than chemical degradation of cyanide, or reaction with metals ions such as iron (III) to produce complexes which do not react with the colour-forming reagents. However, degradation and complexation reactions may still occur to some extent. The assessment of any possible impact of cyanide on the environment made from these experiments must be constrained by the experimental procedures used. Some of the more important factors are now discussed.

There was a clear difference in the loss of cyanide for each type of test container and volume used. Therefore the concentration of the toxicant experienced by the test organism was dependent on the physical shape of the test container. Any further study of cyanide toxicity should only be conducted after careful consideration is given to the design of test containers.

The solubility of HCN in water increases as temperature decreases. The present experiments were performed at 30°C, which approximates the temperature of the South Alligator River from about September to March. During the Dry season, however the temperature of the river may decrease to less than 20°C. The retention of cyanide should then increase, thereby exposing aquatic species to a higher effective concentration of the toxicant. There is some evidence, from duplicate experiments using the isothermal diffusion technique, that HCN is evolved more rapidly from SAR water than from high purity water. After 4 hours about 10% more HCN was lost from the test water than from Super-Q water. This is most likely due to the dissolved salts in the test water reducing the solubility of HCN. If this is the case, the concentration of dissolved salts would have a small effect on the fate of cyanide in the test water. It is possible that evolution of HCN more rapidly from SAR water could be caused by reducing the pH to 6.5 for the isothermal diffusion experiments. This might result in the CO_2 equilibrium between the water and atmosphere being disturbed with the degassing of CO_2 from solution and the simultaneous loss of HCN.

It would seem that iron oxide has limited capacity to react with cyanide on the time scale of the toxicity tests, but its ability to complex free cyanide cannot be wholly excluded. It may, for example be responsible for the small reduction in cyanide concentrations

observed in solutions stored for several hours in sealed bottles with minimal head space. Also, ultraviolet radiation can cause photolytic dissociation of metal-cyanide complexes. Although there is currently no evidence of cyano complexes in the South Alligator River, such compounds may form if free cyanide makes contact with metal ions. These may later release free cyanide if exposed to increased UV radiation. If this were to happen in the SAR, the fate and consequence of cyanide in the environment would become extremely complex.

Any pH excursion above 8 would be expected to alter the toxicity of cyanide. Although HCN would predominate with the average pH conditions of the SAR water (i.e. pH of about 7.0) and is more toxic than the CN^- ion to fish (Lind et al. 1977), an increase in pH of simple cyanide solutions would cause an increase in free cyanide as CN^-. This situation would be unlikely to occur in the SAR system unless ambient conditions were modified due to any mining process. Indeed, the pH would need to rise to about 8.3 before the proportion of free cyanide present as CN^- increased to 10% (calculated from Aylward & Findlay 1974).

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