A reliable, easy, and inexpensive method for determination of molecular hydrocyanic acid (HCN) in solutions of simple and complex metal cyanides is described. The method was used to determine molecular HCN concentrations as low as 0.005 milligram per liter, and can be used for determination of even lower levels. It is a modification of a previously published method. A concentration column of glass beads coated with NaOH is employed, on which HCN displaced by air that has been bubbled through solutions under examination is trapped and concentrated for measurement of cyanide by a conventional analytical method. The apparatus could easily be modified for use in both field and laboratory situations where only limited facilities are available.

Time periods required for attainment of equilibria upon dilution of solutions of metal-cyanide complexes, and also when metal salts
and free cyanide are combined, were quite variable and ranged from several hours for the silver-cyanide complex to many months for iron-cyanide complexes kept in the dark. In solutions in which CuCN and NaCN were combined so that the molar ratio of CN to Cu was either 2.5 to 1 or 3 to 1, constancy of the HCN concentration usually was not attained even 110 days after preparation. The time to attainment of equilibrium through dissociation of the nickelocyancne complex ions generally was longer than that required for equilibrium to be attained in comparable experiments on complex formation, and it increased as the pH or the total cyanide concentration decreased; it is directly related to the percentage of total cyanide present as HCN at equilibrium. Results obtained at high total cyanide concentrations in nickelocyancne formation experiments were anomalous but verifiable by bioassay with fish. The HCN concentrations were at first unexpectedly low and then increased very slowly to the higher equilibrium levels.

Cumulative dissociation constants (K_D) at 20°C for the Ag(CN)_2^-, Cu(CN)_2^-, Ni(CN)_4^{2-}, Fe(CN)_6^{4-}, and Fe(CN)_6^{3-} complex ions, calculated from equilibrium levels of HCN, are 1.94 ± 2.82 x 10^{-19}, 3.94 ± 1.75 x 10^{-24}, 1.00 ± 0.37 x 10^{-31}, approximately 10^{-47}, and approximately 10^{-52}, respectively. The calculated constants for the tetracyanoni-celate (II) and dicyanoargentate (I) complex ions inexplicably varied somewhat, increasing slightly with increase in total cyanide concentration and pH. Those for the tetracyanonickelate (II) and
dicyanocuprate (I) complex ions showed close agreement with values recently reported in the literature, whereas the constants for the dicyanoargentate (I) and hexacyanoferrate (II) and (III) complex ions were materially different from presently accepted values. Possible unreliability of presently accepted stepwise constants for the cuprocyanide complex ions also was indicated.

The acute toxicity of solutions of the different metal-cyanide complexes was generally found to be a function of the molecular HCN level, which increases with increase of total cyanide concentration and with decrease of pH. In some solutions however, a metal-cyanide complex ion per se was shown to be the major toxic component. The 48-hour median tolerance limits for bluegills of the dicyanoargentate (I) and dicyanocuprate (I) ions at 20°C were found to be approximately 9 and 4 mg/l as CN, respectively. The metallo cyanide complex ions studied can be arranged in order of decreasing toxicity as follows:

\[ \text{Cu(CN)}_2^-, \text{Ag(CN)}_2^-, \text{Ni(CN)}_4^{2-}, \text{and Fe(CN)}_6^{3-} \text{ or Fe(CN)}_6^{4-}. \]

A published empirical relationship between pH and 48-hour median tolerance limits of the nickelocyanide complex for a fish, determined without assurance that equilibria had been attained in test solutions, was compared with a calculated, theoretical relationship. Considerable divergence of the empirical and theoretical curves at pH values less than about 7.2 is ascribable mostly to the introduction of fish into test solutions long before equilibria had been attained in the
solutions of low pH. Divergence at pH values greater than about 7.8 is attributable largely to moderate toxicity of the Ni(CN)$_4$\(^2^-\) complex ion itself.

Slightly alkaline solutions of the silver cyanide complex, Ag(CN)$_2^-$, become more toxic to sticklebacks with increase of chlorinity. The high toxicity in saline solutions, as compared with the toxicity in fresh water, is clearly attributable, at least in part or in some instances, to a molecular HCN content of the saline solutions much greater than that of comparable solutions prepared with fresh water. The two ligands CN\(^-\) and Cl\(^-\) compete for the silver ion, with which both ligands form complexes, and dissociation of the Ag(CN)$_2^-$ ion, with production of HCN, consequently increases as the Cl\(^-\) ion concentration increases. Additional reasons for the observed increase of toxicity of solutions of the complex with increase of chlorinity can be an observed increase of the toxicity of HCN and a possible, similar increase of the toxicity of the complex ion.

Experiments with $^{14}$C-labeled cyanide complexed with nickel showed that the complex does not penetrate readily into the body of a bluegill. The $^{14}$C accumulated in gill tissues much more markedly than it did in the blood and in tissues of internal organs sampled. When bluegills were exposed to solutions of the cyanide complexes of copper (I) and silver (I), considerable amounts of the metals accumulated in the blood and in tissues of internal organs, but little accumulation in
gill tissues was observed. These results indicate that the cuprocyanide and silver-cyanide complexes enter the body of a bluegill much more readily than does the much less toxic nickelocyanide complex. The silver cation, however, apparently enters even more readily than does the silver-cyanide complex anion, the silver accumulating most markedly in the gill tissues of bluegills exposed to silver nitrate solutions, but also in their internal organ tissues.
Determination of Molecular Hydrocyanic Acid in Water
and Studies of the Chemistry and Toxicity to Fish
of Metal-Cyanide Complexes

by

Steven James Broderius

A THESIS

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INTRODUCTION

Cyanides have long been recognized as substances highly toxic to fish. Cyanides are extensively employed in industry and occur in effluents from many different industrial processes such as the case hardening of steel, the extraction of precious metals, electroplating and metal-finishing, and the scrubbing of coke-oven or blast-furnace gases. Sodium cyanide is a raw material used in the manufacture of Prussian blue, other cyanide complexes, nitriles, aldehydes, fertilizers, rodenticides, explosives, and methyl acrylate resins. A very large increase in the annual production of acrylonitrile, which decomposes to produce hydrocyanic acid, has occurred since the early 1950's. Many industrial effluents and receiving waters therefore contain the cyanide radical.

In past years, numerous studies have been made to determine the toxicity to various fish species of simple alkali-metal cyanides and the influence of factors such as pH, temperature, length and weight of the fish, water hardness, oxygen concentration, etc., on this toxicity. In general, it can be concluded that the toxicity of simple cyanide solutions to fish increases with increasing temperature, any reduction in dissolved oxygen below the 100 percent saturation level,
and decreasing pH. But there is apparently no relationship of toxicity to alkalinity and hardness of the dilution water. The minimum lethal (threshold) concentrations of free cyanide for brook trout (Karsten, 1934), rainbow trout (Herbert and Merkens, 1952), brown trout (Burdick, Dean and Harris, 1958), smallmouth bass (Burdick et al., 1958), two-spotted cichlids (Brockway, 1963), and bluegills (Doudoroff, Leduc and Schneider, 1966) are reported to be 0.05, 0.07 (0.06 determined concentration), 0.09, 0.104, 0.12, and 0.15 mg/l as CN, respectively.

Many of the cyanide-bearing wastes, as well as other industrial wastes discharged to the same waters, contain heavy metals, which also can endanger aquatic life. It has long been known that simple cyanides readily react with certain heavy-metal salts, forming complex cyanides (double salts). Hydrocyanic acid is formed in solutions of these salts through dissociation or decomposition of the complex metalocyancide anions and hydrolytic reaction with water of the cyanide ions so liberated. Doudoroff (1956) presented indirect evidence that the toxicity to fish of dilute solutions containing some metal-cyanide complexes was due primarily to the presence of molecular hydrocyanic acid (HCN). Direct evidence presented by Doudoroff et al. (1966) established that the acute toxicity of solutions of various simple cyanides and metal-cyanide complexes to fish is determined by the concentration of undissociated molecular
hydrocyanic acid (HCN) virtually alone, and not of the cyanide ion (CN)\(^-\) nor of most metallocyanide anions. In general, they observed that "median immobilization times" for the bluegills in these solutions varied independently of total cyanide concentrations but were closely related to molecular HCN levels between 0.18 and 1.0 mg/l, decreasing as the HCN levels increased.

Very little of the published work on the toxic effects of cyanides on fish life has dealt with the metallocyanides. Even chemical knowledge concerning the complex cyanides formed when solutions of simple alkali-metal cyanides and heavy metals are combined is still quite limited. Therefore, a study designed to investigate not only the toxicity of metallocyanide solutions to fish but also the various chemical reactions that cyanides and heavy-metals undergo when combined in an aqueous medium and the rates of these reactions was deemed warranted.

**Toxicity of Metal-Cyanide Complexes**

The toxicity of different metal-cyanide complex solutions has been shown to vary widely in that some of the complexes are relatively nontoxic whereas others approach HCN in toxicity. Stumm, Woker and Fischer (1954) noted that the relative toxicities to fish of the metal-cyanide complexes in comparable solutions generally tend to decrease as the stabilities (or the formation constants) of the complexes increase.
Environmental factors such as pH of the solution and degree of exposure to sunlight also influence the extent of dissociation or decomposition of the complexes and resultant release of toxic hydrocyanic acid. A comprehensive review of the literature on the toxicity of free cyanide and metal-cyanide complexes to fish is included in a paper by Doudoroff et al. (1966).

**Zinc- and Cadmium-Cyanide Complexes**

Cyanide complexed with some metals can be potentially as dangerous to fish as free cyanide. Computations using the dissociation constants of approximately $10^{-17}$ and $10^{-19}$, which have been determined for the zinc ($\text{Zn(CN)}_4^{2-}$) and cadmium ($\text{Cd(CN)}_4^{2-}$) cyanide complexes, respectively, show that in very dilute solutions virtually complete dissociation of these complex ions is to be expected at any pH value.

Doudoroff (1956) performed acute toxicity bioassays with young fathead minnows, *Pimephales promelas* Rafinesque, of experimental solutions prepared by diluting freshly mixed, concentrated solutions of NaCN and ZnSO$_4$ or NaCN and CdSO$_4$ in which the ratio of zinc to cyanide (Zn/CN) by weight was 0.7 and that of cadmium to cyanide (Cd/CN) was 1.2. Since the corresponding ratio for the Zn(CN)$_4^{2-}$ ion is 0.63 and that for the Cd(CN)$_4^{2-}$ ion is 1.08, there was a slight excess of metal ion added in both cases. A few tests were also
performed using a purified potassium zinc-cyanide in which the cyanide to zinc molar ratio was 4 to 1. Doudoroff's experimental data on the toxicity of the zinc-cyanide and cadmium-cyanide complex solutions supported the previously published dissociation constants for the complex ions in that these solutions proved to be exceedingly toxic at low concentrations, their toxicity being apparently due to cyanide. In fact, synergism of the free cyanide and the toxic metallic ions (Zn++, Cd++) liberated through dissociation of the complex ions was indicated, since the estimated 24-, 48-, and 96-hour median tolerance limits (TLm) of the complexes, expressed as mg/l of total cyanide, were lower than that of NaCN alone. Doudoroff (1956) therefore concluded that simple cyanide solutions are not effectively detoxified by the addition of zinc or cadmium salts in quantities insufficient to precipitate most of the cyanide as the insoluble metal-cyanide salt.

Turnbull, DeMann and Weston (1954) also observed a synergistic effect like that mentioned above. They found that a cadmium plating solution had toxicity to bluegills greater than that indicated by its total cyanide concentration. The 24-hour TLm of the plating solution and that of potassium cyanide were equivalent to 0.18 mg/l CN and 0.28 mg/l CN, respectively.

Nickel-Cyanide Complex

Milne (1950) suggested that the free cyanide could be combined
with nickel in effluents from metal-finishing plants in such proportions that most of the cyanide ions would be bound up as a nickel-cyanide complex. The toxic properties of the effluents theoretically would then be eliminated and could be disposed of safely into waters of normal pH values. In so stating, Milne must have believed that the release of cyanide ions through the dissociation of the tetracyanonickelate (II) complex (Ni(CN)$_4^{2-}$), and their subsequent combination with available hydrogen ions to form HCN until equilibrium is reached, is negligible in dilute solutions in which the pH is within the range of values usually found in natural waters. In fact, the dissociation equilibrium for HCN (i.e., HCN $\rightleftharpoons$ H$^+$ + CN$^-$) was not even incorporated into the calculations on which his conclusions were based. However, Milne did perform a bioassay that supported his theoretical conclusion, since a 104 mg/l total cyanide solution at pH 8.1, in which the cyanide to nickel molar ratio was 3.05 to 1, was not lethal to young goldfish during a 24-hour exposure period.

Doudoroff (1956) reported that when approximately 4 moles of free cyanide combined with 1 mole of nickel ion to form the fairly stable Ni(CN)$_4^{2-}$ complex ions, solutions of the complex proved less toxic than solutions with corresponding concentrations of free cyanide, especially at high concentrations and high pH values. Nickel was able to displace zinc and cadmium from their cyanide complexes, forming the more stable tetracyanonickelate (II) complex. Doudoroff stated
that in nickel-cyanide complex solutions only a minute portion of the 
total cyanide is free at pH near and above 7.5 and that the remainder 
exists in the form of the relatively nontoxic tetracyanonickelate (II) 
complex ion. However, total cyanide concentrations even as low as 
0.5 mg/l as CN in the solutions of the nickel-cyanide complex proved 
to be toxic at low pH values that could occur in natural waters, the 
toxicity of the solutions at pH 6.5 approaching that of sodium cyanide 
solutions of the same cyanide content. Doudoroff also reported that 
the toxicity to fathead minnows of a solution containing the tetracyano-
nickelate (II) complex ion increased more than a thousandfold with 
decrease of pH from 8.0 to 6.5; a tenfold to thirteenfold decrease of 
median tolerance limits was observed when the pH was reduced from 
7.8 to 7.5 (i.e., when the hydrogen ion concentration was doubled). 
At pH 6.5, 1.0 mg/l of cyanide as CN combined with nickel proved 
more toxic than did 1,000 mg/l at pH 8.0. The increase of toxicity 
with decrease of pH was explained as being a consequence of great 
increase of dissociation of the Ni(CN)$_4$$^{2-}$ complex ion resulting from 
combination of cyanide ions liberated by this dissociation with hydro-
gen ions to form molecular HCN. It was also demonstrated by 
Doudoroff et al. (1966) that changes in CO$_2$ content and pH of the 
medium occurring at gill surfaces had no appreciable influence on the 
toxicity of the tetracyanonickelate (II) complex. This was interpreted 
to mean that dissociation of the Ni(CN)$_4$$^{2-}$ complex ion is not rapid
enough to increase the molecular HCN concentration at the gill surfaces as the solution is pumped over the gills. Exposure of a dilute nickel-cyanide complex solution to bright sunlight did not result in any photo-decomposition of the complex and consequent increase of toxicity.

With respect to their shape, Doudoroff's (1956) sigmoid curves relating logarithms of 24-, 48-, and 96-hour median tolerance limits (50 percent lethal concentrations) to pH do not agree fully with theoretical curves relating pH and the logarithms of total cyanide concentrations required to produce constant HCN levels that would account for the observed constant levels of toxicity. At very low pH levels, the increase of toxicity was found to be less than that which was theoretically predictable on the basis of the assumptions that molecular HCN was the only toxic component of the test solutions and that equilibria had been attained. With increase of pH to the highest tested level, the decrease of toxicity proved less than that which was predictable on the same basis. The slope of the middle portion of the pH-toxicity curves did not differ greatly from the theoretical slope. Doudoroff (1956) gave a partial but apparently inadequate explanation of the shape of the observed curves. He stated that very dilute solutions with relatively low pH must undergo a considerable, gradual loss of cyanide, and therefore the initial total concentration of cyanide necessary to render the solutions fatal to fish is higher than it would
be if no such loss occurred. Doudoroff also concluded that the high toxicity of the concentrated solutions with high pH probably is not completely referable to HCN and that some ions present in these solutions probably contributed considerably to the toxicity, their relative importance increasing with the concentration and pH of the solutions.

Some unpublished data of Doudoroff's (personal communication) provided verification of the unduly low initial toxicity of dilute and acid nickelocyanide solutions. He also observed that, after aging in sealed vessels, these solutions are much more toxic than freshly prepared solutions, their toxicity apparently approaching the theoretical value. This result indicated slow dissociation of the tetracyanonickelate (II) complex ion.

A major objective of the research here reported was to clarify further the discrepancy between the observed and theoretical toxicities of nickel-cyanide complex solutions, examining in some detail the rates of dissociation and formation of the tetracyanonickelate (II) complex ion in solutions of varying total cyanide concentration and pH. This was done by repeated chemical analysis of specially prepared fresh and aged solutions to determine rates of attainment of chemical equilibria, and also by performing acute toxicity bioassays of the solutions, with fish as test animals.
Iron-Cyanide Complexes

Several investigators have shown that very dilute solutions of metal-cyanide complexes can be highly toxic to fish. Burdick and Lipschuetz (1950) reported that dilute solutions of potassium ferrocyanide and ferricyanide (1-2 mg/l as salt or 0.07-0.16 mg/l as CN), with pH 7.2-7.3, when exposed to bright sunlight, rapidly become highly toxic to fish, because of photo-decomposition of the otherwise highly stable complexes (i.e., complexes with large formation constants) and liberation of cyanide ion. Myers and Iezzi (1950), working independently of Burdick and Lipschuetz, also reported that irradiation increases the toxicities of ferro- and ferricyanide compounds. According to Burdick and Lipschuetz (1950), decomposition of the complexes under the influence of sunlight had been previously reported in the chemical literature to be accelerated in the presence of oxygen. Burdick and Lipschuetz suggested that the slight toxicity of concentrated solutions of these salts kept in diffuse light and in the dark is due to the liberation of cyanide through decomposition or dissociation of the complexes at a very slow rate. They concluded that a large part of the actinic rays producing decomposition are in the ultraviolet wavelengths. No toxic effect having been observed with potassium ferrocyanide solutions with concentrations under 2,000 mg/l as the salt were kept in diffuse light or in the dark, the hexacyanoferrate (II) ion itself evidently has little if any toxicity to fish.
Doudoroff (1956) observed that a 10 mg/l as CN solution of reagent grade potassium ferrocyanide, with a weight ratio of iron to cyanide of 0.36, did not kill fathead minnows in 48 hours. But solutions prepared by first adding NaCN and then FeSO$_4$ to water in amounts equivalent to 0.33 (or 0.5) mg/l CN and 0.13 (or 0.2) mg/l Fe (II) were highly toxic even after 27 hours. Their toxicity apparently was due to residual free cyanide which was not complexed, because much iron was precipitated as iron hydroxide. A solution containing 482 mg/l of cyanide (as CN) and 193 mg/l of iron, prepared by gradually adding a solution of FeSO$_4$ to a solution of NaCN, proved toxic even when much diluted. A concentration of the mixture equivalent to 10 mg/l of cyanide proved fatal to all the test fish within 48 hours, whereas concentrations equivalent to 1.0 to 4.0 mg/l of cyanide were not lethal after a 48-hour exposure. Doudoroff concluded that though some formation of a complex did occur in a concentrated mixture of NaCN and FeSO$_4$ with a weight ratio of iron to cyanide of 0.4, it was evident that very little if any combination of cyanide and ferrous ions occurred when FeSO$_4$ was added to a very dilute NaCN solution.

It has been noted that, in the dark or under diffuse laboratory light, potassium ferro- and ferricyanide did not prove toxic to fish at concentrations under 2,000 mg/l as salt. Assuming that equilibrium had been nearly attained, it is not possible to reconcile these toxicity
data with the presently accepted dissociation constants for the
Fe(CN)$_6^{3-}$ and Fe(CN)$_6^{4-}$ complex ions, namely, $10^{-35.4}$ and $10^{-43.6}$ (Watt, Christensen and Izatt, 1965), respectively. If indeed these were the correct values, the test solutions of the iron-cyanide complexes would have been much more toxic even in the dark than they were reported to have been. Therefore, since numerically smaller dissociation constants are indicated, a need for their re-evaluation was apparent and another objective of this research was to estimate the magnitude of these constants.

**Silver-Cyanide Complex**

Only limited data on the toxicity of the moderately stable silver-cyanide complex has been reported. Doudoroff et al. (1966) observed that, in a test solution of the silver cyanide complex with 10 mg/I of total cyanide, a silver to cyanide (Ag/CN) weight ratio of 2.08 (erroneously reported as 0.48), and pH 7.5, the molecular HCN concentration was barely measurable (about 0.02 mg/I), indicating very little dissociation of the complex. Yet, bluegills lived little longer in this solution than they did in a solution with the same total cyanide content but with pH 6.5 and a molecular HCN level near 0.12 mg/I. The authors regarded this result as an indication that the Ag(CN)$_2^-$ ion itself, unlike the Ni(CN)$_4^{2-}$ ion, has considerable toxicity to fish and that the toxicity of the slightly alkaline solution was attributable
mostly or entirely to this lethal factor. The toxicity of a similar but decidedly acid solution, with pH 6.0, was judged to have been due mostly or entirely to its relatively high HCN content (0.19 mg/l), the fish dying in this solution showing signs of cyanide poisoning. Blue-gills dying in the more alkaline solutions often showed no such signs but only superficial coagulation of mucus suggestive of heavy-metal poisoning.

Doudoroff (personal communication) later found also that the toxicity to bluegills of acid solutions of the silver-cyanide complex could be materially increased by the addition of sodium chloride. The possibility of withdrawal of silver ion from such solutions, through formation of small amounts of insoluble (perhaps colloidal) silver chloride and consequent liberation of cyanide ion from the silver-cyanide complex, had been suggested earlier by Doudoroff et al. (1966). They believed that this reaction probably could result in formation of more HCN in the solutions than would have been present in the absence of chloride ion.

It can be shown that the HCN concentrations found by Doudoroff et al. (1966) in three silver-cyanide complex solutions at various pH values and containing 10 mg/l total cyanide were by about four times greater than the values that can be calculated by using the presently accepted dissociation constant for the Ag(CN)$_2^-$ complex ion of $3.67 \times 10^{-21}$ (Azzam and Shimi, 1963) and the ionization constant $(K_a)$ for
HCN of $4.365 \times 10^{-10}$ (Izatt et al., 1962). Assuming the above $K_a$ for HCN to be correct, a value for the dissociation constant of the complex of $1.75 \times 10^{-19}$ can be calculated from the HCN concentrations determined by Doudoroff et al. (1966). Because of the apparent discrepancy between this and previously accepted values for the constant, another objective of this research was to re-evaluate the dissociation constant for the dicyanoargentate (II) complex ion.

Since the toxicity of the Ag(CN)$_2^-$ ion was found by Doudoroff et al. (1966) apparently to be much greater than that reported for other metal-cyanide complex ions, evaluation of the toxicity of this complex ion was undertaken by me. The effect that the chloride ion has on the toxicity of silver-cyanide solutions through its competition with cyanide for silver ions was also investigated.

**Copper-Cyanide Complexes**

Lipschuetz and Cooper (1955) found that KCN solutions were about two to four times as toxic to blacknose dace (*Rhinichthys atratulus meleagris*) as some potassium cuprocyanide solutions, with corresponding total cyanide content prepared by dissolving cuprous cyanide (CuCN) in KCN solutions. This means that more total cyanide is required to produce a given toxic effect when it is present as cuprocyanide than when it is present as a simple cyanide. The toxicity of these cuprocyanide solutions, prepared with tap water of pH 7.6 to
8.0, decreased as the ratio of gram moles of total cyanide to gram atoms of copper decreased from 4.0 to 3.0.

The toxicity of NaCN and CuSO₄ solutions, in which the copper-to-cyanide weight ratio was 0.8 (i.e., a mole ratio of about 1 to 3), was evaluated by Doudoroff (1956). The fairly concentrated NaCN-CuSO₄ stock solutions that he prepared (533 mg/l total cyanide as CN) had the odor of HCN, and the behavior of fish in lethal dilutions of this mixture suggested poisoning with free cyanide. Bioassays performed with fathead minnows showed that a test solution prepared by diluting this stock solution and containing only 1.0 mg/l of cyanide (as CN) was not lethal in 96 hours. Neither exposure of this solution to bright sunlight for 6 hours nor its prolonged aeration rendered it demonstrably toxic to the fish. When a similar solution was prepared by adding 0.8 mg/l of copper to a solution of NaCN containing 1.0 mg/l of cyanide (as CN), some toxicity apparently due to cyanide was observed; of 20 fish used in four tests only nine survived for 96 hours.

Doudoroff (1956) stated that the behavior and appearance of fish affected or dying in dilute, nearly neutral or slightly acid solutions prepared by diluting a stock NaCN-CuSO₄ mixed solution with distilled or extremely soft waters to a concentration equivalent to 0.5 mg/l of copper and 0.62 mg/l CN indicated that death was attributable to poisoning with copper, rather than cyanide. The toxicity appeared to increase with prolonged, continuous aeration of the solutions, whereby
removal of volatile HCN was accelerated. The removal of HCN presumably caused an increase of the concentration of free copper, which was liberated through dissociation or decomposition of relatively harmless and fairly stable complex ions at the low pH of the solutions.

Doudoroff et al. (1966) proposed an explanation for the observed toxicity in slightly alkaline water of a mixture of NaCN and CuSO₄ solutions like that tested earlier by Doudoroff (1956), which toxicity was apparently attributable to hydrocyanic acid. They theorized that the mixture must soon have a total cyanide concentration lower than that initially present because of oxidation of cyanide to cyanogen with reduction of the copper to the monovalent state (Griffith, 1962). However, because some cyanide must be regenerated by hydrolysis of the cyanogen, the net reaction equation becomes:

$$6\text{CN}^- + 2\text{Cu}^{++} \rightleftharpoons 2\text{Cu(CN)}_2^- + \text{C}_2\text{N}_2$$

$$\text{C}_2\text{N}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCN} + \text{HCNO}$$

$$\text{HCNO} + \text{H}_2\text{O} \rightleftharpoons \text{CO}_2 + \text{NH}_3$$

Complete hydrolysis of C₂N₂ should reduce the overall loss of cyanide to only one-sixth of the initial amount of cyanide added. Thus, assuming essentially complete reduction of Cu(II) to Cu(I), Doudoroff et al. (1966) concluded that, since some portion of the volatile C₂N₂ and HCN must have escaped to the atmosphere and hydrolysis of C₂N₂
may not have been complete, the final amounts of total cyanide in their test solutions were between four-sixths and five-sixths of the original amounts. Because the initial mole ratio of total cyanide to copper \((CN/Cu)\) was approximately 3, the final ratio must have had a value between 2 and 2.5. From calculations made by the authors, using stepwise dissociation constants of the copper complex ions reported by Penneman and Jones (1956), it was concluded that most of the cyanide was present as the \(Cu(CN)_2^-\) ion and only a very small portion of the cyanide present in excess of two gram moles per gram-atom of copper could have been in the form of the \(Cu(CN)_{3}^{2-}\) ion. The excess cyanide present as free cyanide was analytically determined to amount to about one-tenth of the initial amount of cyanide added in preparing the test solution. The results of Doudoroff (1956) show that the toxicity of copper in concentrations less than 1 mg/l can be markedly reduced or eliminated by complexing the copper with cyanide. The presence of zinc, which is synergistic with copper, did not prevent the inactivation of small amounts of copper by means of cyanide.

The chemical reactions in the \(NaCN-CuSO_4\) mixed solutions are complex and apparently are not fully understood. Therefore, the chemistry and toxicity of copper-cyanide complex solutions were investigated by studying only solutions in which cuprous cyanide \((CuCN)\) and sodium cyanide \((NaCN)\) were combined, so that reduction of
copper and oxidation of cyanide did not have to be considered. The rates of dissociation and formation of the cuprocyanide complex ions and HCN equilibrium levels in solutions of varying total cyanide concentration, pH, and cyanide to copper ratios were examined in some detail. This was done by repeated chemical analysis of specially prepared fresh and aged solutions to determine rates of attainment of chemical equilibria. Additional objectives of this research were to determine to what extent the acute toxicity of the solutions at equilibrium is related to determined HCN concentrations and to evaluate the toxicity of the dicyanocuprate (I) complex ion itself.

**Accumulation Rate of Simple Cyanides and Metal-Cyanide Complex Ions**

The toxicity to fish of a water pollutant is not only a function of its mode of action, but is also dependent on the rate at which the toxicant reaches the affected tissues. Some toxicants act superficially, whereas others penetrate and damage the internal tissues of a fish. The three possible routes by which metals and metallocyanide complex ions may enter the bodies of fish are the gills, the body surface, and the alimentary canal. Since fish in fresh water do not ingest much water and because the skin is relatively impermeable to most ions, it is generally believed that most dissolved toxicants that enter fresh water fish from their surrounding medium do so principally by way of the gills.
Only limited information is available on the rates of uptake of simple cyanide by various fish tissues, and all of this knowledge was obtained by somewhat dated histological and colorimetric techniques (Holden and Marsden, 1964; Kariya et al., 1967). In a study of the toxicity of cyanide to salmon and brown trout, Holden and Marsden (1964) observed that at a constant immersion time the cyanide concentration in the gills is proportional to the external aqueous cyanide concentration. The cyanide concentration in tissues was also found to increase with immersion time, high cyanide concentrations being found in the brain and liver of poisoned fish. Trout generally showed a higher concentration in the liver than in the gills, but in salmon the order was reversed. It was also apparent that small fish have a greater resistance to cyanide poisoning than large fish, especially fresh-run salmon. Kariya et al. (1967) observed that the relationship between the rate of CN removal by fish, in μg/g/hr, and the CN concentration in the test solution was linear over the concentration range of 0.53 to 10.67 mg/l.

Data reported by Kariya et al. (1967) indicate very little penetration of the cuprocyanide complex into the bodies of fish from the external medium, but for reasons to be explained later, these observations are not deemed reliable. No other information is available on the extent to which cyanide may enter the body of a fish in the form of a complex and then dissociate, yielding cyanide and metal ions. As
previously noted, Doudoroff (1956) did suggest that the sigmoid curves relating median tolerance limits, in mg/l total cyanide, to pH gave evidence of the presence of some toxic species other than molecular HCN in nickel-cyanide complex solutions. Doudoroff et al. (1966) also presented some toxicity test results that indicate probable penetration of the dicyanoargentate (I) complex ion into fish tissues. Therefore, another objective of the research reported here was to estimate the rates of uptake of hydrocyanic acid and of metal-cyanide complex ions by various tissues of bluegills exposed to solutions of simple and complex metal cyanides by the use of radioactive $^{14}$C-labeled cyanide and determination of the metal by flame atomic absorption spectrophotometry.

**HCN and Its Determination**

It has been in past years and still is to a large extent customary to report cyanides as total cyanides expressed as CN. This chemical measure can be very misleading as an indication of toxicity, since molecular HCN virtually alone determined the acute toxicity to bluegills of various solutions of simple and complex cyanides tested by Doudoroff et al. (1966). Because the complex ions are relatively harmless, the total cyanide concentration of such solutions is not a reliable index of their toxicity.

The free cyanide (i.e., CN$^{-}$ ion and molecular HCN) present in
solutions only of the relatively stable metal-cyanide complex species such as the ferro- and ferricyanide ions probably have been measured fairly accurately. However, the concentration of free cyanide in less stable complex solutions and that of molecular HCN cannot be accurately determined by conventional analytical methods in common use for the determination of cyanide, since they involve marked disturbance of existing equilibria of the system through removal of an indeterminate fraction of the cyanide from metal-cyanide complex ions in solution. It is virtually impossible to compute from the questionable, accepted dissociation constants of the metallocyanide ions the HCN levels in a polluted water, because of uncertainty as to the kinds and concentrations of metallic compounds and complex ions present. Therefore, a specific and sensitive analytical procedure for direct determination of molecular HCN in water, to the exclusion of other cyanide forms, is necessary not only for regulatory purposes but for more in-depth research into the chemistry and toxicity of metal-cyanide complexes.

Various methods have been published for the determination of hydrogen cyanide in aqueous solution. Worley and Browne (1917) were probably the first to determine the concentrations of hydrocyanic acid in solutions of sodium cyanide by comparing the amounts of hydrogen cyanide evolved on passing the same volume of air through the sample and through two standard solutions of hydrogen cyanide, the concentrations of which bracket the unknown. The evolved HCN was collected
in a solution containing 0.2 percent of picric acid and 2 percent of sodium carbonate. The intensity of the reddish-brown color produced in the absorber solution was proportional to the concentration of hydrocyanic acid in the sample. By keeping the amount of absorbed hydrogen cyanide very small, as compared with the total amount in solution, the concentration of hydrocyanic acid in solution could be determined without appreciably disturbing the existing equilibrium.

A more refined analytical procedure for the specific determination of low levels of molecular HCN in water without material disturbance of ionic equilibria was developed by Schneider (1962) and used in the work of Doudoroff et al. (1966). This method, based on the same principle as that employed in Worley and Browne's (1917) method, has been published by Schneider and Freund (1962). A distribution equilibrium is established between the concentrations of HCN in the water and in air bubbled through the water. Analysis of the dispersed air for HCN, which is collected on a concentration column, is accomplished by gas-liquid chromatography incorporating a thermal conductivity detector, permitting determination of HCN in water in the concentration range of $1 \times 10^{-6}$ to $5 \times 10^{-4}$ M (0.027 to 13.5 mg/l HCN). Schneider (1962) reported that the efficiency of his equilibration apparatus with a sintered glass air disperser at a depth of 40 inches was fairly constant when the rate of flow of air varied between 25 and 125 cc/min. Schneider also reported that, with a constant air
flow rate, the efficiency apparently does not vary significantly with disperser depth over the range of 11 to 40 inches.

Claeys (1968) developed a highly sensitive modification of the Schneider method for determination of HCN. This modification has been published by Claeys and Freund (1968). Nanogram quantities of HCN collected on a concentration column were determined by a gas chromatographic procedure, using a flame ionization detector. Twenty-liter test solutions containing as little as 1 μg/l HCN were analyzed in 5 minutes after 30-minute concentration periods, during which air was bubbled through the solutions and HCN collected in a cold trap for gas-chromatographic analysis.

In papers published by the Ministry of Technology (1967), and by Brown, Shurben and Shaw (1970), descriptions were given of a somewhat different analytical method designed for the determination of molecular hydrocyanic acid. A 15-liter sample is placed in a thermally-insulated perspex box covered with an air-tight lid, leaving an air space enclosed above the sample. Hydrogen cyanide is allowed to diffuse from the sample into 50 ml of 0.05 N sodium hydroxide contained in an open, shallow petri dish exposed to the atmosphere above the sample for a fixed period of 1.5 hours. The temperature of the sodium hydroxide solution is the same as that of the water sample. During the 1.5-hour period, about 1 percent (i.e., 1.5 μg HCN) of the total cyanide present in a solution containing 0.01 mg/l of
HCN is absorbed. The concentration of cyanide in the alkali solution is proportional to the concentration of hydrocyanic acid in the sample. The sodium hydroxide solution is then removed and its cyanide content determined by a modification of Epstein's method (American Public Health Association, 1965). The relationship between temperature from 7 to 24°C, and logarithm of the quantity of HCN absorbed from a 0.10 mg/l HCN solution into 50 ml of NaOH solution in 1.5 hours was observed to be linear. Errors, at concentrations of 0.01 mg/l or more, varied from ±2 to ±10 percent, depending on the concentration and on temperature of the sample.

Another method for the determination of molecular hydrocyanic acid is that of Montgomery, Gardiner and Gregory (1969). With this method, the HCN concentration in test solutions containing up to 2 mg/l HCN can be determined, extracting less than 4 percent of the hydrocyanic acid from a 100-ml sample by equilibration of the sample with 35 ml of 1, 1, 1-trichloroethane (methylchloroform). The limit of detection was reported to be less than 0.01 mg/l HCN. The proportion of hydrogen cyanide extracted from a sodium cyanide solution was 3.7 percent at 22°C, this corresponding to a distribution coefficient (HCN in 1, 1, 1-trichloroethane to HCN in water) of 0.11. In the temperature range of 9 to 22°C, the amount of hydrocyanic acid extracted is reduced by an average of 2 percent for each degree C by which the temperature falls below 22°C. The solvent-extracted
hydrocyanic acid is transferred into a sodium pyrophosphate solution, and the determination is completed colorimetrically by a modification of the method of Bark and Higson (1964). Since the method was designed to extract only a small proportion of the HCN, the disturbance of the existing equilibria between hydrocyanic acid, cyanide ion, and complex cyanides during the determination will not occur to any significant extent. Changes in pH value, which would affect the ionization of hydrocyanic acid and the dissociation of some complex cyanides, are not completely avoided but are minimal when the pH value of river water samples is between 7.4 and 8.7. In sewage effluent samples and possibly in some nickel-cyanide complex solutions significant pH changes may have occurred during extraction. But according to Montgomery et al. (1969), unpublished work at the Water Pollution Research Laboratory has shown that carbon dioxide is removed from aqueous solution by aeration more rapidly than hydrogen cyanide, and more severe pH changes are likely to occur in solutions containing high CO₂ concentrations when HCN determinations are made by the Schneider method. The method of Montgomery et al. is convenient for field use, since extractions can be carried out in the field, and procedures have been established for bringing extracted samples back to the laboratory for colorimetric analysis.

It appears that the theoretical basis of the proposed extraction method is sound, since good agreement was observed between the
predicted and observed concentrations of HCN in sodium cyanide solutions at various pH values between 8 and 10. However, only indirect evidence was presented that the method is valid for the determination of HCN in solutions of complex cyanides. Good agreement between expected and determined HCN concentrations in zinc- and cadmium-cyanide complex solutions was observed, but this is to be expected since dissociation is for all practical purposes complete at concentrations of $2.15 \times 10^{-5}$ M total cyanide in tested solutions. Hydrocyanic acid concentrations in solutions of potassium ferro- and ferricyanide complexes and of copper-cyanide complexes, prepared by combining dilute solutions of CuSO$_4$ and KCN in a CN to Cu molar ratio of 4 to 1, can not be accurately calculated and the validity of determined HCN concentrations was not substantiated. Only for a few solutions of the nickel-cyanide complex, prepared by combining dilute solutions of NiSO$_4$ and KCN in a CN to Ni molar ratio of 4 to 1, did the HCN concentration determined by the extraction method show reasonable agreement with the theoretical, expected values calculated by using $5.4 \times 10^{-31}$ as the cumulative dissociation constant for the tetracyanonicelate complex ion at 25°C (Schneider and Freund, 1962) and $6.17 \times 10^{-10}$ as the dissociation constant of hydrocyanic acid at 25°C (Izatt et al., 1962). The authors reported that significant discrepancy was indeed apparent in four of the 13 nickelocyanide test solutions, and my calculations show that in only about three of the remaining nine
solutions was fair agreement between observed and theoretical HCN concentrations attained. The validity of the extraction method for determination of HCN in nickel-cyanide complex solutions therefore was not demonstrated. When results obtained by this method and by the diffusion method (Ministry of Technology, 1967) in testing a biologically treated sewage effluent and two samples of river water were compared, the two methods for HCN determination were found to be in fair agreement, the extraction method generally giving somewhat higher HCN concentrations. Comparison with the method of Schneider was not made. In experiments with sea water (pH 7.8, chlorinity 17.7°/oo), the HCN concentrations determined by the extraction method were equal to 71 percent of the added cyanide, expressed as HCN. The discrepancy was explained by the authors as resulting from HCN being ionized to a greater extent in sea water than in distilled water at the same pH value.

The most recent method for the specific determination of undisassociated molecular hydrocyanic acid in water without material disturbance of existing ionic equilibria was developed by Nelson and Lysyj (1971). This method uses the vapor phase equilibration technique of Schneider to strip, with 1 liter of nitrogen sparged from a coarse-porosity fritted-glass gas disperser at a rate of 35 cc/min, a small portion of the undisassociated HCN from a 2-liter water sample. The displaced HCN is trapped in a bubbler containing 1.0 mg of 0.07 M
NaOH solution and then measured polarographically in a cell containing a stationary platinum cathode and rotating gold anode (Miller et al., 1964). The method was found to be applicable not only to KCN solutions prepared with distilled water but also to solutions prepared with a natural water. Among a number of possible interfering compounds only sulfide introduced an error, which was significant when the $H_2S$ concentration exceeded that of HCN. The present limit of detection of HCN by this method is about 2 $\mu g/l$ HCN, but the authors believed that it is possible to apply the procedure to samples with lesser concentrations of HCN.

In the development or selection of an analytical method for the determination of HCN, the required sensitivity and precision, as well as the nature and cost of equipment and facilities required, are important considerations. A review of the literature on the chronic toxicity of cyanide, as revealed by such indices as impaired swimming performance or growth (Neil, 1957; Leduc, 1966; Broderius, 1970), leads to the conclusion that a method for determination of molecular HCN which is suitable for wide application in the field of water pollution control must be sensitive enough to measure HCN in the microgram per liter range. The method of Schneider, and Claeys' very sensitive modification thereof, each requires costly analytical apparatus which is not easily transported and can be operated only by specially trained personnel. The concentration step also requires use of a
desiccant for removal of the water vapor from the gaseous sample and cooling of an HCN collecting column in a dry ice-acetone mixture.

The presence of natural, volatile organics in some waters may also present problems in the measurement of HCN. These features of the methods tend to limit their application, for they can be used only in well equipped and well staffed laboratories. Doudoroff et al. (1966) stated that a method which is usable in field situations and which requires less expensive apparatus is needed for practical application in the area of water quality control, and they suggested some possible approaches to the problem. Accordingly, my first objective in this research was to modify the method developed by Schneider so it could be used by persons with limited technical training and laboratory facilities and yet remain sufficiently accurate and precise for studying the toxicity and chemistry of simple and complex metal-cyanides and for the prediction or explanation of acute or chronic effects on fish in waters receiving waste cyanides.

Since research on development of my modification of Schneider's method was essentially completed before the solvent extraction method was published, and because of limited time available, I did not attempt to employ the extraction method. This method seems promising, however, and its applicability to the determination of HCN in solutions of metal-cyanide complexes should be further evaluated.
Hydrocyanic Acid

Hydrocyanic acid is formed in solutions of simple and complex cyanides through dissociation of the simple salts and dissociation or decomposition of complex ions and through hydrolytic reaction of cyanide ions so formed with water. Sodium or potassium cyanide being the salt of a strong base (NaOH or KOH) and a weak acid (HCN) will hydrolyze when in solution and the solution will be noticeably alkaline in reaction. A distilled water solution of NaCN or KCN with pH below about 8.5 is essentially a solution containing alkali metal and hydroxide ions and hydrocyanic acid as its predominant constituents. As the pH of these solutions is increased the percentage of the total cyanide present as the CN⁻ ion is increased, so as to satisfy the equilibrium reaction of HCN ⇌ H⁺ + CN⁻. The most reliable dissociation constant for HCN (Kₐ), selected from various values presented in Appendix A, is 4.365 × 10⁻¹⁰ at 20°C (Izatt et al., 1962). Since the pKₐ (pKₐ = -log Kₐ) for hydrocyanic acid is 9.36 ± 0.1 at 20°C, then in simple cyanide solutions one-half of the total cyanide exists as HCN and one-half as CN⁻ ion at pH of 9.36.

Thermodynamically, the CN radical presents a certain similarity to the halogens and, like the latter, can exist as three degrees of oxidation: -1 in HCN, 0 in C₂N₂, and +1 in HCNO. The oxidation of
HCN can thus take place to $C_2N_2$ or to HCNO. According to Deltombe and Pourbaix (1955), oxidation of the system HCN-CN$^-$ probably takes place according to a stepwise scheme. The first stage of oxidation is thought to be of the formation of gaseous $C_2N_2$, which then reacts with water to give a mixture of cyanide and cyanate:

\[
C_2N_2 + 2H^+ + 2e^- \rightarrow 2HCN \text{ (aq)}
\]

\[
C_2N_2 + 2H_2O \rightarrow 2HCNO \text{ (aq)} + 2H^+ + 2e^-
\]

\[
2C_2N_2 + 2H_2O \rightarrow 2HCN \text{ (aq)} + 2HCNO \text{ (aq)}
\]

Deltombe and Pourbaix (1955) also stated that, in an acid medium, this disproportionation of $C_2N_2$ is relatively slow, while in a very alkaline medium it is very rapid. Cyanate therefore does not form by direct oxidation of the cyanide, but results from a transformation of cyanogen, $C_2N_2$. It was further reported that HCNO is completely unstable and decomposes immediately according to the secondary reaction:

\[
HCNO + H_2O \rightarrow CO_2 + NH_3
\]

This is contradictory to observed results of Resnick, Moore and Ettinger (1958), since they reported that cyanates are stable in aerobic water at pH 7 and 20°C for at least 10 days, eventually undergoing hydrolysis to yield ammonium carbonate. They reported that in anaerobic solutions cyanates are reduced not to cyanide but to a mixture of ammonia and formic acid.
Solutions of HCN may be decomposed slowly with the formation of ammonium formate by the hydrolysis reaction:

\[ \text{HCN} + 2\text{H}_2\text{O} \rightarrow \text{HCO}_2^- + \text{NH}_4^+ \]

This hydrolysis is very slight in acid media, but rather more significant in alkaline media. Any condition contributing to the loss of NH\textsubscript{3} should shift the equilibrium from left to right, this leading to a more complete decomposition of cyanide.

**Equilibrium Constants and Reaction Mechanisms**

According to Ford-Smith (1964) there are about 28 elements that are able to form complex cyanides. Since under each element there are various oxidation states, altogether there are more than 64 oxidation states of metals which may form complex cyanides. The structure of these complexes is such that the hexacyano complexes are all octahedral, the tetracyano complexes are either tetrahedral or square planar, the tricyano complexes are planar, and the dicyano complexes are linear. The stabilities of the complex cyanides vary greatly from one element to another and from one oxidation state to another. The formation constants for some of the transitional metal complexes are so great that only an approximation of their magnitude can be attempted by indirect methods (Ford-Smith, 1964).

In natural waters there are many metallic ions present that are
capable of forming complexes with cyanide and others may be introduced as pollutants. When a metal salt is added to a contaminated water containing a metallo-cyanide complex, replacement of the metal in the original complex may occur. If the formation constant for the original metal-cyanide complex is much greater numerically than the formation constant for the possible second complex, then no effective replacement of the first metal ion by the second metal ion will take place. If, on the other hand, the formation constant for the original metal-cyanide complex is much smaller numerically than the formation constant for the possible second complex, then the metal in the initial complex will be displaced by the second metal and the displaced metal will precipitate as an insoluble cyanide, hydroxide, or carbonate.

Doudoroff's (1956) explanation for the influence of pH on the toxicity to fish of the nickel-cyanide complex (NaCN combined with NiSO₄) involved a consideration of two equilibria. One equilibrium was for the dissociation of the \( \text{Ni(CN)}_{4}^{2-} \) ion and the other was for the dissociation of HCN. In the year 1956, however, the assumption that the toxicity of a sodium nickelocyanide solution was due to the molecular HCN present there could not be substantiated by calculations based on the generally accepted dissociation constant of the \( \text{Ni(CN)}_{4}^{2-} \) complex ion, namely 10⁻²² (Latimer, 1952). This discrepancy prompted Doudoroff to suggest that the value for the dissociation constant may
be incorrect and to estimate that a value of the order of $10^{-30}$ would be more nearly in accord with his toxicological theory and findings.

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stated that equilibrium constants and enthalpy data are adequately explained by assuming the nickel to be present only as $\text{Ni}^{++}$ and $\text{Ni(CN)}_{4}^{-2}$. It is possible that species intermediate between $\text{Ni}^{++}$ and $\text{Ni(CN)}_{4}^{-2}$ exist in small concentrations, although, as indicated by Freund and Schneider (1959), no such species have been reported.

Nickel-cyanide complex species with a mole ratio of cyanide to nickel that is greater than 4 to 1 have been reported by Blackie and Gold (1959) and by Penneman et al. (1963). The latter authors gave formation constants for $\text{Ni(CN)}_{5}^{-3}$ and $\text{Ni(CN)}_{6}^{-4}$ as follows:

$$\text{Ni(CN)}_{4}^{-2} + \text{CN}^{-} \rightarrow \text{Ni(CN)}_{5}^{-3} \quad K_5 = 0.279 \text{ mole}^{-1}$$

$$\text{Ni(CN)}_{5}^{-3} + \text{CN}^{-} \rightarrow \text{Ni(CN)}_{6}^{-4} \quad K_6 = 0.095 \text{ mole}^{-1}$$

Therefore, for all practical purposes the $\text{Ni(CN)}_{5}^{-3}$ and $\text{Ni(CN)}_{6}^{-4}$ species are only present at very high CN$^{-}$ concentrations, and in dilute nickelocyanide solutions in which the cyanide to nickel molar ratio is 4 to 1, the $\text{Ni(CN)}_{4}^{-2}$ ion is the only significant stable complex species.

There appears to have been some confusion in the literature as to the form in which copper exists in cuprocyanide solutions (e.g., Rothbaum, 1957). In the system CuCN-KCN-H$_2$O, Penneman and Jones (1956) have observed infrared absorption spectra of three distinct complex ions: $\text{Cu(CN)}_{2}^{-}$, $\text{Cu(CN)}_{3}^{-2}$, and $\text{Cu(CN)}_{4}^{-3}$. They concluded that the $\text{Cu(CN)}_{4}^{-3}$ species is the principal ion present at high cyanide to copper ratios. In the system CuCN-KCN-H$_2$O at 25°C it
was not possible for them to prepare a stable solution of appreciable concentration having a CN/Cu molar ratio below approximately 2.8. However, with a CuCN-NaCN-H₂O system they were able to prepare solutions having lower CN/Cu ratios.

Rothbaum (1957) suggested that the following equilibria exist in fairly concentrated cuprocyanide solutions:

\[
\begin{align*}
\text{Cu}^+ + 2\text{CN}^- & \overset{K_1}{\longrightarrow} \text{Cu(CN)}_2^- \\
\text{Cu(CN)}_2^- + \text{CN}^- & \overset{K_2}{\longrightarrow} \text{Cu(CN)}_3^- \\
\text{Cu(CN)}_3^- + \text{CN}^- & \overset{K_3}{\longrightarrow} \text{Cu(CN)}_4^- 
\end{align*}
\]

He also determined that \( K_1 > K_2 > K_3 > 1 \), which implies that most of the copper is always present in the form with the highest CN to Cu molar ratio compatible with the amount of cyanide present. Therefore, copper is in the form of \( \text{Cu(CN)}_3^- \) as long as sufficient cyanide is present, and only at smaller cyanide to copper ratios are lower complexes formed. Thus, where this ratio is about 3 to 1, according to Rothbaum \( \text{Cu(CN)}_3^- \) is the principal ion present and at smaller ratios \( \text{Cu(CN)}_2^- \) begins to form.

Iron (II) and (III) form a number of complexes, mostly of the octahedral type. Iron forms two complexes with cyanide, the diamagnetic (weakly repelled by magnets) hexacyanoferrate (II) ion \( (\text{Fe(CN)}_6)^{-4} \) and the paramagnetic (weakly attracted to magnets)
hexacyanoferrate (III) ion \((\text{Fe(CN)}_6^-)^3\). Adamson, Welker and Volpe (1950) postulated that the concentrations of intermediates such as \(\text{Fe(CN)}_5^-\), \(\text{Fe(CN)}_4^1\), and \(\text{Fe(CN)}_3^-\) must be very small.

Iron-cyanide complexes are extremely stable compounds, but it has long been known that both \(\text{Fe(CN)}_6^4^-\) and \(\text{Fe(CN)}_6^3^-\) are decomposable to a large extent under the influence of light, more readily so in the presence of oxygen. Baudisch and Bass (1922) suggested a mechanism for the photo-decomposition of potassium ferrocyanide with the release of one-sixth of the cyanide. Mitra et al. (1963) stated that neutral solutions of \(\text{K}_4\text{Fe(CN)}_6\) have been known to become alkaline when irradiated by ultraviolet light. They also emphasize that photolysis of \(\text{Fe(CN)}_6^4^-\) occurs at first, yielding \(\text{Fe(CN)}_5^3^-\) and \(\text{CN}^-\), and the latter then hydrolyzes producing free alkali:

\[
\text{Fe(CN)}_6^4^- \rightleftharpoons \text{Fe(CN)}_5^3^- + \text{CN}^- \\
\text{CN}^- + \text{H}_2\text{O} \rightleftharpoons \text{OH}^- + \text{HCN} \\
\text{Fe(CN)}_5^3^- + \text{H}_2\text{O} \rightleftharpoons \text{Fe(CN)}_5(\text{H}_2\text{O})^3^- \\
\text{Fe(CN)}_6^4^- + 2\text{H}_2\text{O} \rightleftharpoons \text{Fe(CN)}_5(\text{H}_2\text{O})^3^- + \text{OH}^- + \text{HCN}
\]

Photo-decomposition of potassium ferrocyanide therefore produces hydrogen cyanide resulting from the release of one-sixth of the cyanide. When the light source is discontinued, all three reactions are reversed and the original pH is restored. After prolonged exposure, the reaction becomes irreversible with precipitation of ferrous
hydroxide in aerated basic solutions and formation of Prussian blue, \( \text{FeFe(CN)}_6^3 \), in aerated acid solutions, implying a further decomposition of the primary reaction product.

A number of reports have appeared since the latter part of the 19th century on the photochemistry of aqueous solutions of \( \text{Fe(CN)}_6^3 \). Nevertheless, Balzani and Carassiti (1970) have stated that the photochemical behavior of this complex is not yet completely understood.

Alich, Haworth and Johnson (1967) observed that reduction of the \( \text{Fe(CN)}_6^3 \) ion by \( \text{CN}^- \) is pH dependent; at higher pH values the \( \text{OH}^- \) enters into the reduction reaction, possibly by oxidizing the \( \text{CN}^- \) ion:

\[
2\text{Fe(CN)}_6^3 + 2\text{H}_2\text{O} \rightleftharpoons 2\text{Fe(CN)}_5(\text{H}_2\text{O})^{2-} + 2\text{CN}^- \\
2\text{Fe(CN)}_5(\text{H}_2\text{O})^{2-} + \text{CN}^- + 2\text{OH}^- \rightleftharpoons 2\text{Fe(CN)}_5(\text{H}_2\text{O})^{3-} + \text{CNO}^- + \text{H}_2\text{O} \\
2\text{Fe(CN)}_6^3 + \text{H}_2\text{O} + 2\text{OH}^- \rightleftharpoons 2\text{Fe(CN)}_5(\text{H}_2\text{O})^{3-} + \text{CN}^- + \text{CNO}^- \\
2\text{Fe(CN)}_5(\text{H}_2\text{O})^{3-} + 2\text{CN}^- \rightleftharpoons 2\text{Fe(CN)}_6^4 + \text{H}_2\text{O} \\
2\text{Fe(CN)}_6^3 + 2\text{OH}^- + \text{CN}^- \rightleftharpoons 2\text{Fe(CN)}_6^4 + \text{H}_2\text{O} + \text{CNO}^- 
\]

Moggi et al. (1966) showed that the photochemical behavior of \( \text{Fe(CN)}_6^3 \) was qualitatively the same regardless of the wavelength of irradiation (254, 313, or 405 nm light). Spectral changes suggested that \( \text{Fe(CN)}_5(\text{H}_2\text{O})^{2-} \) was formed as the main product. In the dark, the complex slowly underwent an oxidation-reduction reaction which was accelerated by the presence of \( \text{CN}^- \) and produced \( \text{Fe(CN)}_5(\text{H}_2\text{O})^{3-} \).

Upon irradiation, the rate of the above reaction increased, \( \text{Fe}^{2+} \) ions
formed, and the pH of the solution initially increased. The authors concluded that for Fe(CN)$_5$(H$_2$O)$_2^{-2}$, a photochemical aquosubstitution reaction occurred simultaneously with the oxidation-reduction reaction which yielded Fe(CN)$_5$(H$_2$O)$_3^{-3}$. After long irradiation periods, the products produced were Fe(OH)$_3$ in alkaline solution and some ferrous-ferric complex (Prussian blue) in acid solution.

In summary, it can be stated that several authors generally agree that photolysis of ferricyanide and ferrocyanide solutions forms pentacyanoaquoferrates and that ferricyanide is reduced to ferrocyanide.

A review of the literature has revealed essentially no information on the chemical behavior of iron-cyanide complexes in the dark.

Formation constants for Fe(CN)$_6^{-4}$ and Fe(CN)$_6^{-3}$ complexes are presented in Appendix A.

From my discussion so far, it can be generalized that the formation of complexes always appears to occur in a stepwise fashion, with the stabilities of the various species characterized by a series of mass action constants. Similarly, it seems to be generally accepted that the stable cyano-complexes of silver are Ag(CN)$_2^{-}$ and Ag(CN)$_3^{-2}$ and that the 4-coordinated complex is not stable (Randall and Halford, 1930; Jones and Penneman, 1954; Azzam and Shimi, 1963). The Ag(CN)$_3^{-2}$ species is only present at high CN$^{-}$ concentration and is stable only in concentrated solutions. Therefore, the Ag(CN)$_2^{-}$
complex is the predominant stable form in dilute silver-cyanide complex solutions.

Formation constants for the various Ag(I)-cyanide complexes are presented in Appendix A.

Cyanide Exchange

Many different mechanisms have been proposed for the exchange of CN\(^-\) in a metal-cyanide complex, but most authors believe that there are two possible exchange paths:

(1) A reversible, rapid dissociation of the complex, or

(2) a substitution or inversion type process whereby one group leaves as the other enters.

A survey of published research on the exchange of CN\(^-\) in metal-cyanide complex ions with the simple cyanide ion or HCN in solution led to the conclusion that this exchange is consistent with a mechanism which proceeds in the following way:

\[
\text{Ni(CN)}_2^2^- + \text{H}_2\text{O} \rightleftharpoons \text{Ni(CN)}_3\text{H}_2\text{O}^- + \text{CN}^-.
\]

It should also be pointed out that there are six generally accepted observations pertaining to such an exchange:

(1) The rate of the exchange reaction with \(^{14}\text{C}\)-labeled radio-cyanide is zero order with respect to the concentration of free cyanide in the exchange system. According to MacDiarmid and Hall (1954), this is to be expected if the
rate of exchange is governed by a step involving the slow
dissociation of the complex cyanide.

(2) A decrease in pH increases the rate of exchange.

(3) Those complexes which exhibit an increase of dissociation
with illumination also exhibit acceleration of their exchange
reactions when exposed to light.

MacDiarmid and Hall (1954) have observed that the rate
of dissociation of Fe(CN)$_6^{-4}$ and Fe(CN)$_6^{-3}$ is increased by
light.

(4) The following complex ions exhibit slow exchange with
cyanide: Fe(CN)$_6^{-4}$, Fe(CN)$_6^{-3}$.

(5) The following complex ions exhibit immeasurably rapid
exchange with cyanide: Ni(CN)$_4^{-2}$, Cu(CN)$_3^{-2}$, Ag(CN)$_2^{-}$.

The fact that a complex is only slightly dissociated at equilibrium
is of no importance whatsoever. The important factor is the rate at
which the equilibrium can be attained, and if this is rapid, isotopic
exchange should also be rapid.

Adamson et al. (1950) pointed out that the overall thermodynamic
instability constants are misleading in predicting exchange. The
fundamental point is that the thermodynamic stability of a complex
should not necessarily be expected to show a relationship with its rate
of exchange. It can be argued that the rate of exchange of a complex
should increase with decrease of thermodynamic stability, since the
chance of a bond gaining sufficient energy to break will then be proportionally increased. This would be the case only if the activation energy for the breaking of the bond were the same for all complexes. It has therefore been proposed by MacDiarmid and Hall (1954) that the rate of exchange is governed by the magnitude of the activation energy necessary to break the bond, which is in turn governed mainly by the electronic structure of the complex ion. In the group of fast-exchanging complexes there is only the comparatively low-energy outer orbital binding.

It appears from the above discussion that reversible, rapid dissociation of a complex is of minor importance in accounting for the exchange behavior of the complex ions, but substitution alone could account for the above observations.

Long (1951) studied the exchange of the nickel in tetracyano-nickelate (II) ion with (hydrated) nickel ion. He observed that at pH values ranging from 4 to 8 there is no exchange of nickel.

Spectrophotometric Absorption

During the course of spectrophotometric investigations, characteristic absorption curves have been obtained for various complex cyanides. Since concentrations of some metal-cyanide complexes of the order of micrograms per milliliter give highly absorbing solutions, spectrophotometric measurements can be utilized for the qualitative
and sometimes for quantitative determination of these metal-cyanide complex ions.

The molar extinction coefficient values in the infrared, visible, and ultraviolet spectrum region for the various metal-cyanide complex ion species are presented in Appendix B.

The two main absorption peaks that Soine (1957) observed for \( \text{Ni(CN)}_2^{2-} \) were at 267.5 nm and at 285 nm. Soine observed that the presence of \( \text{NaOH-H}_3\text{BO}_3 \) buffers and an increase of pH above 9.5 had no apparent effect on the absorbance of the tetracyanonickelate (II) complex. However, in the range of pH below 9.5, the absorbance decreased slightly with decreasing pH.

McCullough, Jones and Penneman (1960) observed that the infrared spectra of aqueous solutions containing the tetracyanonickelate (II) ion show a characteristic absorption peak at 2124 cm\(^{-1}\). In the presence of a large excess of cyanide ion, a solution containing tetracyanonickelate (II) ion shows a single new absorption peak at 2102 \( \pm \) 2 cm\(^{-1}\) characteristic of the pentacyanonickelate (II) ion, \( \text{Ni(CN)}_5^{3-} \). But no infrared absorption by a higher complex, e.g., \( \text{Ni(CN)}_6^{4-} \), was detected even in a solution nearly saturated with NaCN and containing 0.4 M \( \text{Ni(CN)}_4^{2-} \). Penneman et al. (1963) presented results agreeing quite well with those of McCullough et al. (1960), except that they observed that at very high free cyanide concentrations some hexacyanonickelate (II) ion is formed, its absorption band being close to the
position of the pentacyano ion. Even with a 10- to 20-fold excess of sodium cyanide, most of the nickel remains in the form Ni(CN)\(^{-2}\), absorbing at 2124 cm\(^{-1}\). Therefore, the formation constant of a higher complex cannot be large.

In the system CuCN-KCN-H\(_2\)O, Penneman and Jones (1956) have observed infrared absorption spectra of three distinct complex ions: Cu(CN)\(^{-2}\) at 2125 cm\(^{-1}\), Cu(CN)\(^{-3}\) at 2094 cm\(^{-1}\), and Cu(CN)\(^{-4}\) at 2076 cm\(^{-1}\). Simpson and Waind (1958) measured the absorption spectra of copper (I) and cyanide solutions in water. They observed that the spectrum at low CN\(^{-}\) concentrations corresponds to that of Cu(CN)\(^{-2}\) (234 nm). This passes to that of Cu(CN)\(^{-3}\) (238 nm) with increasing CN\(^{-}\), and with further increases of CN\(^{-}\) moves towards that of Cu(CN)\(^{-4}\) (235 nm).

Iron cyano complexes do not show sharp absorption, but Adamson (1952) observed that the optical density at 420 nm is a direct measure of the hexacyanoferrate (III) concentration. Hexacyanoferrate (II) has a high absorption at 350 nm.

Jones and Penneman (1954) measured the absorption spectra of silver (I) and cyanide solutions in water. They observed that the spectrum at low CN\(^{-}\) concentrations corresponds to that of Ag(CN)\(^{-2}\) (2135 ± 1 cm\(^{-1}\)). This passes to that of Ag(CN)\(^{-3}\) (2105 ± 1 cm\(^{-1}\)) with increasing CN\(^{-}\) and moves towards that of Ag(CN)\(^{-4}\) (2092 ± 1 cm\(^{-1}\)) with further increases of CN\(^{-}\).
The molar extinction coefficient values at most wavelengths for CN\(^-\), CNO\(^-\), and HCO\(_2\)^- are too small to introduce error into this kind of spectrophotometric investigation.
MATERIALS

Experimental Fish

Young bluegills, *Lepomis macrochirus* Rafinesque, and adult marine threespine sticklebacks, *Gasterosteus aculeatus aculeatus* Linnaeus, were used as test organisms to study the toxicity of solutions containing simple cyanides or metal-cyanide complexes.

The young bluegills were seined from small ponds in the Willamette Valley near Corvallis, Oregon. Bluegills used for acute toxicity bioassays averaged about 50 or 60 mm in total length, respective wet weights being about 1.8 and 3.4 g. Those used in carbon-14, copper, and silver absorption experiments were much larger, averaging 80, 88, or 107 mm in total length and 8, 10, or 21 g in wet weight, respectively.

The euryhaline threespine sticklebacks were collected from small channels and potholes in tidal-flat sloughs adjacent to Yaquina Bay, Newport, Oregon. Fish averaging 43 mm in total length and 0.9 g in wet weight were collected and placed in glass aquaria. One group was acclimated to fresh water and the other groups to sea water having a chlorinity of approximately 2, 4.4, 8.8, 11.4, and 17 parts per thousand and a temperature of 20°C. They were acclimated to these conditions for about one week before use in bioassays. Sticklebacks acclimate rapidly to salinity and temperature changes, and a longer acclimation period was deemed unnecessary.
All fish were held in Plexiglas or glass aquaria placed in a 20°C constant-temperature room. The freshwater aquaria contained a continuously renewed and aerated supply of filtered but not otherwise treated water from a spring-fed stream or dechlorinated city tap water. The water was maintained at a constant temperature of 20°C by heating the incoming water with an immersion heater in a constant-head box. The group of marine sticklebacks acclimated to various sea water dilutions were held in 15-gallon glass aquaria placed in a 20°C constant-temperature room. About 5 gallons of the sea water were replaced with new water every other day during the acclimation period.

In the laboratory, all stocks of fish were fed at least a daily maintenance ration of tubificid worms, finely ground and frozen beef heart, and/or marine euphausiads (*Euphausia pacifica*), a pelagic shrimplike crustacean approximately 1 inch in length. All fish increased in size and remained apparently in good health throughout the period of experimentation.

**Experimental Water**

Five types of water were used in the various experiments. They included water supplied from a small spring-fed stream and sand-filtered, water from a 110-foot well, dechlorinated city tap water, sea water and various dilutions prepared with distilled water, and distilled water having a specific conductivity of about 1 micromho.
Available data on the mineral content of a sample of well water taken at a time when this research was in progress and analyzed by the U.S. Geological Survey are given in Table 1. The untreated well water was used to prepare essentially all solutions for experiments with bluegills.

Table 1. Dissolved mineral content of a sample of well water.

<table>
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<tr>
<th>Date</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Specific conductance (25°C)</td>
<td>260 micromhos/cm</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
</tr>
<tr>
<td>Silica (SiO₂)</td>
<td>36 mg/l</td>
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<tr>
<td>Calcium (Ca)</td>
<td>33 &quot;</td>
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<tr>
<td>Magnesium (Mg)</td>
<td>9.4 &quot;</td>
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<td>Sodium (Na)</td>
<td>8.6 &quot;</td>
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<tr>
<td>Potassium (K)</td>
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<tr>
<td>Bicarbonate (HCO₃)</td>
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<td>Carbonate (CO₃)</td>
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</tr>
<tr>
<td>Sulfate (SO₄)</td>
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</tr>
<tr>
<td>Chloride (Cl)</td>
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<td>Residue on evaporation at 180°C</td>
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<td>Hardness as CaCO₃</td>
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</tr>
<tr>
<td>Noncarbonate hardness</td>
<td>0 &quot;</td>
</tr>
<tr>
<td>Color</td>
<td>5</td>
</tr>
</tbody>
</table>

Data on the mineral content of samples of the stream water, taken in late summer and fall, have been reported by Doudoroff et al. (1966).
The sand-filtered and ultraviolet irradiated sea water was obtained from the Marine Science Center at Newport, Oregon. The water was stored at a temperature of about 4°C in two 55-gallon barrels lined with two heavy polyethylene bags. Over a 3-week period, this water was used for acclimation of threespine sticklebacks and in preparation of simple and metal-cyanide complex experimental solutions.
APPARATUS AND METHODS

Analytical Method

Outline of Method

The method used for determination of low-level molecular HCN in solution is my modification of that of Schneider (1962), as published in Schneider and Freund (1962). Finely dispersed compressed air is passed (bubbled) continuously, at a precisely regulated rate, through a large volume (approximately 20 liters) of the test solution as the solution circulates through a special glass bubbler. The equilibrated air is then passed through a concentration column containing glass beads coated with 6 N NaOH solution, in which the HCN is trapped. Subsequent repeated washing of the concentration column with distilled water and collection of the washings in a 25-ml volumetric flask permits measurement of the trapped HCN by means of the pyridine pyrazolone colorimetric method for determination of cyanide. The HCN concentration in the tested solution is obtained from a calibration curve relating the quantity (in μg) of HCN displaced per liter of gas dispersed to the concentration of HCN (in mg/l) present in standard solutions of known HCN content. Because the loss of HCN due to bubbling of air through a large volume of test solution in the course of each determination is very small, equilibria are not materially disturbed.
**Apparatus**

The apparatus used for determination of molecular HCN is pictured in Figure 1.

The flow rate of compressed air from a cylinder is maintained by means of a two-stage gas regulator with pressure reducing valve, and also a constant differential flow controller and metering valve equipped with a vernier adjustment. It has been determined that adequate flow regulation can be accomplished with the two-stage gas regulator alone; however, the vernier adjustment metering valve used in addition to this flow regulator permitted more precise control. The gas is sparged through a 16-inch-high column of test solution in a bubbler immersed in a 20-liter Pyrex glass carboy. The bubbler has been described by Schneider and Freund (1962). Schneider (1962) determined that an 11-inch height of the solution column was adequate for attaining equilibrium distribution of HCN between air and water. The bubbler was designed so that the rising bubbles cause circulation of the test solution in the container, preventing significant local depletion of cyanide. A medium porosity sintered glass disc (30 mm diameter) produces the desired bubble size.

A spray trap is inserted between the bubbler and the concentration column to ensure that no droplets of test solution are carried over in the air and deposited on the concentration column.
Figure 1. Apparatus used for distribution between water and air and concentration of molecular hydrocyanic acid. Insert shows top view of buret stopcock end of the concentration column.
The use of a new concentration column was my most important modification of the Schneider method. The column is a 26-cm section of 10-mm diameter borosilicate glass tubing to which a three-way buret stopcock has been fused and which is packed with glass beads of 3 mm diameter. The actual concentration section of the column containing the beads is 18 cm long. To facilitate installation and removal of the concentration column, ground glass joints were fused to each end. Tests with columns of various lengths showed that 100 percent absorption of HCN from the dispersed air could be obtained with a column having a glass bead packing as short as 7 cm. The capillary tip of the three-way buret stopcock facilitates complete delivery of the column washings into a 25-ml volumetric flask.

A 10-liter water-displacement bottle is used to determine the volume of air dispersed in the solution and passed through the concentration column. The bottle is inverted and mounted on a supporting frame. A graduated glass tube of 8 mm diameter is inserted into a 3/4-inch hole drilled in the bottom of the bottle. The glass tube extends to within about 2 mm of the rubber stopper inserted in the neck of the bottle. An inlet tube for air and an outlet tube for displaced water are inserted into the stopper closing the neck of the bottle. A two-way teflon stopcock controls the flow of air into the bottle. The bottle need not be removed from the support except for cleaning purposes, because it can be filled through the 3/4-inch hole after each run.
By proper manipulation of the various stopcocks, the dispersed air from the bubbler can be passed through the concentration column and then to the water displacement bottle. By manipulation of three stopcocks, the air flow can be diverted from one concentration column to another and at the same time from one water displacement bottle to another, permitting virtually continuous determinations.

**Procedure**

The solution whose molecular HCN content is to be determined is placed in a 20-liter solution bottle and the circulating glass bubbler is immersed in the solution. The bubbler is connected to the spray trap, which is connected to the No. 1 three-way teflon stopcock; all connections are made by means of ball and socket ground-glass joints. The gas regulator on the compressed air cylinder is opened and its pressure adjusted to 12 pounds per square inch. The vernier metering valve is then adjusted so the compressed air is bubbled through the solution at a regulated flow rate of approximately 50 cc per minute. The No. 1 three-way teflon stopcock is positioned so that the equilibrated air from the bubbler will not pass through the arm of the stopcock to which the concentration column is to be connected, but instead will escape into the atmosphere through the third arm, which at that time is open to the atmosphere. Compressed air is then bubbled through the test solution for 30 minutes to ensure equilibrium in the
system before collection of HCN is begun. The concentration column is prepared by coating the glass beads with 6 N NaOH. This is done with the concentration column held in a vertical position, with the stopcock end down, by a buret clamp fastened to a ring stand. A separatory funnel, containing 6 N NaOH, is connected to the buret stopcock at the lower end of the column by means of tygon tubing and a ball and socket ground-glass joint. By raising and lowering the separatory funnel, the column can be filled and drained. When this procedure has been repeated three times, the ball and socket joint is disconnected and the excess NaOH is allowed to drip out of the column. The small section of glass tubing between the ground-glass ball joint and buret stopcock barrel is then washed several times with distilled water and acetone and allowed to dry. By weighing the concentration column before and after coating, the weight of 6 N NaOH adhering to the glass beads and column wall can be determined. The specific gravity of a 6 N NaOH solution was found to be 1.2091. From the weight of 6 N NaOH solution on the column and its known specific gravity, the milliliters of NaOH solution remaining on the column after each coating can be calculated. Results to be reported will show that the intensity of color development, when the pyridine pyrazolone method is used for determination of cyanide, is related to the volume of concentrated NaOH solution rinsed from the column. The column is then placed in position between the No. 1 and No. 2
three-way teflon stopcocks, and the ball and socket ground-glass joint at each end of the column is secured with a metal clamp. The concentration column is connected in series with a water displacement bottle by rotating, in the appropriate manner, the three-way buret stopcock and the No. 2 and No. 3 teflon stopcocks, and by opening the two-way teflon stopcock on the air inlet tube of the water displacement bottle. The No. 1 three-way teflon stopcock then is rotated so the equilibrated air coming from the bubbler passes through the concentration column and then continues through the system to displace water from the displacement bottle. In the concentration column, the HCN in the air reacts with the NaOH to form NaCN.

At the end of the concentration period, the column is removed from its collecting position and rinsed three times with distilled water into a 25-ml volumetric flask, using 5 ml of water each time, to remove the NaCN. The combined washings are then analyzed for cyanide (CN) by the pyridine pyrazolone method (American Public Health Association et al., 1965). This method was first described by Epstein (1947). Color intensity is determined with a Beckman DB, or Gilford Model 2400 spectrophotometer, and the concentration of CN in the volumetric flask from the combined column washings is determined by reference to a calibration curve. Since the quantity of CN collected on the column is directly related to the HCN concentration in the test solution, the latter concentration can be determined by reference to
another calibration curve. Only one concentration column is used at a time, but continuous HCN determinations can be made by rotating the stopcocks at the end of each concentration period so as to channel the equilibrated air through a newly prepared concentration column and into a displacement bottle that has been refilled with water.

In making an HCN determination, it is necessary to know precisely the amount of air that has passed through the concentration column. Since gas regulators do not always deliver precisely the desired, sustained flow, I decided to measure the air volume by means of the water displacement bottle (Figure 1). The inverted bottle is filled through the 3/4-inch hole in its bottom with water that has been thoroughly aerated at 20°C. The graduated glass tube is then inserted into this 3/4-inch hole and the rubber cap is removed from the S-shaped outlet tube. The water in the graduated glass tube drops to the level of the discharge end of the outlet tube and the displaced water is discarded. The two-way stopcock on the inlet line now can be opened and the air passing through the concentration column is allowed to enter the displacement bottle. The displaced water flows out through the S-shaped outlet tube and is collected. At the end of the concentration period, the two-way inlet stopcock is closed and a rubber cap is placed over the outlet tube opening. The displaced water is measured in a volumetric flask and small graduated cylinder and corresponds to the total uncorrected volume of gas.
dispersed. Water is then introduced into the graduated tube by means of a 100-ml buret until the water level in the tube rises to the water level in the 10-liter displacement bottle. The amount of water added by way of this tube is referred to here as the "crude correction volume." To obtain the "true correction volume," one must determine how much water would have been required to just fill the graduated tube to the level of water in the displacement bottle if the correction tube were sealed off at the bottom. This value is called the "tube correction" and will never exceed 10 ml. The true correction is equal to the crude correction minus the tube correction. When the level of water in the tube equals the level of water in the displacement bottle, the gas above the water must be at atmospheric pressure, because the correction tube is open to the atmosphere at the top. Therefore, the total volume of gas dispersed is equal to the total uncorrected volume of gas dispersed minus the true correction volume.

**Standardization**

The pyridine pyrazolone method for cyanide determination was based on that of Epstein (1947) and is presented in the twelfth edition of *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association *et al.*, 1965). A number of authors have noted that significant variations in salt concentration in sample and standard solutions result in changes of absorbance of the
developed color. It has also been shown that the color reaction is reproducible in solutions of pH varying from 3 to 8 (Ludzack, Moore and Ruchhoft, 1954). However, the pH values of the final reaction mixtures after addition of reagents were observed to be close to 7 for these samples with initial pH values ranging between 3 to 8. Because of the probable importance of acetic acid and sodium hydroxide concentrations in relation to color development by the pyridine pyrazolone method, their relationship to absorbance was determined and the results are presented in Figures 2 and 3. All absorbance values are based on 25-ml final volumes containing 3.9 µg HCN and read at 620 nm. Acetic acid was added in the ratio of 1.48 ml to 1 ml of 6 N NaOH to cyanide solutions in 25-ml volumetric flasks in order to define the relationship between absorbance and milliliters of 6 N NaOH. The pH of these solutions, after addition of acetic acid and before addition of color development reagents, was adjusted to a value that randomly ranged between 5.9 and 6.9.

By reference to the negative slopes of the straight lines relating absorbance to milliliters of 25 percent acetic acid and 6 N NaOH, one notes that the effect on color development is 2.78 times as great for a unit change in acetic acid as for a unit change in sodium hydroxide. Therefore, in defining standard curves relating absorbance to cyanide concentration, and for solutions of unknown cyanide concentration, one must know the NaOH concentration in the sample, but it is more
Figure 2. The effect of acetic acid (pH) on color development by the pyridine-pyrazolone method in solutions containing 3.9 μg cyanide and 0.44 ml 6 N NaOH diluted to a total volume of 25 ml.
Figure 3. The effect of 6 N NaOH (salt concentration) on color development by the pyridine-pyrazolone method in solutions containing 3.9 μg cyanide and acetic acid in the ratio of 1.48 ml to 1 ml of 6 N NaOH diluted to a total volume of 25 ml.
Y = 0.7484 - 0.1773 X
important to control the pH of the sample before addition of the color development reagents by adding an amount of 25 percent acetic acid precisely appropriate for each determination.

Calibration curves relating absorbance at 620 nm and cyanide concentrations (expressed as HCN) in the presence of various amounts of 6 N NaOH and 25 percent acetic acid were defined for the Beckman DB and Gilford 2400 spectrophotometers. These relationships were linear over the range 0 to 6 µg HCN per 25-mI total volume, with linear correlation coefficients greater than 0.999 in all instances. The linear regression equations defining these relationships are presented in Table 2.

Table 2. Linear regression equations for calibration curves relating absorbance at 620 nm (Y) and hydrocyanic acid concentration (X) in µg per 25-mI total volume in the presence of various amounts of 6 N NaOH and 25 percent acetic acid.

<table>
<thead>
<tr>
<th>6 N NaOH (ml)</th>
<th>25% Acetic acid (ml)</th>
<th>Linear regression equations $Y = b + mX$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Beckman DB</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$b$</td>
</tr>
<tr>
<td>0.40</td>
<td>0.65</td>
<td>-0.0206</td>
</tr>
<tr>
<td>0.42</td>
<td>0.65</td>
<td>-0.0044</td>
</tr>
<tr>
<td>0.44</td>
<td>0.65</td>
<td>-0.0047</td>
</tr>
<tr>
<td>0.46</td>
<td>0.72</td>
<td>-0.0054</td>
</tr>
<tr>
<td>0.47</td>
<td>0.71</td>
<td>-0.0128</td>
</tr>
<tr>
<td>0.56</td>
<td>0.85</td>
<td>-0.0070</td>
</tr>
</tbody>
</table>

|               |                      | Gilford 2400                           |
|               |                      | $b$ | $m$                          |
| 0.40          | 0.65                 | -0.0199 | 0.1681                       |
| 0.42          | 0.65                 | -0.0042 | 0.1741                       |
| 0.44          | 0.65                 | -0.0052 | 0.1733                       |
| 0.46          | 0.72                 | -0.0061 | 0.1619                       |
| 0.47          | 0.71                 | -0.0031 | 0.1755                       |
| 0.56          | 0.85                 | -0.0015 | 0.1697                       |
The amount of HCN in μg (X) collected in a concentration column during a defined bubbling period can then be calculated from the appropriate regression equation when the absorbance (Y) at 620 nm is determined for the 25-ml total volume solution, when the milliliters of 6 N NaOH remaining in the concentration column after coating are calculated, and the milliliters of 25 percent acetic acid added before color development are known.

The calibration curve in Figure 4 shows the relationship between the known quantity of HCN in solution and the amount (μg) of HCN collected in the concentration column per liter of air dispersed. The known HCN concentrations were obtained by adding known amounts of sodium cyanide to distilled water and lowering the pH of the solution with 20 percent H₂SO₄ to about 5.0. At this pH, virtually all cyanide is in the form of HCN. Each point in Figure 4 represents the mean of a series of determinations (Table 3) and the slight deviation of the points from the regression lines fitted to these data indicates the precision of the method. One can also represent the precision of the method by computing the standard deviation of the mean and the coefficient of variability of the amounts of HCN, in μg, displaced per liter of dispersed air for each of the various HCN concentrations in the test solutions. A summary of these calculations is presented in Table 3. In general, one can conclude that there is a decrease in the coefficient of variation with an increase in HCN concentration in the
Figure 4. Calibration curves relating the concentration of HCN in solution with the amount of HCN collected in the concentration column per liter of air dispersed through the solution.
Table 3. Analysis of data used to define calibration curves relating various known HCN concentrations in test solutions to the amounts of HCN in dispersed air.

<table>
<thead>
<tr>
<th>Mean HCN concentration in test solution (mg/l)</th>
<th>Number of consecutive tests</th>
<th>Bubbling time required to collect each sample 1/ (min)</th>
<th>Mean HCN in ug per liter of dispersed air</th>
<th>Standard deviation (s)</th>
<th>Standard deviation of Mean (sx)</th>
<th>Coefficient of variability 2/ (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00480</td>
<td>2</td>
<td>400</td>
<td>0.01324</td>
<td>0.01265 - 0.01382</td>
<td>0.000827</td>
<td>0.000585</td>
</tr>
<tr>
<td>0.00950</td>
<td>5</td>
<td>400</td>
<td>0.02728</td>
<td>0.02541 - 0.03012</td>
<td>0.002012</td>
<td>0.000900</td>
</tr>
<tr>
<td>0.00955</td>
<td>5</td>
<td>400</td>
<td>0.02889</td>
<td>0.02628 - 0.03259</td>
<td>0.002731</td>
<td>0.001221</td>
</tr>
<tr>
<td>0.02411</td>
<td>4</td>
<td>160</td>
<td>0.08658</td>
<td>0.07518 - 0.09815</td>
<td>0.009413</td>
<td>0.004707</td>
</tr>
<tr>
<td>0.02411</td>
<td>4</td>
<td>160</td>
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<td>0.07033 - 0.1045</td>
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</tr>
<tr>
<td>0.04326</td>
<td>5</td>
<td>100</td>
<td>0.1849</td>
<td>0.1677 - 0.1986</td>
<td>0.01338</td>
<td>0.005985</td>
</tr>
<tr>
<td>0.04327</td>
<td>5</td>
<td>100</td>
<td>0.1773</td>
<td>0.1705 - 0.1899</td>
<td>0.008863</td>
<td>0.003964</td>
</tr>
<tr>
<td>0.1380</td>
<td>2</td>
<td>60</td>
<td>0.5013</td>
<td>0.4922 - 0.5104</td>
<td>0.01287</td>
<td>0.009100</td>
</tr>
<tr>
<td>0.1432</td>
<td>5</td>
<td>80</td>
<td>0.4997</td>
<td>0.4738 - 0.5365</td>
<td>0.02482</td>
<td>0.01110</td>
</tr>
<tr>
<td>0.1432</td>
<td>5</td>
<td>80</td>
<td>0.5005</td>
<td>0.4517 - 0.5409</td>
<td>0.03790</td>
<td>0.01695</td>
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<td>0.1924</td>
<td>2</td>
<td>60</td>
<td>0.6972</td>
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<td>0.001202</td>
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</tr>
<tr>
<td>0.2400</td>
<td>2</td>
<td>60</td>
<td>0.7925</td>
<td>0.7825 - 0.8025</td>
<td>0.01414</td>
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<tr>
<td>0.2413</td>
<td>5</td>
<td>60</td>
<td>0.8845</td>
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<tr>
<td>0.2413</td>
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<td>60</td>
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<td>0.7303 - 0.9706</td>
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<td>40</td>
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<td>1.5543 - 1.8002</td>
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<td>40</td>
<td>1.7064</td>
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<td>40</td>
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<td>5</td>
<td>30</td>
<td>2.5172</td>
<td>2.4608 - 2.5429</td>
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<td>30</td>
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<tr>
<td>0.9560</td>
<td>5</td>
<td>30</td>
<td>3.3085</td>
<td>3.1473 - 3.3979</td>
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<td>0.04477</td>
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<tr>
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<td>5</td>
<td>30</td>
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<td>2.9519 - 3.3285</td>
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<td>0.06059</td>
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<tr>
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<td>30</td>
<td>3.3523</td>
<td>3.2371 - 3.4446</td>
<td>0.1056</td>
<td>0.06098</td>
</tr>
</tbody>
</table>

1/ The compressed air was dispersed through the solution at a rate of approximately 50 cc/min.

2/ \( CV = \frac{100 \cdot s}{\bar{y}} \) where \( s \) = sample standard deviation

\( \bar{y} \) = sample mean
test solution. One can also observe that the standard deviations of the means of the amounts of HCN, in μg, displaced per liter of dispersed air increase with increase in HCN concentration in the test solution.

Examination of data in Table 3 used to define the calibration curve in Figure 4 led to the conclusion that the relationship between the mean known HCN concentration in the test solution (mg/l) and the mean amount of HCN, in μg, collected in the concentration column per liter of air dispersed could for practical purposes best be defined by two linear regression equations:

\[
Y = -0.00784 + 3.9120 \times X \\
Y = 0.02844 + 3.4146 \times X
\]

These equations apply to "Y" values of HCN displaced per liter of dispersed air ranging between 0 to 0.1849 and 0.1849 to 3.3523 μg/l, respectively. The overall linear regression equation for all mean HCN concentrations in the various test solutions was determined to be \( Y = 0.01537 + 3.4341 \times X \). The linear correlation coefficients for the data used to define the linear regression equations were in all three cases greater than 0.9980, demonstrating that a straight-line relationship is closely approached.

The lowest HCN concentration in a test solution used to obtain the calibration curve in Figure 4 was 0.0048 mg/l HCN. It is reasonable to assume that lower concentrations of HCN could be determined by increasing the volume of air dispersed through the test
solution. The only limiting factor is the time required to disperse this air through the solution, for the longer the test takes the more likely is the HCN concentration to change during the collecting period. This difficulty would be of little consequence if one were only interested in determining HCN in solutions in a state of equilibrium. This problem may be overcome by increasing the air flow rate from 50 cc/min to 100 cc/min, thus reducing the collecting time by one half. New calibration curves for the greater flow rate then would need to be determined. Data for one solution containing 0.2580 mg/l HCN showed that increasing the dispersion rate from 50 to 75 and 100 cc/min reduced the amount of HCN displaced per liter of dispersed air to 99 and 93 percent of that displaced at 50 cc/min, respectively. This indicates that the air dispersion rate may be increased to values even higher than 100 cc/min without reduction of the HCN displacement efficiency too great to permit reliance on a new calibration curve obtained at the greater flow rate.

Doudoroff et al. (1966) tested the reliability of the analytical method of Schneider and Freund (1962) under various pollutional conditions. They prepared three test solutions containing the tetra-cyanonickelate (II) complex and contaminated with municipal raw sewage, spent calcium-base sulfite pulp mill waste liquor, or a mixture of potassium dichromate (20 mg/l as Cr) and zinc sulfate (1.0 mg/l as Zn). These were compared with test solutions having the
same cyanide and nickel content and the same pH but without the above contaminants. It was concluded that no serious interference with HCN determinations by the various pollutants was apparent, even when pronounced foaming occurred inside the bubbler immersed in the sulfite waste liquor. I have further observed that the efficiency of displacement of HCN by bubbling compressed air through a test solution is only slightly increased when the solution is prepared with sea water of 8.8 and 17 parts per thousand chlorinity instead of freshwater. This average increase of 2.3 percent probably results from finer dispersion of the air in sea water which permits equilibrium to be more nearly attained. It was also noted that the HCN displacement rate was essentially the same when nitrogen, oxygen, and compressed air were used as the carrier gases.

All tests were performed in a constant temperature room at 20°C. The air temperature in this room was observed to fluctuate between 19.5 and 20.5°C on a 15-minute cycle. With this degree of air temperature control, the water temperature of test solutions located in this room was assumed to fluctuate only slightly about the desired temperature of 20°C.

Dissociation, Formation, and Toxicity of Metal-Cyanide Complexes

Preparation of Test Solutions

Chemicals of reagent or chemically pure grade and distilled
water were used without further purification in the preparation of all stock solutions.

The pH values of the various test solutions prepared in 20-liter carboys were controlled by adding 200 ml of the appropriate phosphate buffer and by adding, as necessary, concentrated solutions of strong acid (20 percent H₂SO₄) or base (6 N NaOH). The buffers were prepared by combining in various proportions 0.067 M solutions of KH₂PO₄ and Na₂HPO₄ dissolved in distilled water (Kolthoff and Rosenblum, 1937).

Test solutions containing a metal-cyanide complex were prepared by three methods. To study dissociation of the complex and its toxicity, a stock solution of NiSO₄, or of AgNO₃ or some granular CuCN was added to a continuously agitated concentrated solution of NaCN. The cyanide concentration in the NaCN solution was determined by titration with 0.02 N standard silver nitrate solution to a visually determined endpoint with paradimethyl-aminobenzalrhodamine as the indicator. The solutions of Ni (II) and Ag (I) salts were added until a definite and persistent turbidity due to precipitated metal-cyanide could be observed. The molar ratios of nickel to cyanide (Ni/CN) and silver to cyanide (Ag/CN) in the respective metal-cyanide complex solutions were consistently near the theoretical values of 1/4 and 1/2. The formation of a slight but persistent precipitate indicates that virtually all the free cyanide has been complexed. Appropriate
amounts of these solutions were then added to buffered water (distilled or well water) to give the desired total cyanide concentrations in the test solutions. The Cu (I) metal-cyanide complex stock solution containing 200 mg/l total cyanide was prepared in a continuously stirred solution by combining solid CuCN and NaCN in a Cu/CN molar ratio of 1/2 and by maintaining the pH of the solution at approximately 8.59. After 3 days of continuous agitation the undissolved CuCN was then removed through decantation and filtration and the concentrations of dissolved copper and cyanide species were calculated by assuming certain constants presented in Appendix F and a $1/\beta_2$ Cu(CN)$_2$ constant of $3.94 \times 10^{-24}$ determined during the course of this research. The percentage of total cyanide present in the stock solution as HCN, Cu(CN)$_2^-$, Cu(CN)$_3^{2-}$, and CuCN was calculated to be 0.031, 84.73, 11.40, and 3.84, respectively.

To study the formation of the Ni (II), Cu (I), and Ag (I) complexes, test solutions containing Ni (II) and Ag (I) were prepared by adding the NaCN stock solution and then the metallic salt or a stock solution of it to buffered experimental water in a 20-liter carboy in the proportion determined to be appropriate for the dissociation experiments. The same ratio of metal to cyanide in the differently prepared solutions is thus ensured. Test solutions containing Cu (I) were prepared by adding solid CuCN and a NaCN stock solution so as to produce a final solution containing the desired total cyanide concentration and
a CN to Cu molar ratio of either 2, 2.5, or 3 to 1. All test solutions were agitated with a magnetic stirrer during their preparation and equilibration.

Commercially prepared reagent grade potassium metal-cyanide complexes were used for Fe (II) and Fe (III) test solutions because preparation of these complexes in an aqueous medium without precipitation of iron hydroxide is difficult.

Procedure

All pH values were determined with a digital Radiometer Model PHM 52 or a Corning Model 12 pH meter. These instruments were calibrated with two KH$_2$PO$_4$ and Na$_2$HPO$_4$ primary standard buffer solutions of pH 6.881 and 7.429 prepared according to procedures outlined by the National Bureau of Standards (Bates, 1964).

To define rates at which equilibria are attained at 20°C, the test solutions of varying pH and concentration were allowed to stand in completely filled, stoppered, 20-liter Pyrex glass carboys, and determinations of the molecular HCN content of these solutions were made after defined time intervals. Only a few successive determinations of HCN were made on each solution in a given carboy to reduce the possibility of removing enough cyanide as HCN to alter materially the ratio of cyanide to metal. By varying both the pH and the total cyanide concentration in diluted solutions of a formed metal-cyanide
complex and testing various combinations of these, the influence of each of these two factors on the time necessary for attainment of equilibrium after dilution of the complex and pH adjustment was determined and their effects were compared. The time necessary for attainment of HCN equilibrium is a measure of the rate of dissociation of the complex. To compare the time necessary for formation of the metal-cyanide complex, corresponding experiments were performed in which uncomplexed cyanide ions and metal ions are combined in dilute solutions.

If HCN equilibrium is attained, one can calculate the dissociation constant for a metal-cyanide complex by the procedures outlined in Appendices C through I. A comparison can then be made with values obtained from the literature (Appendix A). On the other hand, if decomposition of the complex is observed, there should be a gradual increase in HCN values with time, apparently continuing indefinitely unless the liberated HCN is converted to some other cyanide species.

Most of the metal-cyanide complex ion species have a characteristic absorption peak in their absorption spectra (Appendix B). In order to define the actual species present and even in some instances their concentrations at appropriate times during the equilibration of a test solution, UV absorption spectra were determined with a Beckman DB spectrophotometer and Densicord recorder using distilled water as the reference.
In conjunction with the analytical method for the determination of HCN, standing water bioassays for the evaluation of acute toxicity described by Doudoroff et al. (1951; 1966) were used to determine the toxicity of test solutions. The various complex and sodium cyanide solutions were prepared with buffered well water. The concentration of total cyanide, cyanide/metal ratio, pH, and temperature were controlled so as to produce in each case the desired concentration of molecular HCN. The "median resistance time" (arithmetic mean of the survival times for the fifth and sixth fish to succumb in a sample of 10 fish) of fish exposed to such solutions or the TL$_m$ values of the solutions were evaluated, and at the same time when possible the molecular HCN concentrations were determined. Fish used in these bioassays were not fed for at least 1 day before a test. A fish was considered dead when it was completely and finally immobilized. At this point erratic quivering of fins and opercles stopped. A marked color change, especially in the iris of the eye, from dark to light and back to dark, occurred about 2 minutes before the defined death point.

Most tests were performed under artificial fluorescent illumination; however, a few experiments with Fe (II) and Fe (III) cyanide complexes at various pH levels and total cyanide concentrations were performed in the dark.
Penetration of Cyanide and Metal-Cyanide Complexes

To study the entry of molecular hydrocyanic acid and metal-cyanide complex ions, radioactive $^{14}\text{C}$ as HCN or Ni(CN)$_2^-$ and non-radioactive Cu as Cu(CN)$_2^-$ and Cu(CN)$_3^-$, and Ag as Ag(CN)$_2^-$ were employed. Bluegills were exposed to sodium cyanide and metallo-cyanide solutions for varying periods of time, after which tissues such as the blood, liver plus gall bladder, spleen plus heart, and/or gill arches plus filaments were removed for analysis. $^{14}\text{C}$ disintegrations per minute were counted in a tri-carb liquid scintillation spectrometer, manufactured by Packard Instrument Co., Inc. Copper and silver concentrations were determined with a Jarrel Ash flame atomic absorption spectrophotometer, model no. 82-516.

Preparation of Test Solutions

The radioactive tracer was obtained from Nuclear-Chicago, Des Plaines, Illinois. The sodium cyanide-$^{14}\text{C}$ had been prepared by the absorption of $^{14}\text{C}$ hydrogen cyanide in an excess of aqueous sodium hydroxide. Technical data concerning this material are as follows:

- 56.2 mCi/mM
- 1.1 mCi/mg sodium cyanide
- 90 percent isotopic abundance of carbon-$^{14}$
- 51 molecular weight of sodium cyanide-$^{14}\text{C}$
The contents of an ampule containing the radioactive cyanide were dissolved and diluted to 1 liter with distilled water. This stock solution of radioactive cyanide was then added to all test solutions requiring labeled cyanide to produce 2.5 or 5.0 μc of $^{14}\text{C}$ per liter of test solution.

The amount of total cyanide required to produce a desired HCN concentration or the HCN concentration produced at a given total cyanide concentration in a test solution of NaCN or a solution containing Ni(CN)$_2^-$ complex ions with a known pH value was calculated from dissociation constants applicable at 20°C, as shown in Appendices J, K, and L. The determination of the dissociation constants used will be explained later, in connection with the presentation of results of experiments on the dissociation and formation of the complex.

For experiments with the simple alkali metal cyanide, the appropriate amounts of NaCN, ranging from 0.0484 to 0.1967 mg/l CN, and then of the radioactive cyanide were added to buffered well water. At the pH of the solutions (pH 6.5 to 7.7) nearly all of the $^{14}\text{C}$ in the NaCN experiments was present in the form of HCN, and only a very small percentage was present as CN$^-$ ion. In the preparation of solutions for experiments on the nickel-cyanide complex, the desired amount of unlabeled NaCN was added to well water in a 20-liter carboy, and then the desired amount of radioactive cyanide was added. Next, NiSO$_4$ was added to form the Ni(CN)$_2^-$ complex.
The ratio of nickel to cyanide by weight was 0.565. The stoichiometric ratio for this complex is 0.564; however, addition of a slight excess of nickel ensures the absence of free cyanide other than the amount that would derive from dissociation of the complex. Test solutions containing 0.50, 25, and 500 mg/l total cyanide were prepared in this manner. The pH was adjusted by adding 200 ml of a phosphate buffer solution. If the buffer could not hold the pH in the desired range, a solution of 20 percent H₂SO₄ or of 6 N NaOH was added to adjust the pH. The test solutions were then allowed to stand undisturbed in a 20°C constant-temperature room in rubber-stoppered 20-liter Pyrex glass carboys until chemical equilibrium was attained. The time to equilibrium was determined from formation rate studies previously described, and it varied from about 1 to 10 days, depending on the pH and total cyanide concentration of the test solution. Through appropriate calculations (Appendices K and L), the percentages of the radioactive cyanide present as free HCN and as Ni(CN)₄²⁻ at equilibrium were determined. It has been noted in the Introduction that Ni(CN)₄²⁻ complex ions exhibit immeasurably rapid exchange with CN⁻, whereas there is no exchange of nickel over the pH range of 4 to 8. However, the rapid exchange of cyanide is of no practical importance when the penetration into various fish tissues of the Ni(CN)₄²⁻ complex ion is studied, because the percentages of radioactive labeled cyanide present in the different cyanide species at equilibrium remain constant.
The method of preparation of solutions for experiments on the absorption of the copper and silver cyanide complexes was the same as that outlined in a previous section on the preparation of test solutions for dissociation experiments.

**Procedure**

Four fish were placed in a 20-liter carboy filled with test solution and one fish at a time was removed after the desired exposure period. Since the duration of exposure to the test solutions was not more than 750 minutes and the volume of water used was large in relation to the total weight of fish, the concentrations of various cyanide ion species were assumed to be constant throughout the exposure periods.

Immediately after removal of a fish from a test solution, various tissues were taken from it for $^{14}$C or metal analysis. A blood sample was obtained by severing the caudal peduncle with a sharp razor blade and drawing the blood by capillary action into a heparinized capillary tube. The sample was then blown into a glass vial for $^{14}$C analysis or into a 15-ml graduated conical test tube for metal analysis. The entire liver plus gall bladder, heart plus spleen, and gill arches plus filaments were removed and each placed in a separate vial or test tube, depending upon which analysis was to be performed. The wet weights of all tissues used for $^{14}$C analyses
were recorded, because it was supposed that heating the tissues to
dryness may drive off some labeled cyanide. The dry weights of
all tissues, except the blood, to be used for metal analysis were
obtained, after drying the tissues in a 105°C oven for at least 24 hours.

Various methods have been described by which tissue and whole
blood can be prepared for assay of beta-emitting isotopes by liquid
scintillation counting. With most of these methods, color quenching
is present to such a degree that accurate counting is difficult. The
method used in these experiments was described by Mahin and Lofburg
(1966). Protein was digested and heme completely decolorized by
heating the sample to moderate temperature (70-80°C) after addition
of 0.50 ml 60 percent perchloric acid and 1.0 ml 30 percent hydrogen
peroxide. In this way, tissue samples of variable size and type could
be dissolved in individual counting vials and assayed with nearly
uniform counting efficiency. The sample vials were heated for 1 hour
in an oven set at 70-80°C. The contents were swirled twice during
this period to ensure thorough mixing with the added chemicals. The
vials were then cooled to room temperature and sample preparation
was completed by the addition of 9 ml of ethylene glycol monoethyl
ether (Cellosolve) and 10 ml of toluene phosphor solution. The phos-
phor solution contained 6.0 g of PPO (2,5-diphenyloxazole) per liter
of toluene. After mixing, the samples were placed in a tri-carb
liquid scintillation spectrometer, manufactured by Packard Instrument
Co., Inc., for counting. The information received was then corrected for background and counting efficiency in order to obtain disintegrations per minute per gram of tissue. The counting efficiency was obtained by dividing the counts per minute for channel 2 by the counts per minute for channel 1, and by reference to the appropriate curve. All samples were counted for 100 minutes to reduce the counting error to about 1 percent.

The various tissues removed from bluegills that had been exposed to metal-cyanide complex solutions were prepared for atomic absorption analysis by wet digestion. One milliliter of concentrated nitric acid was added to a 15-ml graduate conical-tip test tube containing a dried and weighed tissue sample. This initial digestion was allowed to continue overnight at room temperature. The tissues were then further digested in a steam bath for 2 hours, cooled to room temperature, treated with 30 percent hydrogen peroxide by addition of 0.20 ml to each sample, and returned to the steam bath for about 1 hour. After the samples were cooled to room temperature and diluted to a constant volume, they were ready for atomic absorption analysis. The whole blood samples were not digested but were merely diluted with distilled water to a constant volume of 1 ml before analysis. Copper and silver levels were determined respectively at wavelengths of 3247 Å and 3281 Å with a gas mixture of air and acetylene. A standard curve for silver was prepared from appropriate dilutions of a
stock AgNO₃ solution prepared in 1 percent HNO₃, and the standard curve for copper was prepared similarly from Cu metal, dissolved in a minimum volume of HNO₃, diluted with 1 percent HNO₃.
RESULTS AND INTERPRETATIONS

Chemistry and Toxicity of the Nickel-Cyanide Complex

**Dissociation and Formation of Ni(CN)$_4^{2-}$**

In the experiments described below, either the nickelocyanide complex (Ni(CN)$_4^{2-}$) or NaCN and NiSO$_4$ in the CN to Ni molar ratio of 4 to 1, as well as appropriate phosphate buffers, were added to distilled water and the subsequent change with time of the HCN concentration, through dissociation or formation of the tetracyanonickelate (II) complex in the test solutions, was observed.

Figure 5 shows this change in HCN concentration in the solutions with various pH values and total cyanide concentrations. Estimates of time required for equilibrium to be reached in each test solution and of the HCN concentration at equilibrium are given in Table 4.

In the experiments in which the nickel-cyanide complex was added to buffered distilled water and a gradual increase of the HCN concentration resulted from dissociation of the complex, the time to equilibrium generally decreased with any increase of either pH or the total cyanide concentration (Table 4). Neither pH nor total cyanide concentration was clearly the dominant factor determining the time to equilibrium.
Figure 5. Changes of the HCN concentration with time, in solutions of varying pH and total cyanide concentration, presumably due to dissociation or formation of the Ni(CN)$_2$ complex ion. ($CN_T =$ total cyanide as CN)
Table 4. Data on equilibria in solutions with a cyanide to nickel molar ratio of 4 to 1, which were used in experiments on the dissociation and formation of the nickelocyanide complex.
Equilibrium concentrations

Total cyanide

Total
nickel

as CN

(mg/i)
0. 50 F
0.551D

5.0 D
5.0 F
5.0 D
5.0 D
5.0 F
5.0 D

0.019218
0.019218
0.021178

0.048045
0.048045
0.052945

6.500
6.485
6.525

31.623

0.19218
0.19218
0.19218

0. 48045

6.507
6.522
6.580

31.117
30.061
26.303

0.19218

0.48045
0.48045
0.48045

0. 19218

50
50
50

1.9218
1.9218
1.9218

D

500 D
500 F

19.218
19.218

9.6088
9.6088
9.6088

0.48045
0. 48045

29.854

3

0.3446
0.3328
0.4114

0.021173
0.020443
0.025275

0.43040
0.43040

0.2938
0.2939

5.0040
5.0053

1. 9300

7.006
7.006
7.034

0. 43212

0. 2837

4. 8330

0.70266
0.70451
0.70451
0.78073

7.534
7.533
7.532
8.299

0.46280
0.46275
0.46272

0.1037
0.1040

1.7659
1.7704
1.7713

0. 50982

0. 1152

1. 9628

4. 7770
4. 7772

0. 1609

0.1598
0.1639

Ni(CN)
5

)

(mg/i) (M x 10 )

1.4816
1.4703
1.6159

.2901
.2980
.2987

1.0734

2.8039
2.9029

.5402
.5403
.5216

1.9988
1.9992

.1899

3. 2028

1. 1026

1.1052

(mg/i)

4
4

(M x 10 )

1. 1770

.019
.019
.021
.021

3.66
3.67
3.67
3.69

96.33
96.32
96.31
96.29

1,500
1,500
2,700

0.7055
0.6890
0.9873

2. 7399

0. 57

99. 43

2.7215
2.7918

0.56
0.58

99.43
99.42

2,500
1,100
1,500

0.6736
0.6817
0.9389

0.098
0.099

99.90
99.90

600

2,500

0.9659
1.7254

0.076
0.075
0.077

99.92
99.92

300
250

99.94

750

99. 94

1,000

99.96
99.96
99.96

48.045
48.045

7.084
7.138

8.2414
7.2778

9.9536
11.458

.5079
.5163

1.8793
1.9104

781.3
781.3

47.998
47.998

0.2773
0.2821

4.7231
4.8046

24. 022

7.493
7.486

3. 2137

9. 8856

. 1967

0. 72782

3.2659

9.5990

.1941

0.71820

390.7
390.7

0.1068

1.8443
1.8195

24. 022

7. 502

3. 1477

10. 283

. 2004

0. 74151

390. 7

24.004
24.004
24.004

0. 1083

24.022

0. 1103

1. 8795

3.2359

11.934

625.3

38.412

0.1316

2.2416

11. 433

0. 86732

625. 3

38. 412

0. 1290

2. 1969

3.1333

12.072

.2391
. 2344
.2342

0.88471

3. 3113

0.86658

625.3

38.412

0.1290

2.1966

0.058
0.056
0.056

2.1930
2.1038

14.067
14.679
14.888

.1910

0.70673
0.70747
0.67121

781.8
781.8
781.8

48.028
48.028
48.028

0.1058
0.1060
0.1007

1.8020
1.8054
1.7152

0.037
0.037
0.035

15.374

38.435

15. 374

38. 435

D

15.374

38.435

7.490
7.480
7.504

500 D 19.218

48.045
48.045
48.045

7.659
7.677
7.706

1.9679

.1912

.1814

77. 76

77.76
77.75

1/ Letter F designates formation and letter D designates dissociation of the nickelocyanide complex.

4.7765

0.6116
0.6310
0.7465

9,800

6.3308

4.8045

1.0901
1.0827
1.1104

14,000
3,000
17,000

0. 7186

5. 8540
5. 8815

.2926
.3001

x 10

10,000
5,000

8.0353
7.6560

. 2946

D

89.58
89.58
89.94

8. 1283

.1904
.2110

0. 1039

rium

jmin)

10.40
10.40
10.04

7.090
7.095
7.116

4. 8045
4. 8045

. 1904

Estimated Calculated cumulative
time to dissociation constants
for Ni(CN)4-2 at 20°c
equilib-

.015
.015
.017

4. 3801

D

500 D

32. 734

HCN

7. 7804

0. 52945

F

19.218
19.218

(M x 10 ) (M x 10

Percent total cyanide
Ni
at equilibrium as
-2
6
(mg/i) (M x 10 ) CN
Ni(CN)
HCN
4
2.6872
0.1578
.077
55.85
44.07
0. 1620
2.7602
.077
57.37
42. 55
0.1625
2.7670
.076
52.19
47.74

3.6875
3.6633
4.0075

100
100
100

500 F

8

-2

8.3176
8.3946
7.6736

F
D

-

7.080
7.076
7.115
7.109

50
50
50

D

CN

pH

(M x 10 )

0.19218
0.21178

F

H

4
(M x 10 )

3

5.51 D
D

+

-

99. 92

99.94

-

0.8259

1. 4170

-

0. 7338

0.6435
0. 8754

-

1. 1837
0. 9772

1.2146
300

1.4691

2,500

1. 7452

-

1.7546

Ave. = 1.0038
± 0.3746

31


Table 4 shows the percentage of total cyanide that is present as HCN at equilibrium in each of the test solutions together with the approximate time required for attainment of the equilibrium. Figure 6 shows that the logarithms of percentages of total cyanide present as HCN at equilibrium in phosphate buffered distilled water to which the nickelocyanide complex has been added are directly and probably linearly related to the logarithms of time in minutes to attainment of equilibrium through dissociation of the complex. In other words, the time to equilibrium is apparently a function of the ratio of HCN concentration present at equilibrium to the total cyanide concentration.

In the experiments in which NaCN and then NiSO₄ were added to distilled water and the change in HCN concentration was determined, the initial rate of formation of the complex appeared to increase with increasing pH or total cyanide concentration (Figure 5). However, the results of the four experiments at pH 7.1, 7.5, 7.5, and 7.7, in which the total cyanide concentrations were 500, 250, 400, and 500 mg/l respectively, were anomalous. Not only was the time required for attainment of equilibrium unexpectedly long (Table 4), but also the HCN concentration first determined was comparatively low and the concentration then increased to the equilibrium level. These results suggest that in such solutions the Ni(CN)₄⁻² ion and some similar species such as Ni(CN)₅⁻³ are formed very rapidly and this rapid formation of the complexes is followed by their subsequent
Figure 6. Relationship between the percentage of total cyanide present as HCN at equilibrium and the time to attainment of equilibrium, plotted on logarithmic scales.
dissociation. The procedure used in preparing these concentrated solutions, in which anomalous nickelocyanide formation occurs, could have easily allowed for the production of at least small amounts of the pentacyanonickelate (II) ion or some other larger cyanide to nickel molar ratio complex ion. The solutions were prepared by adding the appropriate amount of a concentrated NaCN stock solution to the buffered distilled water, resulting in solutions quite alkaline in pH. Next the Ni (II) salt stock solution was added slowly and in small volumes allowing the formed nickel cyanide precipitate to dissolve before the addition of more nickel. Because the nickel was added slowly, the initial nickel atoms are combined with cyanide in an environment in which the molar ratio of cyanide to nickel was much greater than the final 4 to 1 ratio. Therefore, it is likely that initially some of the nickel combines with cyanide to form nickel-cyanide complex ions such as Ni(CN)$_3^-$, resulting in lower HCN concentrations and increasing the time required for attainment of equilibrium. This phenomenon is not expected to occur to any significant degree in solutions of lower total cyanide concentration since the nickel stock solution could be added to the NaCN solution rapidly and quite often all at once rather than in repeated small volumes. In the four experiments in which the HCN concentrations first determined were greater than the equilibrium concentrations, the time to equilibrium was less than the time required for attainment of
equilibrium in corresponding experiments on the dissociation of the nickel-cyanide complex (Table 4).

Cumulative Dissociation and Formation Constants of Ni(CN)$_4^{2-}$

From the equilibrium concentration of HCN in a nickelocyanide test solution of known temperature, pH, and total cyanide concentration, the cumulative dissociation constant ($K_D$) for the tetracyano-nickelate (II) complex ion can be calculated. An "apparent $K_D$" value corresponding to the equilibrium value for HCN obtained in each of the experiments is presented in Table 4; the procedure used to calculate these values is outlined in Appendix C. The $K_D$ values thus obtained tended to increase with increase in total cyanide concentration.

Theoretically, the apparent $K_D$ should be directly related to ionic strength or should increase with increases in the total cyanide concentration. Approximate calculations have shown that changes in the activity of ionic species, as a result of changes in ionic strength of the test solutions, may indeed account in part at least for the observed variation in calculated dissociation constants. However, the range of calculated $K_D$ values, namely, from $0.6116 \times 10^{-31}$ to $1.7546 \times 10^{-31}$, is within limits that would allow for quite accurate prediction of the equilibrium HCN concentration for nickel-cyanide complex solutions of various total cyanide content. The average
apparent $K_D$ was calculated to be $1.00 \times 10^{-31}$, with a standard deviation of $\pm 0.37 \times 10^{-31}$.

**Absorption Spectra of Ni(CN)$_4^2$**

It has been shown by various authors that most complex cyanides have characteristic absorption curves in the ultraviolet spectrum. Nickelocyanide is one such complex having significant absorption in the milligram per liter range, which makes possible quantitative determination of the tetracyanonickelate (II) ion concentration. The absorption spectrum in Figure 7 for a solution with 10 mg/l total cyanide and 5.64 mg/l total nickel concentrations (CN/Ni molar ratio of 4/1) at pH 6.75, shows two main absorption peaks for Ni(CN)$_4^2$ at 267.5 nm and 285 nm.

McCullough et al. (1960) and Penneman et al. (1963) concluded that aqueous solutions containing tetracyanonickelate (II) together with a large excess of cyanide ion contain also some pentacyanonickelate (II) ion, and at very high free cyanide concentrations Penneman et al. (1963) observed that some hexacyanonickelate (IV) ion is formed. However, Penneman et al. (1963) found that, even with a 10- to 20-fold excess of sodium cyanide, most of the nickel remains in the tetracyanonickelate (II) form. With the latter finding in mind, standard curves relating absorbance and mg/l Ni(CN)$_4^2$ were determined for 267.5 nm and 285 nm wavelengths. The linear regression
Figure 7. Ultraviolet absorption spectra for a 10 mg/l total cyanide as CN tetracyanonickelate (II) solution at pH 6.75, a 10 mg/l sodium cyanide as HCN solution, and a 5 mg/l nickel sulfate as Ni (II) solution. The spectra were determined with a Beckman DB spectrophotometer using distilled water as the reference.
10 mg/liter NiCN\(^{-2}\) as CN

10 mg/liter NaCN as HCN

5 mg/liter NiSO\(_4\) as Ni(II)
equations for these relationships at the corresponding wavelengths were \( Y = 0.0038 + 0.07187 X \) and \( Y = 0.0026 + 0.02803 X \). In these equations, \( Y \) is absorbance as determined with a Gilford 2400 spectrophotometer, and \( X \) is the Ni(CN)\(_4\)\(^{-2}\) concentration in mg/l.

The alkaline tetracyanonickelete (II) solutions were prepared by diluting with distilled water a stock solution of nickel-cyanide complex that contained a small excess of free cyanide, thus insuring the complete complexation of nickel. It was observed that increasing the excess free cyanide concentration to as much as four times that required for complexation with the nickel to form Ni(CN)\(_4\)\(^{-2}\) did not affect the absorbance of the nickel-cyanide solutions when these were compared to a solution in which the CN to Ni ratio was at the theoretical 4 to 1 value.

The standard curve at 267.5 nm applies to solutions with tetracyanonickelate (II) concentrations ranging between 0 and 16 mg/l Ni(CN)\(_4\)\(^{-2}\), and the curve at 285 nm applies to solutions with Ni(CN)\(_4\)\(^{-2}\) concentrations up to 33 mg/l. Higher Ni(CN)\(_4\)\(^{-2}\) concentrations resulted in absorbance values greater than 1, the accurate determination of which is questionable. Concentrations of Ni(CN)\(_4\)\(^{-2}\) solutions greater than those defined by the above regression equations can be determined by diluting the original solutions with distilled water so as to produce a concentration in the above defined ranges. It was determined that diluting concentrated nickel-cyanide complex solutions
containing more than 33 mg/l Ni(CN)$_4^{2-}$ by as much as 100-fold did not affect the accuracy of the determination of the Ni(CN)$_4^{2-}$ concentration in the concentrated solutions.

**Toxicity of Nickel-Cyanide Complex Solutions**

The results of all nickelocyanide bioassays are summarized in Table 5. In Figure 8, the median survival time of bluegills, as determined in tests nos. 1 through 5 (Table 5) of solutions with approximately 0.20 mg/l HCN but varying concentrations of nickelocyanide complex and pH values, are plotted against the total cyanide concentration, expressed in mg/l CN. It can be seen that, although the HCN concentration is constant, there is an inverse and linear relationship between total cyanide concentration as CN and median survival time. This observation is direct evidence that some constituent other than the HCN molecule contributes to the toxicity to bluegills of solutions with high concentrations of the nickelocyanide complex. It indicates that the Ni(CN)$_4^{2-}$ complex ion itself may be toxic, because the CN$^-$ ion concentration is very low in these test solutions and by calculation can be shown to increase only by 0.0023 mg/l CN with an increase in pH from 7.1 to 7.6 (Table 5, tests nos. 1 and 5). By reference to a curve relating median resistance time in minutes to the concentration of molecular HCN in milligrams per liter that was
Table 5. The total cyanide and molecular HCN concentrations, and the median survival times for bluegills exposed to various NaCN and Ni(CN)$_{4}^{-2}$ complex test solutions.

<table>
<thead>
<tr>
<th>Test number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical or mixture tested</td>
<td>NaCN</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>Ni(CN)$_{4}^{-2}$</td>
<td>NaCN</td>
<td>NiSO$_{4}$</td>
<td>NiSO$_{4}$</td>
<td>NiSO$_{4}$</td>
</tr>
<tr>
<td>Mean pH</td>
<td>7.10</td>
<td>7.10</td>
<td>7.34</td>
<td>7.45</td>
<td>7.60</td>
<td>6.50</td>
<td>6.50</td>
<td>7.10</td>
<td>7.10</td>
<td>7.10</td>
<td>7.10</td>
<td>7.10</td>
<td>7.60</td>
</tr>
<tr>
<td>Total cyanide concentration (mg/l)</td>
<td>0.2395</td>
<td>9.99</td>
<td>100</td>
<td>248</td>
<td>500</td>
<td>0.2316</td>
<td>0.2316</td>
<td>0.2316</td>
<td>5.0</td>
<td>5.0</td>
<td>1.935</td>
<td>9.99</td>
<td>500</td>
</tr>
<tr>
<td>Time from preparation of solution to beginning of bioassay (min)</td>
<td>30</td>
<td>1,440</td>
<td>1,440</td>
<td>1,440</td>
<td>1,440</td>
<td>7,050</td>
<td>11,370</td>
<td>29,580</td>
<td>315</td>
<td>1,750</td>
<td>210</td>
<td>60</td>
<td>45</td>
</tr>
<tr>
<td>Determined HCN concentration during test period (mg/l)</td>
<td>0.20</td>
<td>0.20</td>
<td>0.21</td>
<td>0.20</td>
<td>0.20</td>
<td>0.08</td>
<td>0.11</td>
<td>0.20</td>
<td>0.075</td>
<td>0.19</td>
<td>0.17</td>
<td>0.35</td>
<td>0.09</td>
</tr>
<tr>
<td>Median survival time (min)</td>
<td>260</td>
<td>257</td>
<td>226</td>
<td>196</td>
<td>140</td>
<td>&gt;480$^{2/}$</td>
<td>&gt;480$^{2/}$</td>
<td>240</td>
<td>&gt;600$^{2/}$</td>
<td>308</td>
<td>351</td>
<td>174</td>
<td>208</td>
</tr>
</tbody>
</table>

$^{1/}$ First value represents the initial determination and the second value the final determination made during the course of the experiment.

$^{2/}$ Test was discontinued at this time with all fish still alive.
Figure 8. Median survival times of bluegills exposed to 0.20 mg/l HCN in solutions with varying concentrations of Ni(CN)$_2$ ion and varying pH, as related to the total cyanide concentration.
determined by Doudoroff et al. (1966), one can see that this increase in CN\(^{-}\) would result in a negligible decrease in median resistance time of the bluegills. Thus, the observed differences in toxicity of the solutions cannot be ascribed to differences in CN\(^{-}\) ion content. It is apparent, however, that when the total cyanide concentration is not much greater than 10 mg/l CN, the toxicity of the Ni(CN)\(_{4}\)\(^{-2}\) complex is quite negligible, and that the toxicity is then a function of the molecular HCN concentration only, for all practical purposes.

Dissociation curves (Figure 5) for the nickel-cyanide complex give information on the course of dissociation of the complex in solutions with various pH values and total cyanide concentrations. From these curves one can conclude that the toxicity of the test solutions should increase with time until equilibria are attained, and more slowly when the solutions are very dilute and acid than when they are more concentrated and slightly alkaline. Various acute toxicity bioassays were performed in order to verify this conclusion. These are reported as tests nos. 6 through 10 (Table 5). Ten bluegills were used in each test. The median survival times of bluegills in test solutions containing 0.2316 mg/l total cyanide and with pH 6.5 were greater than 480 minutes when the time intervals from preparation of the test solutions to the beginning of the bioassays were 7,050 or 11,370 minutes; when the time interval was 29,580 minutes, the median survival time was only 240 minutes. In test solutions containing
5.0 mg/l total cyanide and with pH 7.1, bluegills of average resistance lived for more than 600 and for 308 minutes when the times from preparation of the test solutions to the beginning of the bioassays were 315 and 1,750 minutes, respectively. The toxicity of nickel-cyanide complex solutions evidently increased with time, and as the pH and total cyanide content of the test solutions increased, the time required for equilibrium to be attained decreased.

Tests nos. 12 and 13 were performed to verify by bioassay some of the observed differences of molecular HCN concentration in different solutions where NaCN was combined with NiSO₄ to form the nickel-cyanide complex. In test no. 12, the median survival time of bluegills should be shorter than that observed in test no. 2, the corresponding Ni(CN)⁴⁻ complex ion dissociation experiment, because of the relatively high molecular HCN concentration that was expected and observed in the former test. Indeed this was the result; the median survival times were 174 minutes in test no. 12 and 257 minutes in test no. 2. In test no. 13, the median survival time should be longer than that observed in test no. 5, the corresponding Ni(CN)⁴⁻ complex ion dissociation experiment, because of the surprisingly low initial concentration of molecular HCN that was observed in relatively concentrated solutions such as that used in test no. 13. Indeed this was the result; the median survival times were 208 minutes in test no. 13 and 140 minutes in test no. 5. These tests show that the toxicity of
the solutions varied in accordance with variations in molecular HCN concentration determined by chemical analysis. The confirmation by bioassay of the anomalous, immediate reduction of molecular HCN to a very low level that was observed upon combination of 500 mg/l of CN\(^-\) ion with nickel ion at pH 7.1 and 7.7 is especially noteworthy.

**Chemistry and Toxicity of the Silver-Cyanide Complex**

**Dissociation and Formation of Ag(CN)\(_2^-\)**

In the experiments described below, either the silver-cyanide complex (Ag(CN)\(_2^-\)) or NaCN and AgNO\(_3\) in a CN to Ag molar ratio of 2 to 1, as well as the appropriate phosphate buffers, were added to distilled water or well water and the subsequent change with time of the HCN concentration, through dissociation or formation of the silver-cyanide complex in the test solutions, was observed. HCN equilibration in the various silver-cyanide complex dissociation and formation experiments was quite rapid, requiring less than 2 days in all solutions. The exact time to equilibrium was not determined, since it was relatively short as compared with the time required by other metal-cyanide complexes. The HCN concentration in all Ag(CN)\(_2^-\) dissociation and formation tests, determined within a few hundred minutes after preparation of the test solutions, very closely approximated the equilibrium HCN levels. The time to equilibrium did not
show any apparent correlation with pH nor with total cyanide concentration.

Cumulative Dissociation and Formation Constants for Ag(CN)$_2^-$

From the equilibrium concentration of HCN in a silver-cyanide complex test solution of known temperature, pH, and total cyanide concentration, the cumulative dissociation constant ($1/\beta_2$) for the dicyanoargentate (I) complex ion can be calculated. An "apparent $1/\beta_2$" value corresponding to the equilibrium HCN concentration obtained in each of the experimental solutions is presented in Table 6; the procedure used to calculate these values is outlined in Appendix D. The $1/\beta_2$ values thus calculated ranged between $0.2672 \times 10^{-19}$ and $2.7440 \times 10^{-19}$ and average $0.8628 \pm 0.6833 \times 10^{-19}$ for solutions with total cyanide ranging between 0.5 and 20 mg/l. The dissociation constants calculated for the 100 and 200 mg/l total cyanide solutions are $8.7629 \times 10^{-19}$ and $8.0796 \times 10^{-19}$, respectively. No explanation other than increase in ionic strength can be given for this apparent increase in $1/\beta_2$ with increase in total cyanide concentration. A similar phenomenon was observed with nickelocyanide solutions. The overall average apparent $1/\beta_2$ value calculated for all total cyanide solutions ranging between 0.5 and 200 mg/l was $1.94 \pm 2.82 \times 10^{-19}$. By reference to Appendix A, it can be seen that the above $1/\beta_2$ value
Table 6. Data on equilibria in solutions with a cyanide to silver molar ratio of 2 to 1, which were used in experiments on the dissociation and formation of the silver-cyanide complex.

<table>
<thead>
<tr>
<th>Total cyanide as CN (mg/l)</th>
<th>Silver as Ag (mg/l)</th>
<th>pH</th>
<th>CN⁻</th>
<th>HCN</th>
<th>Ag(CN)₂⁻</th>
<th>Ag⁺</th>
<th>Percent total cyanide at equilibrium as</th>
<th>Calculated cumulative dissociation constants for Ag(CN)₂⁻ at 20°C (K₁ x 10⁻¹⁹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 F 0.38435</td>
<td>0.19218</td>
<td>7.714</td>
<td>0.09609</td>
<td>4.8654</td>
<td>0.00582</td>
<td>0.21535</td>
<td>1.519</td>
<td>0.094989</td>
</tr>
<tr>
<td>1.0 F 0.38435</td>
<td>0.19218</td>
<td>7.715</td>
<td>0.09608</td>
<td>2.9831</td>
<td>0.00396</td>
<td>0.13173</td>
<td>3.062</td>
<td>0.19150</td>
</tr>
<tr>
<td>5.0 F 1.9218</td>
<td>0.96088</td>
<td>5.998</td>
<td>0.09604</td>
<td>2.3992</td>
<td>0.010804</td>
<td>1.2997</td>
<td>15.13</td>
<td>0.94594</td>
</tr>
<tr>
<td>7.0 D 2.6905</td>
<td>1.3452</td>
<td>8.024</td>
<td>0.09624</td>
<td>11.607</td>
<td>0.00685</td>
<td>0.25161</td>
<td>21.49</td>
<td>1.3439</td>
</tr>
<tr>
<td>10 D 3.8435</td>
<td>1.9218</td>
<td>5.982</td>
<td>0.09204</td>
<td>5.8210</td>
<td>0.1666</td>
<td>5.1645</td>
<td>30.24</td>
<td>1.8909</td>
</tr>
<tr>
<td>10 D 3.8435</td>
<td>1.9218</td>
<td>6.235</td>
<td>0.09210</td>
<td>2.8857</td>
<td>0.1040</td>
<td>3.8462</td>
<td>30.42</td>
<td>1.9025</td>
</tr>
<tr>
<td>10 D 3.8435</td>
<td>1.9218</td>
<td>6.416</td>
<td>0.09371</td>
<td>2.6013</td>
<td>0.0618</td>
<td>2.2867</td>
<td>30.55</td>
<td>1.9103</td>
</tr>
<tr>
<td>10 D 3.8435</td>
<td>1.9218</td>
<td>6.516</td>
<td>0.09479</td>
<td>2.9410</td>
<td>0.0555</td>
<td>2.0536</td>
<td>30.57</td>
<td>1.9115</td>
</tr>
<tr>
<td>10 D 3.8435</td>
<td>1.9218</td>
<td>7.498</td>
<td>0.09176</td>
<td>6.2533</td>
<td>0.0123</td>
<td>0.45512</td>
<td>30.69</td>
<td>1.9194</td>
</tr>
<tr>
<td>10 D 3.8435</td>
<td>1.9218</td>
<td>7.695</td>
<td>0.09204</td>
<td>7.4414</td>
<td>0.0093</td>
<td>0.34411</td>
<td>30.70</td>
<td>1.9200</td>
</tr>
<tr>
<td>10 D 3.8435</td>
<td>1.9218</td>
<td>8.522</td>
<td>0.09061</td>
<td>17.192</td>
<td>0.0032</td>
<td>0.11840</td>
<td>30.72</td>
<td>1.9211</td>
</tr>
<tr>
<td>20 F 7.6870</td>
<td>3.8435</td>
<td>5.003</td>
<td>0.34055</td>
<td>4.5556</td>
<td>0.05885</td>
<td>3.3276</td>
<td>30.62</td>
<td>1.9221</td>
</tr>
<tr>
<td>100 F 38.435</td>
<td>19.218</td>
<td>7.100</td>
<td>0.79433</td>
<td>26.392</td>
<td>0.1298</td>
<td>4.5028</td>
<td>306.9</td>
<td>19.193</td>
</tr>
<tr>
<td>200 F 76.870</td>
<td>38.435</td>
<td>7.503</td>
<td>0.31405</td>
<td>43.986</td>
<td>0.08553</td>
<td>3.1647</td>
<td>614.3</td>
<td>38.419</td>
</tr>
</tbody>
</table>

Ave. = 1.9426 ± 2.8190

1/ Letter F designates formation and letter D designates dissociation of the silver-cyanide complex.
is approximately 1 to 3 orders of magnitude larger than the values recently reported in the literature. This is a significant difference, so that use of the presently accepted dissociation constants would yield an inaccurate prediction of the equilibrium HCN concentration in silver-cyanide complex solutions in which the CN to Ag molar ratio is 2 to 1.

As determined by Jones and Penneman (1954), the best estimate for the stepwise formation constants for $\text{Ag(CN)}_3^{-2}$ (i.e., $\text{Ag(CN)}_2^+ + \text{CN}^- \rightleftharpoons \text{Ag(CN)}_3^{-2}$) and $\text{Ag(CN)}_4^{-3}$ (i.e., $\text{Ag(CN)}_2^+ + \text{CN}^- \rightleftharpoons \text{Ag(CN)}_4^{-3}$) are $5.0119 \times 10^{-2}$ and $7.4131 \times 10^{-2}$, respectively. The calculated molar CN$^-$ ion concentration for all silver-cyanide complex experimental solutions ranged between $10^{-9}$ and $10^{-7}$. Therefore, the estimated $\text{Ag(CN)}_2^-$ concentration is by $10^6$ to $10^8$ times larger than the calculated $\text{Ag(CN)}_3^{-2}$ concentration and by $10^{14}$ to $10^{18}$ times larger than the calculated $\text{Ag(CN)}_4^{-3}$ concentration. The $\text{Ag(CN)}_3^{-2}$ and $\text{Ag(CN)}_4^{-3}$ ion concentrations are therefore presumed to be negligible when compared with the $\text{Ag(CN)}_2^-$ ion concentration and are disregarded in all calculations.

**Effect of Chloride on the Silver-Cyanide Complex Equilibrium**

The effects of adding chloride ion as NaCl on the equilibrium levels of HCN in silver-cyanide complex solutions, in which the CN to Ag molar ratio is 2 to 1, at various total cyanide concentrations and
pH values are presented in Table 7 and plotted in Figure 9. It is observed that, in all instances, as the molarity of added chloride increased the HCN concentration also increased. This increase seems to be less dependent on the pH and total cyanide concentration of the test solution than on the equilibrium HCN concentrations for respective silver-cyanide complex test solutions before the addition of NaCl. The increase in HCN concentration with increase of total chloride added to respective silver-cyanide complex test solutions followed a pattern of initially increasing at a decreasing rate up to a chloride concentration of about $1 \times 10^{-2}$ M, after which the rate of HCN increase was quite linear.

A qualitative explanation for the observed phenomena involves the realization that when two ligands such as the $\text{CN}^-$ and $\text{Cl}^-$ ions are present in a solution containing silver ion, there is competition for the $\text{Ag}^+$ ion. This competition is due to certain reactions of silver ion not only with cyanide ion but also with chloride ion. It has been shown that, besides combining to form solid (precipitated) $\text{AgCl}$ according to the solubility equilibrium equation

$$\text{AgCl}_\text{(s)} \rightleftharpoons \text{Ag}^+ + \text{Cl}^- \quad \left( K_{\text{so}} @ 20-25^\circ \text{C} = 1.78 \times 10^{-10} \right)$$

silver ions and chloride ions combine to form neutral molecules according to the following equations:
Table 7. The effect of successive additions of chloride ion, as NaCl, on the determined equilibrium levels of HCN in various solutions of the silver-cyanide complex in which the cyanide to silver molar ratio was 2 to 1. ($CN_T = \text{total cyanide as CN}$)

<table>
<thead>
<tr>
<th>pH</th>
<th>HCN concentration (mg/l)</th>
<th>Chloride concentration ($M \times 10^2$)</th>
<th>pH</th>
<th>HCN concentration (mg/l)</th>
<th>Chloride concentration ($M \times 10^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.0</td>
<td>mg/l CN_T</td>
<td></td>
</tr>
<tr>
<td>7.715</td>
<td>.00356 $^1/$</td>
<td>-</td>
<td>5.998</td>
<td>.08084 $^1/$</td>
<td>-</td>
</tr>
<tr>
<td>7.717</td>
<td>.01683</td>
<td>8.39</td>
<td>5.999</td>
<td>.1064</td>
<td>0.172</td>
</tr>
<tr>
<td>7.712</td>
<td>.02780</td>
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</tr>
<tr>
<td>7.729</td>
<td>.05160</td>
<td>58.7</td>
<td>5.996</td>
<td>.1611</td>
<td>0.690</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.001</td>
<td>.2246</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.004</td>
<td>.3086</td>
<td>4.14</td>
</tr>
<tr>
<td>20 mg/l CN_T</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6.499</td>
<td>.1442</td>
<td>0.545</td>
<td>6.499</td>
<td>.2728</td>
<td>2.18</td>
</tr>
<tr>
<td>6.503</td>
<td>.1950</td>
<td>0.981</td>
<td>6.488</td>
<td>.3576</td>
<td>4.36</td>
</tr>
<tr>
<td>6.496</td>
<td>.3576</td>
<td></td>
<td>7.503</td>
<td>.08553 $^1/$</td>
<td></td>
</tr>
<tr>
<td>100 mg/l CN_T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.499</td>
<td>.1442</td>
<td>0.545</td>
<td>7.100</td>
<td>.1298 $^1/$</td>
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<tr>
<td>6.503</td>
<td>.1950</td>
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<td>7.097</td>
<td>.3212</td>
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<tr>
<td>200 mg/l CN_T</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7.503</td>
<td>.08553 $^1/$</td>
<td></td>
<td>7.508</td>
<td>.3514</td>
<td>5.45</td>
</tr>
</tbody>
</table>

$^1/$ Equilibrium HCN concentration before the addition of NaCl.
Figure 9. The relationship between determined HCN concentration in mg/l and molarity of total chloride as NaCl added to various silver-cyanide complex solutions in which the CN to Ag molar ratio is 2 to 1.
\[ \text{Ag}^+ + \text{Cl}^- \rightleftharpoons \text{AgCl}_{(aq)} \quad \left( K_1 @ 20-25^\circ C = 5.01 \times 10^2 \right) \]

\[ \text{Log } K_1 = 2.7 \]

in which the notation \((aq)\) means that this is a molecule in aqueous solution and not a solid \((s)\). As the concentration of chloride ion is increased, additional chloride ions combine with the \(\text{AgCl}_{(aq)}\) molecules to form the complex ions \(\text{AgCl}_2^-\) and \(\text{AgCl}_3^{2-}:\)

\[ \text{AgCl}_{(aq)} + \text{Cl}^- \rightleftharpoons \text{AgCl}_2^- \quad \left( K_2 @ 20-25^\circ C = 6.31 \times 10^1 \right) \]

\[ \text{Log } K_2 = 1.8 \]

\[ \text{AgCl}_2^- + \text{Cl}^- \rightleftharpoons \text{AgCl}_3^{2-} \quad \left( K_3 @ 20-25^\circ C = 2 \right) \]

\[ \text{Log } K_3 = 0.3 \]

The solubility product and stepwise formation constants were obtained from Kolthoff et al. (1969).

The extent to which the dicyanoargentate \((I)\) or the various silver-chloride complexes are formed when both chloride and cyanide ions are present in silver solutions depends upon the magnitude of the formation constants for the complex ions and the concentrations of the two ligands. Previous results indicate that the best estimate for the cumulative formation constant for the dicyanoargentate \((I)\) complex is \(5.15 \times 10^{18}\). By calculations using the various formation constants, it can be shown that the \(\text{Ag(CN)}_2^-\) ion will be the predominant silver complex species in a solution in which the CN to Ag molar ratio is 2 to 1 and to which chloride has been added. As the \(\text{Cl}^-\) molar
concentration increases, the dissociation of the $\text{Ag(CN)}_2^-$ complex is enhanced because of the increased competition of the $\text{Cl}^-$ ion for the $\text{Ag}^+$ ion to produce silver-chloride complex ions. At a given pH, with increased dissociation of the $\text{Ag(CN)}_2^-$ complex, the HCN concentration must also increase. The manner in which the HCN concentration changes with increase in chloride ion will not be quantitatively discussed since fairly complicated mathematical calculations are necessary to adjust for increases in ionic strength, especially for yet to be discussed silver-cyanide complex solutions prepared in various strengths of sea water. However, with increasing tendency of the higher silver-chloride complexes to be formed with increase in the $\text{Cl}^-$ molar concentration in silver-cyanide complex solutions, an accompanying decrease in the rate of increase in HCN production should be observed. This is apparent if we note that as the $\text{Ag(CN)}_2^-$ ion dissociates to yield the $\text{Ag}^+$ and $\text{CN}^-$ ions, more $\text{Cl}^-$ ions per $\text{Ag}^+$ ion are necessary to produce the higher silver-chloride complex species. Therefore, as the $\text{Cl}^-$ molar concentration in a silver-cyanide complex solution, at a constant pH and constant total cyanide concentration, is increased, the HCN produced should approach an asymptote.

**Absorption Spectra of Silver-Cyanide Complex Solutions**

The dicyanoargentate (I) complex ion shows a significant
ultraviolet absorption in the milligram per liter range. However, quantitative determination of the $\text{Ag(CN)}_2^-$ ion concentration is difficult because there is no peak absorption by this ion at a specific ultraviolet wavelength. The absorption spectra in Figure 10 for silver-cyanide complex solutions with total cyanide concentrations ranging from 0.5 to 200 mg/l and a CN to Ag molar ratio of 2 to 1, show this lack of any absorption peaks. However, a linear standard curve relating calculated $\text{Ag(CN)}_2^-$ ion concentration and absorbance can be defined at appropriate wavelengths and for a specific $\text{Ag(CN)}_2^-$ ion concentration range. With these restrictions in mind, standard curves relating absorbance and mg/l $\text{Ag(CN)}_2^-$ were defined for 220 nm and 237 nm wavelengths. The linear regression equations for these relationships at the respective wavelengths were $Y = 0.0226 + 2.4369 \times 10^{-2}X$ and $Y = 0.0234 + 1.4215 \times 10^{-3}X$, with linear correlation coefficients greater than 0.9990 in both cases, indicating a good "fit" of the data to a straight line. These two equations apply only to $\text{Ag(CN)}_2^-$ concentrations ranging from 0 to 10 and 10 to 200 mg/l, respectively. In these equations, $Y$ represents absorbance as determined with a Beckman DB spectrophotometer, while $X$ is the calculated $\text{Ag(CN)}_2^-$ concentration in mg/l.

The absorption spectrum of silver-cyanide complex solutions is significantly altered upon the addition of NaCl with accompanying formation of silver-chloride complex ions. The change was directly
Figure 10. Ultraviolet absorption spectra for silver-cyanide complex solutions at various pH values and total cyanide concentrations, and with a CN to Ag molar ratio of 2 to 1. The spectra were determined with a Beckman DB spectrophotometer using distilled water as the reference.
related to the amount of chloride added and produced a shift in the spectrum to the right with increased absorbance at ultraviolet wavelengths of 200 to 220 nm.

**Toxicity of Silver-Cyanide Complex Solutions**

The theoretical effect of Cl\(^-\) ions on dissociation of the Ag(CN)\(_2^-\) complex ion through competition for the Ag\(^+\) ions to form silver-chloride complex ions, with a resultant increase in the HCN concentration, has previously been discussed. These considerations suggest that silver-cyanide complex solutions should show an increased toxicity in sea water when compared with solutions prepared in fresh water of low chloride concentration.

Toxicity bioassays were performed with the marine threespine stickleback, *Gasterosteus aculeatus aculeatus* Linnaeus, in order to define this double ligand competition effect on the toxicity of silver-cyanide complex solutions in various dilutions of sea water (approx. 17\(^\circ\)/oo chlorinity). The results of these bioassays are summarized in Table 8. In Figure 11, the median survival times of 10 sticklebacks and the determined HCN concentrations for respective bioassay solutions are plotted against the determined parts per thousand chlorinity in various 10 mg/l total cyanide solutions with a CN to Ag molar ratio of 2 to 1 and at pH values near 7.7. It should be noted that, as the chlorinity in the 10 mg/l total cyanide bioassay solutions is increased,
Table 8. Properties and toxicity to the threespine stickleback of NaCN solutions and of solutions of the silver-cyanide complex with cyanide to silver molar ratio of 2 to 1, prepared with fresh water and with various dilutions of sea water.

<table>
<thead>
<tr>
<th>Total cyanide as CN (mg/l)</th>
<th>Percentage sea water</th>
<th>Conductivity (milli-ohm's)</th>
<th>Chlorinity 1/ 0/00</th>
<th>Salinity 2/ 0/00</th>
<th>Average pH during HCN determination</th>
<th>Time to beginning 3/ of HCN analysis (min)</th>
<th>Determined HCN concentration (mg/l)</th>
<th>Survival time (min)</th>
<th>Mean size of fish</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>NaCN Solutions</td>
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<tr>
<td>0.17</td>
<td>4/</td>
<td>0.166</td>
<td>-</td>
<td>-</td>
<td>7.722 7.716</td>
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<td>-</td>
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<td>-</td>
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<td>8.73</td>
<td>15.77</td>
<td>7.670 7.666</td>
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<td>240</td>
<td>0.1807</td>
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<td>23.6</td>
<td>8.85</td>
<td>15.99</td>
<td>7.696 7.708</td>
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<td>0.2256</td>
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<td>17.44</td>
<td>31.51</td>
<td>7.779 7.733</td>
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<td>0.1595</td>
<td>504</td>
<td>506.8</td>
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<tr>
<td>0.21</td>
<td>100</td>
<td>42.9</td>
<td>17.13</td>
<td>30.95</td>
<td>7.720 7.721</td>
<td>240</td>
<td>0.1875</td>
<td>350</td>
<td>356.4</td>
</tr>
<tr>
<td>0.27</td>
<td>100</td>
<td>39.4</td>
<td>15.59</td>
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<td>0.2254</td>
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<td>200.8</td>
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<tr>
<td>Ag (CN)₂ Solutions</td>
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<tr>
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<td>40.2</td>
<td>15.94</td>
<td>28.80</td>
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<td>16.25</td>
<td>29.36</td>
<td>- 7.932</td>
<td>-</td>
<td>-</td>
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<tr>
<td>6.0</td>
<td>0</td>
<td>0.181</td>
<td>-</td>
<td>-</td>
<td>- 7.720</td>
<td>-</td>
<td>-</td>
<td>22 hr</td>
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</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>- 7.738</td>
<td>-</td>
<td>-</td>
<td>15 hr</td>
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</tr>
<tr>
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<td>0.179</td>
<td>-</td>
<td>-</td>
<td>- 7.695 7.695</td>
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<td>0.0939</td>
<td>770</td>
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</tr>
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<td>10.0</td>
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<td>7.91</td>
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<td>473.7</td>
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<td>10.0</td>
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<td>7.669 7.676</td>
<td>5,640</td>
<td>0.2422</td>
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<td>301.7</td>
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<tr>
<td>10.0</td>
<td>75</td>
<td>29.6</td>
<td>11.36</td>
<td>20.52</td>
<td>7.708 7.702</td>
<td>5,640</td>
<td>0.2326</td>
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<tr>
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<td>43.0</td>
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<td>7.747 7.733</td>
<td>4,110</td>
<td>0.2415</td>
<td>140</td>
<td>143.9</td>
</tr>
</tbody>
</table>

1/ Parts per thousand chlorinity for sea water test solutions were calculated from measured conductivity values by use of the following equation derived by Park and Burt (1965):

\[
\text{conductivity in ohm}^{-1} \text{cm}^{-1} = 3.0191 \times 10^{-3} \text{Cl}^{-1} + 5.6253 \times 10^{-8} \text{Cl}^{-1} + 2.181 \times 10^{-6} \text{Cl}^{-1} - 3.804 \times 10^{-9} \text{Cl}^{-1}
\]

2/ Parts per thousand salinity = \( 1 \times 80635 \text{parts per thousand chlorinity} \) (Wooster, Lee, and Dietrich, 1969).

3/ Measured from time of preparation of the test solution. Fish were introduced at about the same time analysis was begun.

4/ Solutions with zero percent sea water were prepared with fresh water of a low chloride concentration (Table 1).
Figure 11. The relationships of median survival time of 10 sticklebacks and the determined HCN concentration in bioassay solutions to the determined chlorinity in various silver-cyanide complex solutions containing 10 mg/l total cyanide and pH near 7.7.
the HCN concentration increases in a linear manner until a chlorinity value of about 8.5/00, that of 50 percent sea water, is reached. With continued increase in chlorinity, the HCN concentration remains constant at about 0.24 mg/l. At low chlorinity values below 2/00 and not acutely toxic HCN concentrations near 0.05 mg/l or less, the median survival time is independent of chlorinity remaining constant at about 780 minutes. This is direct evidence that some constituent other than the HCN molecule contributes to the toxicity of solutions with fairly low concentrations of the silver-cyanide complex. It can be surmised that the Ag(CN)\textsubscript{2} ions are themselves toxic, since the CN\textsuperscript{-} and Ag\textsuperscript{+} ion concentrations are quite low in these test solutions. Although the HCN concentration did not increase with increase in chlorinity from 8.5 to 17 parts per thousand, the median survival time of sticklebacks did decrease from 259.5 minutes at a chlorinity of 8.6/00 and HCN concentration of 0.2422 mg/l to 140 minutes at a chlorinity of 17.18/00 and HCN concentration of 0.2415 mg/l. This suggests that either the HCN molecule becomes more toxic to sticklebacks with increase in chlorinity or that as the HCN concentration remains constant and chlorinity increases the Ag(CN)\textsubscript{2} ions become more toxic.

The first possibility, namely that HCN becomes more toxic with increase in chlorinity was examined by performing bioassays with sticklebacks exposed to NaCN solutions prepared with sea water dilutions of varying chlorinity. The relationships between median survival
time in minutes and determined HCN concentration for bioassays performed in fresh water and sea water of 8.8 and 17 parts per thousand chlorinity near pH 7.7 are presented in Table 8 and plotted in Figure 12. The three curves are essentially linear over the applicable HCN concentration range; however, as the chlorinity of the NaCN test solutions is increased, the median survival time at a given HCN concentration in some instances decreases. There is not much effect of an increase in chlorinity to 8.8\(^0/\)\(\text{o}\) on the survival time of sticklebacks in NaCN solutions containing up to 0.24 mg/l HCN. On the other hand, a significant decrease in survival time occurs when the chlorinity of the test solutions is increased from 8.8 to 17\(^0/\)\(\text{o}\). When the data presented in Figure 12 are plotted on arithmetic coordinate paper, it is observed that the rate of decrease in median survival time is linear and about the same for all three curves over the determined HCN concentration range. However, the degree of decrease in median survival time at a specific HCN concentration increases with increasing chlorinity. The anomalous decrease in median survival time of sticklebacks exposed to silver-cyanide complex solutions of approximately equal HCN concentration but increasing chlorinity can therefore be attributed at least in part to the increased toxicity of HCN at higher chlorinities.

The open data points plotted in Figure 12 represent the median survival times of sticklebacks at determined HCN concentrations from
Figure 12. The relationships of median survival time of 10 sticklebacks and the determined HCN concentration in NaCN bioassay solutions prepared with fresh water or seawater of 8.8 or 17 °/oo chlorinity and pH near 7.7. The open data points represent bioassay results of 10 mg/l total cyanide silver-cyanide complex solutions of specified chlorinities.
bioassays of 10 mg/l total cyanide silver-cyanide complex solutions of specified chlorinities. It is apparent that the toxicity of the solutions with high HCN concentrations of about 0.24 mg/l can be attributed essentially to the HCN molecule, since each of the points obtained at the high HCN levels would fall approximately on a projection of the toxicity curve for NaCN solutions of the same chlorinity. The one observation for the 4.4%o chlorinity solution, in which the median survival time was 473 minutes and the HCN concentration 0.1031 mg/l, falls far below projections of the NaCN toxicity curves obtained at both higher and lower chlorinities. Therefore, it appears that, at low HCN concentrations, the toxicity of silver-cyanide complex test solutions is mainly caused by Ag(CN)$_2^-$ ions.

To further substantiate the increased toxicity of the silver-cyanide complex solutions in sea water, 24-hour median tolerance limits for sticklebacks of the complex ion solutions (with CN to Ag molar ratios of 2 to 1) prepared with sea water of approximately 17%o chlorinity and with fresh water were determined. These TL$_m$ values were found to be approximately 3.0 mg/l total cyanide at pH 7.932 and 6.0 mg/l total cyanide at pH 7.720 for the sea water and fresh water solutions, respectively. The sea water bioassay solutions contained less than 0.0955 mg/l HCN, while the fresh water solutions contained less than 0.0093 mg/l HCN.

The toxicity of fresh water solutions of the silver-cyanide
complex to bluegills was found to be considerably less than their toxicity to sticklebacks. The median survival time of 10 bluegills exposed to a 10 mg/l total cyanide solution at pH 7.498 with a determined HCN concentration of 0.0123 mg/l was approximately 31 hours. The median survival time for a similar solution at pH 8.523, in which the determined HCN concentration was 0.0032 mg/l, was approximately 29 hours. The median survival time of 10 bluegills exposed to a 7 mg/l total cyanide solution at pH 8.024 and with a determined HCN concentration of 0.0068 mg/l was approximately 87 hours. In the above solutions the Ag$^+$ ion concentration is calculated to be 0.02 mg/l or less. However, it should be pointed out that at the relatively high total cyanide concentrations of approximately 10 mg/l and low pH values of about 6.0, the Ag$^+$ ion concentration derived from dissociation of the Ag(CN)$_2^-$ complex ion, could significantly contribute to the observed toxicity of solutions of the silver-cyanide complex (Table 6).

Chemistry and Toxicity of the Iron-Cyanide Complexes

\[
\text{Dissociation of Fe(CN)$_6^{4-}$ and Fe(CN)$_6^{3-}$}
\]

In the experiments described below, either reagent grade K$_4$Fe(CN)$_6 \cdot 3$H$_2$O or reagent grade K$_3$Fe(CN)$_6$ and the appropriate phosphate buffers were added to distilled water and the subsequent changes with time of the HCN concentration through dissociation of the
iron-cyanide complexes in test solutions kept in the dark were observed. Figure 13 shows these changes in HCN concentration in the solutions with pH values of 6.8 and 7.1 and total cyanide concentrations of 5.0 and 500 mg/l, respectively.

In the ferricyanide solution containing 5 mg/l total cyanide, HCN concentrations found after as many as 444.8 thousand minutes (309 days) after preparation were not appreciably different from the initial concentration of 0.012 mg/l. In the absence of verification, the significance of the single relatively high HCN value of 0.020 mg/l obtained after 14.4 thousand minutes in testing the solution with the lower total cyanide concentration of 5 mg/l is uncertain, and this value has been disregarded in fitting the curve to the data in Figure 13. The ferricyanide solution containing the higher total cyanide concentration of 500 mg/l exhibited the slower equilibrium. The HCN concentration was initially 0.016 mg/l and it increased to an apparent equilibrium level of about 0.067 mg/l.

In both of the ferrocyanide solutions, a material increase of the HCN concentration with time after preparation of the solutions was observed. In Figure 13, it can be seen that the initial HCN concentrations in ferro- and ferricyanide solutions of equal total cyanide concentration differed little. However, in the ferrocyanide solution containing 500 mg/l total cyanide, the HCN concentration increased greatly with time, reaching an equilibrium value of about 0.2652 mg/l.
Figure 13. The relationships of changes in HCN concentrations with time in ferro- and ferricyanide solutions at pH values of 6.8 and 7.1 and total cyanide concentrations of 5.0 and 500 mg/l, respectively.
In the ferrocyanide solution containing 5 mg/l total cyanide the HCN level first increased sharply to an apparent maximum of 0.0565 mg/l, observed after about 90.5 thousand minutes, and then rapidly decreased to an average equilibrium concentration of 0.0109 mg/l. It is important to note that the latter equilibrium HCN concentration is only slightly greater than the equilibrium HCN level observed in the corresponding ferricyanide solution. The significance of this observation will be discussed later.

A ferrocyanide solution containing 500 mg/l total cyanide and with initial pH of 7.1 was allowed to remain undisturbed and in the dark at 20°C for approximately 450 days. After this long period, the HCN concentration of the solution, which then had a pH of 7.230, was determined to be 0.2565 mg/l. This HCN concentration is approximately equal to the equilibrium level of 0.2652 mg/l determined for the similar solution on which repeated HCN determinations were performed, whose pH at the time of the last two HCN determinations averaged 7.084.

Cumulative Dissociation Constants

for Fe(CN)$_6^{4-}$ and Fe(CN)$_6^{3-}$

From the equilibrium concentrations of HCN in ferro- and ferricyanide test solutions of known temperature, pH, and total cyanide concentration, the cumulative dissociation constants ($1/\beta_6$) for the
hexacyanoferrate (II) and hexacyanoferrate (III) complex ions can be calculated. An "apparent $1/\beta_6$" value corresponding to the equilibrium HCN concentration obtained in each of the experimental solutions is presented in Table 9; the procedure used to calculate these values is outlined in Appendix E. It is apparent that there is much variability in the calculated dissociation constants, and therefore, only a rough estimate of the true value can be proposed.

The HCN concentration in the 5 mg/l ferrocyanide solution shows an initial increase and then rapid decline (Figure 13) as reaction time progresses, with a subsequent equilibrium concentration only slightly greater than that found in a 5 mg/l ferricyanide solution. It is believed that oxidation of iron (II) to iron (III) may account for the observed rapid decline, after about 90.5 thousand minutes, of the HCN concentration in the ferrocyanide solution containing 5 mg/l total cyanide. Changes in ultraviolet absorption spectra, presented in the following section, give support to this hypothesis. Conversion of ferrocyanide ion to ferricyanide ion in the dilute ferrocyanide solution would explain why its equilibrium HCN concentration was approximately the same as that found in the corresponding ferricyanide solution, with the result that nearly the same $1/\beta_6$ dissociation constants (about $10^{-57}$) were calculated from data obtained with the two solutions. The best estimate of the $1/\beta_6$ dissociation constant for the hexacyanoferrate (II) complex ion can be calculated from the apparent HCN equilibrium
Table 9. Data pertaining to experiments on the dissociation of the hexacyanoferrate (II) and (III) complex ions in solution.

<table>
<thead>
<tr>
<th>Total cyanide</th>
<th>Total iron</th>
<th>Equilibrium concentrations</th>
<th>Percent total cyanide at equilibrium as</th>
<th>Calculated cumulative dissociation constants for Fe(CN)_6^{4-} or Fe(CN)_3^{3-} at 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>at CN (mg/l)</td>
<td>(M x 10^{-3})</td>
<td>(M x 10^{-8})</td>
<td>H^+ (M x 10^{-3})</td>
<td>CN^- (M x 10^{-3})</td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>-----------------------------</td>
<td>--------------------------------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Ferrocyanide Solutions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 0.19218</td>
<td>32.029</td>
<td>6.796</td>
<td>15.996</td>
<td>5.707</td>
</tr>
<tr>
<td>5 0.19218</td>
<td>32.029</td>
<td>6.796</td>
<td>15.996</td>
<td>5.707</td>
</tr>
<tr>
<td>5 0.19218</td>
<td>32.029</td>
<td>6.796</td>
<td>15.996</td>
<td>5.707</td>
</tr>
<tr>
<td>500 19.218</td>
<td>32.029</td>
<td>7.104</td>
<td>7.870</td>
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</tr>
<tr>
<td>500 19.218</td>
<td>32.029</td>
<td>7.104</td>
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<td>500 19.218</td>
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<td>32.029</td>
<td>6.796</td>
<td>15.996</td>
<td>5.707</td>
</tr>
</tbody>
</table>

\[1/\] Letters I, M, and E respectively refer to the initial, maximum, and equilibrium determined HCN concentrations in the various experimental solutions.
concentration determined in the 500 mg/l total cyanide solutions. The HCN concentration of 0.2565 mg/l determined in a 500 mg/l ferrocyanide solution at pH 7.230, which was allowed to remain undisturbed in the dark for 450 days, is approximately equal to the equilibrium HCN concentration of 0.2652 mg/l determined for a similar solution at pH 7.084 on which repeated HCN determinations were performed. Since the HCN equilibrium concentration of the solution to which air was repeatedly added is approximately equal to the HCN concentration in the solution on which only one HCN determination was made, it is believed that conversion of the hexacyanoferrate (II) ion to the hexacyanoferrate (III) ion through oxidation of iron in these test solutions was slight. Ultraviolet absorption spectra presented in the following section give support to this view. The best estimate of the $1/\beta_6$ dissociation constant that can be calculated from the available data for the hexacyanoferrate (II) complex ion is about $10^{-47}$.

The $1/\beta_6$ dissociation constants for the hexacyanoferrate (III) ion computed from data obtained with the ferricyanide solutions containing 5 and 500 mg/l total cyanide and with pH 6.8 and 7.1 were calculated to be approximately $10^{-57}$ and $10^{-52}$, respectively. No explanation can be given for this apparent increase of the dissociation constant with increase in total cyanide concentration; however, similar though much smaller increases were observed in experiments with both the nickel-cyanide and the silver-cyanide complexes. As previously
stated, the $1/\beta_6$ dissociation constant calculated for the more dilute ferrocyanide solution was approximately equal to the constant calculated for the corresponding ferricyanide solution. If oxidation of iron is the correct explanation, the experimental results obtained with iron-cyanide solution containing 5 mg/l total cyanide support a $1/\beta_6$ dissociation constant of $10^{-57}$ for the hexacyanoferrate (III) complex ion. Referring to Table 9, it can be seen that if the initial HCN concentration of 0.0161 mg/l in the 500 mg/l total cyanide solution, rather than the apparent equilibrium concentration, is used in calculating a hexacyanoferrate (III) dissociation constant, a value of about $10^{-56}$ is obtained. No explanation other than dissociation of the complex can be given for the observed increase in HCN from the initial concentration to the higher equilibrium value. Therefore, the best estimate of the $1/\beta_6$ dissociation constant of the hexacyanoferrate (III) complex ion, comparable with the constant of $10^{-47}$ calculated for the hexacyanoferrate (II) complex ion from data obtained with test solutions containing 500 mg/l total cyanide, appears to be $10^{-52}$.

Information in Appendix A shows that the above $1/\beta_6$ values of $10^{-47}$ and $10^{-52}$ are by approximately 11 and 9 orders of magnitude smaller than values recently reported in the literature. This is a highly significant difference, and calculations using the presently accepted dissociation constants would yield much higher equilibrium HCN concentrations than those actually found in the ferro- and
ferricyanide solutions kept in the dark, concentrations that in all cases would be rapidly fatal to fish.

**Absorption Spectra of**

$\text{Fe(CN)}_6^{4-}$ and $\text{Fe(CN)}_6^{3-}$

The hexacyanoferrate (II) and hexacyanoferrate (III) complex ions show measurable ultraviolet absorption in the milligram per liter range. The ultraviolet absorption spectra of test solutions containing these two complex ions are markedly different. The absorption spectra in Figure 14 for ferro- and ferricyanide solutions with total cyanide concentrations of 5 and 500 mg/l show that the $\text{Fe(CN)}_6^{4-}$ complex ion has a peak absorption at about 217 nm, whereas the $\text{Fe(CN)}_6^{3-}$ complex ion has no significant ultraviolet absorption peak.

The 500 mg/l total cyanide solutions were diluted 100-fold with distilled water just before each absorption determination so as to produce a solution whose maximum absorbance could be accurately measured.

The absorption spectra of ferrocyanide solutions containing 5 and 500 mg/l total cyanide, prepared by dissolving $\text{K}_4\text{Fe(CN)}_6 \cdot 3\text{H}_2\text{O}$ in distilled water, are presented in Figures 14A and 14B. As the time after preparation of these test solutions increases, it is observed that the ultraviolet absorption spectra shift, so that the absorbance at the lower wavelengths of 210 to 230 nm is decreased while that at the
Figure 14. Ultraviolet absorption spectra for ferro- and ferricyanide solutions with total cyanide concentrations of 5 and 500 mg/l (latter solutions diluted 100-fold with distilled water) and respective pH values of 6.8 and 7.1. The spectra and their progressive shifts with time after solution preparation, as indicated by the arrows, were determined with a Beckman DB spectrophotometer using distilled water as the reference.
A
5 mg/liter CN\textsubscript{T}  pH 6.8

B
500 mg/liter CN\textsubscript{T}  pH 7.1

C
5 mg/liter CN\textsubscript{T}  pH 6.8

D
500 mg/liter CN\textsubscript{T}  pH 7.1

WAVELENGTH (nm)
higher wavelengths of 290 to 320 nm is slightly increased. This shift of absorption spectra was greatest in the 5 mg/l total cyanide solution; that in the 500 mg/l total cyanide solution diluted 100-fold just before each absorbance determination was much less pronounced. The absorption spectra of ferricyanide solutions containing 5 and 500 mg/l total cyanide, prepared by dissolving K₃Fe(CN)₆ in distilled water, are presented in Figures 14C and 14D. As the time since preparation of these test solutions increases, their ultraviolet absorption spectra remain essentially unchanged. This constancy of the spectra and of the determined HCN concentrations and pH values in ferricyanide test solutions, over extremely long test periods timed from solution preparation, is direct evidence of immutability of the hexacyanoferrate (III) complex ion in an aqueous medium and in the dark. On the other hand, the significant changes in absorption spectra and of determined HCN concentrations in ferrocyanide test solutions is direct evidence that, in an aqueous medium, the Fe(CN)₆⁻⁴ complex ion undergoes slow alteration even in the absence of light.

It has been pointed out that the equilibrium HCN concentrations in dilute ferro- and ferricyanide test solutions, with 5 mg/l total cyanide, were approximately equal. The change in ultraviolet absorption spectra of the dilute ferrocyanide solution (Figure 14) is such that the final spectrum very closely approximates that determined for the dilute ferricyanide test solution. It is well known that ferrous ion
is quite susceptible to oxidation in the presence of atmospheric oxygen:

\[
\begin{align*}
\text{Reaction} & \quad \text{Standard electrode potentials at } 25^\circ \text{C} \\
O_2 + 4H^+ + 4e^- & \rightarrow \ 2H_2O \quad \rightarrow 1.229 \text{ volts} \\
Fe^{+++} + e^- & \rightarrow \ Fe^{++} \quad \rightarrow 0.771 \text{ volt}
\end{align*}
\]

The cell reaction is given by:

\[
\begin{align*}
O_2 + 4H^+ + 4e^- & \rightarrow \ 2H_2O \quad \rightarrow E_{\text{cell}} = E_{\text{reduction}} - E_{\text{oxidation}} \\
4Fe^{++} & \rightarrow 4Fe^{+++} + 4e^- \quad \rightarrow E_{\text{cell}} = 1.229 - 0.771 \\
O_2 + 4Fe^{++} + 4H^+ & \rightarrow 4Fe^{+++} + 2H_2O \quad \rightarrow E_{\text{cell}} = 0.458
\end{align*}
\]

The rate of this reaction is minimal in 0.5 to 1.0 N sulfuric acid solutions, but is accelerated in both neutral and more strongly acidic solutions. Therefore, in the presence of adequate dissolved oxygen and at an approximately neutral pH, the Fe (II) ion is oxidized to Fe (III). The role of H\(^+\) ions in this oxidation reaction was reflected in the observed slight increase in pH of ferrocyanide test solutions that occurred as reaction time progressed. A small volume of a 20 percent sulfuric acid solution was added as needed throughout the experimental period to lower the pH of the test solutions to the appropriate value. No significant change in pH was observed in the ferricyanide solutions; the average pH and standard deviations for the 5 and 500 mg/l total cyanide solutions, over the entire 309-day test period, were 6.793 ± 0.021 and 7.098 ± 0.021, respectively.

Through a substitution or inversion type process in which one
ion leaves as the other enters, Fe(II) - Fe(III) ionic exchange between iron-cyanide complex species conceivably could occur. Thompson (1948) has shown that there was no exchange between Fe (II) or Fe (III) and either ferrocyanide or ferricyanide over a 6-day period at room temperature and in the absence of light. Dodson, Eimer and Medalia (1951) further reported that there is no effect of deaeration on the Fe (II) - Fe (III) exchange. A measurably slow exchange in the dark between 14C-labeled cyanide and both complexes in alkaline solutions was observed over a period of 100 hours by Adamson, Welker and Volpe (1950). Admittedly, in the dark this proposed exchange process is very slow. However, regardless of previously published observations, it can be concluded that in the dilute ferrocyanide solution at pH 6.8, which was repeatedly aerated during HCN determinations, the hexacyanoferrate (II) complex ion was almost completely transformed into the hexacyanoferrate (III) complex ion in approximately 100 days.

Ultraviolet absorption spectra for 100-fold dilutions of the ferrocyanide solution with 500 mg/l total cyanide (Figure 14B) indicate that a measurable amount of transformation of the hexacyanoferrate (II) ion to the hexacyanoferrate (III) ion does occur over a time period of about 309 days. During this time, the ultraviolet absorbance at 217 nm was observed to decrease from an initial maximum of about 0.70 to a final minimum of about 0.60. This apparent reduction in Fe(CN)\textsubscript{6}^4\textsuperscript{−} ion concentration was not considered in calculating the 1/\(\beta_6\)
dissociation constant of the hexacyanoferrate (II) complex ion. It should also be pointed out, however, that if the oxidation of \( \text{Fe(CN)}_6^{4-} \) to form the more stable \( \text{Fe(CN)}_6^{3-} \) indeed occurred, the observed HCN equilibrium concentration in this test solution was somewhat lower than that which would have been determined if no such oxidation had taken place. Since the \( \text{CN}^- \) concentration to the sixth power used in calculating the \( 1/\beta_6 \) dissociation constant would have been correspondingly greater and the \( \text{Fe(CN)}_6^{4-} \) concentration only slightly greater than if no oxidation had occurred, the actual dissociation constant for the hexacyanoferrate (II) complex ion presumably is slightly greater than the estimated value of \( 10^{-4.7} \). A more accurate calculation of this constant could have been accomplished had nitrogen been used as the carrier gas for making HCN determinations on previously deoxygenated ferrocyanide test solutions kept in the dark.

A toxicity bioassay of the ferricyanide solution with 500 mg/l total cyanide, performed at the conclusion of the HCN equilibration experiment, confirmed the reported low toxicity of the \( \text{Fe(CN)}_6^{3-} \) ion. Ten bluegills lived for longer than 48 hours in this solution, which was determined to have an HCN concentration of 0.067 mg/l and a calculated \( \text{Fe(CN)}_6^{3-} \) ion concentration of 679 mg/l. The median survival time of 10 bluegills exposed to the ferrocyanide solution with 500 mg/l total cyanide, which was prepared with distilled water and was determined to have an HCN concentration of 0.2669 mg/l, was only
145 minutes. This survival time is approximately equal to that which one would expect for a solution of NaCN prepared with distilled water and having the same HCN concentration. Therefore, the toxicity of the ferro- and ferricyanide solutions appears to be directly related to the HCN concentration and is essentially independent of the hexacyanoferrate (II) and hexacyanoferrate (III) ion concentrations.

Chemistry and Toxicity of Cuprocyanide Complexes

Formation and Dissociation of Cuprocyanide Complexes

In formation experiments, CuCN and NaCN in CN to Cu molar ratios of 2, 2.5, and 3 to 1, as well as the appropriate phosphate buffers, were added to distilled or well water and the subsequent change with time of the HCN concentration, through formation of cuprocyanide complexes in the repeatedly stirred test solutions, was observed.

Figure 15 shows the changes of HCN concentration in some of the cuprocyanide formation experiments at various pH values, total cyanide concentrations, and CN to Cu ratios. Equilibrium was attained within 2 or 3 days in all solutions in which the CN to Cu ratio was 2 to 1, excepting one at pH 6.5 and with 0.20 mg/l total cyanide, in which equilibration was less rapid (Figure 15A). No effort was made to determine the exact time necessary for attainment of equilibrium, which doubtless depends on the amount of stirring. In solutions in
Figure 15. Changes in HCN concentration with time in cuprocyanide formation solutions at varying pH and total cyanide concentrations with CN to Cu molar ratios of 2, 2.5, and 3 to 1. (CN$_T$ = total cyanide as CN)
which the CN to Cu molar ratio was either 2.5 or 3 to 1, constancy of the HCN concentration usually was not attained even after as many as 110 days following their preparation (Figure 15C-F). Under these circumstances, it is possible that decomposition of cyanide gradually reduced the total cyanide concentrations in the various experimental solutions, thereby changing the CN to Cu ratio and preventing attainment of equilibrium. However, it can be seen that in a solution with a 3 to 1 CN to Cu ratio, at pH 7.1, and with a low total cyanide concentration of 0.25 mg/l, equilibrium was attained rapidly and the determined HCN concentration remained constant for about 48 days indicating no cyanide decomposition (Figure 15B).

Figure 15A shows that, in the three solutions at pH 6.5 with different CN to Cu molar ratios but all containing 0.20 mg/l total cyanide, the initially determined HCN concentrations were low and with time increased to the equilibrium values. It can be calculated that the initially determined HCN concentrations were less than that which the added NaCN would have produced at pH 6.5 if no copper-cyanide complexation had occurred. Therefore, we can assume that some copper-cyanide complexation had already occurred. It is also important to note that, in all three cases, the molar equilibrium HCN concentrations were greater than the initial molar concentration of cyanide introduced as NaCN. Therefore, some of the cyanide initially present as CuCN must have been liberated to form HCN. Calculations
appearing in Appendix I show that the solubility product for CuCN is not exceeded, and therefore, no CuCN remained in these three solutions at equilibrium. The excess unbound copper ion released into these solutions must be present in some form other than the Cu (I) ion. Copper (I) is not stable in aqueous solution, so it is likely that some of the Cu (I) ions transfer electrons to other Cu (I) ions. Disproportionation (self oxidation-reduction) with the formation of elemental copper and cupric ion is known to occur in aqueous solutions of cuprous compounds (Sienko and Plane, 1966). It is also reasoned, on the basis of calculations appearing in Appendix I, that the cyanide not occurring as CN\(^-\) or HCN was mainly present as the cuprocyanide complex, Cu(CN)\(\_\_\)\(_2\). This supposition is strengthened by ultraviolet absorption curves indicating the presence of cuprocyanide complex ions in the solutions.

With some modifications, the calculations appearing in Appendix I apply also to the experimental solution at pH 7.1 with 0.25 mg/l total cyanide and with a CN to Cu molar ratio of 3 to 1. In this solution, the determined HCN concentration was initially high and decreased rapidly to the equilibrium level (Figure 15B). Calculations show the initially determined HCN concentration, as well as the equilibrium concentration, to have been less than that which could have derived from the added NaCN at pH 7.1 if no copper-cyanide complexation had occurred. However, ultraviolet absorption curves indicated the
presence of cuprocyanide complex ions in this solution at the time of initial as well as equilibrium HCN determinations. Calculations appearing in Appendix I indicate that the solubility product for CuCN cannot be exceeded and therefore no undissolved CuCN remained in the solution at equilibrium. Subtraction of the CN\(^-\) + HCN molar concentration \((4.3158 \times 10^{-6})\) from the initial total cyanide (\(\text{CN}_T\)\) concentration \((9.6088 \times 10^{-6})\) shows \(5.2930 \times 10^{-6}\) mole/l of cyanide to have been present in combined form (Table 11). The initial CuCN concentration was \(3.2029 \times 10^{-6}\) gram mole per liter of solution. Since we know that no undissolved CuCN was remaining at equilibrium, the copper initially added as CuCN could have been complexed only if the \(\text{CN}_T - (\text{CN}^- + \text{HCN})\) concentration were at least twice the initial CuCN concentration. Therefore, some excess, unbound copper must have been released into the solution and present in a form other than the Cu (I) ion, most likely as Cu (II) and elemental copper.

A decanted and filtered portion of a 200 mg/l total cyanide stock solution prepared by combining CuCN and NaCN in a CN to Cu molar ratio of 2 to 1 was diluted with distilled water and the appropriate phosphate buffer added for dissociation experiments. Table 10 shows the change of HCN concentration in these cuprocyanide dissociation experimental solutions at various pH values and total cyanide concentrations. Calculations verified by the results of the formation experiments showed that the filtered stock solution used in the dissociation
Table 10. Data pertaining to experiments on the dissociation of the cuprocyanide complex in solutions with various pH values and total cyanide concentrations and with cyanide to copper molar ratio of 2.083 to 1.

<table>
<thead>
<tr>
<th>Total cyanide as CN (mg/l)</th>
<th>Time since preparation to midpoint of analysis (min)</th>
<th>Average pH during determination&lt;sup&gt;1/&lt;/sup&gt;</th>
<th>Determined HCN concentration&lt;sup&gt;1/&lt;/sup&gt; (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>-</td>
<td>0.0017&lt;sup&gt;2/&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,170</td>
<td>6.472</td>
<td>0.0034</td>
</tr>
<tr>
<td></td>
<td>6,840</td>
<td>6.500</td>
<td>0.1374</td>
</tr>
<tr>
<td></td>
<td>21,240</td>
<td>6.490</td>
<td>0.2924</td>
</tr>
<tr>
<td></td>
<td>29,964</td>
<td>6.487</td>
<td>0.3181</td>
</tr>
<tr>
<td></td>
<td>44,946</td>
<td>6.491</td>
<td>0.3203</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.489</td>
<td>0.3192</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>-</td>
<td>0.0051&lt;sup&gt;2/&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,170</td>
<td>7.092</td>
<td>0.1180</td>
</tr>
<tr>
<td></td>
<td>6,840</td>
<td>7.088</td>
<td>0.1614</td>
</tr>
<tr>
<td></td>
<td>21,240</td>
<td>7.064</td>
<td>0.1502</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.081</td>
<td>0.1558</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>-</td>
<td>0.0084&lt;sup&gt;2/&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,350</td>
<td>7.064</td>
<td>0.1843</td>
</tr>
<tr>
<td></td>
<td>6,990</td>
<td>7.066</td>
<td>0.2268</td>
</tr>
<tr>
<td></td>
<td>21,390</td>
<td>7.049</td>
<td>0.2064</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.060</td>
<td>0.2166</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>-</td>
<td>0.0169&lt;sup&gt;2/&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,350</td>
<td>7.476</td>
<td>0.1632</td>
</tr>
<tr>
<td></td>
<td>6,990</td>
<td>7.459</td>
<td>0.1578</td>
</tr>
<tr>
<td></td>
<td>21,390</td>
<td>7.452</td>
<td>0.1564</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.462</td>
<td>0.1591</td>
</tr>
</tbody>
</table>

<sup>1/</sup> Numbers below horizontal lines are averages of values determined after equilibrium had been attained.

<sup>2/</sup> Calculated initial HCN concentration in test solution upon dilution of filtered stock solution.
experiments contained the $\text{Cu(CN)}_2^-$ and $\text{Cu(CN)}_3^{2-}$ complex cyanide species in significant concentrations. The CN to Cu molar ratio in the filtered stock and diluted experimental solutions was calculated to be 2.083 to 1. At the high pH of 8.59, the HCN concentration in the stock solution was calculated to be 62 $\mu$g/l. Upon dilution of the filtered stock solution, the calculated initial HCN concentrations in all four solutions were considerably less than the determined equilibrium values and, for practical purposes, the only copper-cyanide complexes present were the $\text{Cu(CN)}_2^-$ and $\text{Cu(CN)}_3^{2-}$ species. The subsequent increase with time of the HCN concentration, through dissociation of $\text{Cu(CN)}_2^-$ and $\text{Cu(CN)}_3^{2-}$ in the test solutions, was observed by repeated measurements. For three of the four solutions equilibrium was attained within 6 days. The exception was the pH 6.5-5 mg/l total cyanide solution which required about 21 days to equilibrate. The excess unbound copper (I), released through dissociation of the cupro-cyanide complex ionic species, must have been present in a form other than the Cu (I) ion, most likely as Cu (II) and elemental copper.

The determined equilibrium HCN concentrations for the various dissociation experimental solutions with pH and total cyanide concentrations (as mg/l CN) of 6.5-5, 7.1-15, 7.1-25, and 7.5-50 were respectively, 0.319, 0.156, 0.217, and 0.159 mg/l. For the corresponding formation experimental solutions the determined equilibrium HCN concentrations were respectively, 0.223, 0.135, 0.198, and
0.157 mg/l. It is observed that the equilibrium HCN concentrations for the dissociation experiments were consistently greater than the equilibrium HCN concentrations for the corresponding formation experimental solutions. Only for the concentrated solutions of 50 mg/l total cyanide with pH 7.5 were the equilibrium HCN concentrations essentially the same. In more dilute solutions, the difference between the equilibrium HCN concentration for the dissociation and formation experimental solutions becomes progressively larger as the total cyanide concentration of the test solutions decreases. Therefore, it can be concluded that the equilibrium HCN concentration in solutions prepared by diluting a decanted and filtered cuprocyanide stock solution, in which the CN to Cu molar ratio is 2 to 1, will be greater at least for fairly dilute solutions than equilibrium HCN concentrations of corresponding experimental solutions in which undissolved CuCN remains. It is also important to emphasize that the time to equilibrium for dilute dissociation experimental solutions is quite long at pH values that could easily occur in natural waters.

In the formation experimental solutions containing the cuprocyanide complex ionic species and undissolved CuCN, the concentration of free cyanide depends on both the dissociation of the complex ions and the solubility of the CuCN. The concentration of free cyanide in the diluted solutions in which the undissolved CuCN has been removed by decanting and filtering will be controlled by the dissociation constants
of the complex cuprocyanide ions. Neither copper nor complex ions are available from solution and ionization of solid CuCN, so the solubility product constant of CuCN will no longer affect the equilibrium.

**Dissociation and Formation Constants for Cuprocyanide Complexes**

When certain determined and calculated parameters are known for various cuprocyanide test solutions, formation constants for $\text{Cu}^+ + 2\text{CN}^- \rightleftharpoons \text{Cu(CN)}_2^-$ and $\text{Cu(CN)}_2^- + \text{CN}^- \rightleftharpoons \text{Cu(CN)}_3^-$ can be calculated. An "apparent $1/\beta_2$" value corresponding to the equilibrium HCN concentration observed in each of the experimental solutions in which the CN to Cu molar ratio was 2 to 1, is presented in Table 11. The steps used to calculate these values are outlined in Appendix F. Table 11 shows that $1/\beta_2$ values thus calculated for solutions with a CN to Cu molar ratio of 2 to 1 varied only slightly; the range of $1.4766 \times 10^{-24}$ to $7.7999 \times 10^{-24}$ is within limits of acceptable experimental error. The average apparent $1/\beta_2$ value is $3.94 \times 10^{-24}$ with a standard deviation of $\pm 1.75 \times 10^{-24}$. It is apparent that I was able quite accurately and consistently to estimate a dissociation constant for the dicyanocuprate (I) complex in solutions having a wide range of pH values and total cyanide concentrations. Therefore, the concentrations of various copper and cyanide species in solutions in which NaCN and CuCN were combined in the CN to Cu molar ratio of 2 to 1
Table I. Data pertaining to experiments on the formation of cyanoporphyrins complexes in solutions containing copper in the water ratio of \(L_1 \times L_2 \times L_3 \times \ldots \times L_{n0} \).

<table>
<thead>
<tr>
<th>Total cyanide</th>
<th>Initial</th>
<th>Equilibrium</th>
<th>Calculated formation of</th>
<th>Concentrations of</th>
<th>Calculated dissociation of</th>
</tr>
</thead>
<tbody>
<tr>
<td>as ( \text{CN}^\text{-} )</td>
<td>as ( \text{CN}^\text{-} )</td>
<td>as ( \text{CN}^\text{-} )</td>
<td>CuCN</td>
<td>CuCN</td>
<td>CuCN</td>
</tr>
<tr>
<td>( \text{mg/l} )</td>
<td>( \text{mg/l} )</td>
<td>( \text{mg/l} )</td>
<td>( \text{mg/l} )</td>
<td>( \text{mg/l} )</td>
<td>( \text{mg/l} )</td>
</tr>
</tbody>
</table>

** footnote:**

1. **The copper as potentially present, ratio between free base CuCN** and CuCN.  
2. **Column 10 refers to the calculated monomer, and the determined CN concentration in the various experimental solutions.**
can be calculated quite accurately. Reference to Appendix A reveals that the above average $1/\beta_2$ value is close to those reported by Vladimirova and Kakovskii (1950) and Penneman and Jones (1956), namely $1.9 \times 10^{-24}$ and $5.0 \times 10^{-24}$, respectively. It is significantly different, however, from the value of $2.0 \times 10^{-22}$ reported by Rothbaum (1957).

In most of the test solutions in which NaCN and CuCN were combined in the CN to Cu molar ratios of 2.5 and 3 to 1, equilibrium was not attained, and "apparent $1/K_3$" constants therefore cannot be accurately calculated for these solutions. However, by the procedures outlined in Appendices G and H, rough estimates of the $1/K_3$ constant can be calculated for solutions with determined HCN concentrations using the initial and the final HCN values. The initial HCN concentrations were determined 2,910 to 22,770 minutes after the preparation of the various test solutions; the final concentrations were determined 54,360 to 102,180 minutes after preparation of the solutions. Obviously, the initial and final times since preparation are not the same for all test solutions, but we are not trying to make precise quantitative comparisons, and it was only hoped that something could be learned by comparing these $1/K_3$ estimates with the presently accepted equilibrium constant.

For solutions in which NaCN and CuCN were combined in the CN to Cu molar ratios of 2.5 to 1 and 3 to 1, the $1/K_3$ estimates
corresponding to initial HCN determinations ranged from about 1.21 x 10^{-6} to 2.91 x 10^{-6} and from 3.96 x 10^{-6} to 6.15 x 10^{-6}, respectively (Table 11). But the 1/K_3 estimates corresponding to the final HCN determinations ranged from 2.42 x 10^{-7} to 6.81 x 10^{-7} for the 2.5 to 1 solutions and from 2.13 x 10^{-8} to 4.92 x 10^{-8} for the 3 to 1 solutions (Table 11). The estimated 1/K_3 values corresponding to the initial HCN determinations are greater than the values calculated when the final HCN concentrations are used. The 1/K_3 values corresponding to initially determined HCN concentrations for both the 2.5 and 3 to 1 CN to Cu molar ratio solutions are approximately equal to the value of 4.55 x 10^{-6} determined by Baxendale and Westcott (1959), but are materially different from the values reported by Penneman and Jones (1956) and by Rothbaum (1957), namely, 2.57 x 10^{-5} and 2.51 x 10^{-5}, respectively (Appendix A). The discrepancy between 1/K_3 values calculated from final HCN concentrations and those reported in the literature can not be fully explained at this time. Results presented in the previous section indicated that decomposition of cyanide is not a major factor contributing to the slow decline in HCN concentration, although the possibility of some is not ruled out. It is more probable that some reaction or reactions, perhaps with the phosphate buffer or involving formation of other cuprocyanide complex species not assumed in this study and incorporated into the calculations of 1/K_3, can account for the generally observed slow reduction in HCN
concentrations. It is also important to emphasize that since the $1/K_3$ values reported in the literature were most likely based on observations from cuprocyanide test solutions made quite soon after their preparation, it is not surprising that these values are approximately equal to my estimated $1/K_3$ values based on initial HCN concentrations. The presently accepted $K_3$ and $K_4$ cuprocyanide stepwise formation constants reported in the literature may be incorrect having been determined from data pertaining to solutions which were not at true equilibrium. It is also possible that the slow decline in HCN is actually a reflection of the slow formation reactions for $\text{Cu(CN)}_2^+ + \text{CN}^- \rightleftharpoons K_3 \text{Cu(CN)}_3^-$ and $\text{Cu(CN)}_3^+ + \text{CN}^- \rightleftharpoons K_4 \text{Cu(CN)}_4^-$. The $K_4$ stepwise formation constant, as reported in the literature (Appendix A), is about $10^{1.74}$ to $10^{2.3}$. The calculated molar CN$^-$ concentrations for all experimental cuprocyanide solutions ranged between $10^{-9}$ and $10^{-7}$. Therefore, assuming the presently accepted $K_4$ constant to be approximately correct, the predicted $\text{Cu(CN)}_4^-$ concentration is from $10^{-7}$ to $10^{-4}$ times smaller than the calculated $\text{Cu(CN)}_3^-$ concentration and is considered to be negligible in all calculations.

Absorption Spectra of Cuprocyanide Solutions

Cuprocyanide complex ions in the milligram per liter concentration range show significant ultraviolet absorption which permits
determination of their concentrations. However, since the molar extinction or molar absorptivity coefficient is nearly the same for Cu(CN)\(_2^-\), Cu(CN)\(_3^{2-}\), and Cu(CN)\(_4^{3-}\) complex ions at approximately 234 nm (Appendix B), it is not possible from UV absorption data to determine the concentrations of particular complex ions in test solutions. The absorption spectra in Figure 16 for solutions with various total cyanide and total copper concentrations and with CN to Cu molar ratios of 2, 2.5, and 3 to 1 show two main absorption peaks at 210 and 234 nm. The curves defining the relationship between determined absorbance values up to 1.222 and various calculated Cu(CN)\(_2^-\) molar concentrations (Table 11) were linear at 210 and 234 nm wavelengths in cuprocyanide complex formation experiments, regardless of the total cyanide concentration, pH, and the CN to Cu molar ratio. The regression equations for the linear portions of these curves were

\[ Y = 0.0160 + 1.8863 \times 10^4 X \] at 210 nm and

\[ Y = 0.0107 + 1.0972 \times 10^4 X \] at 234 nm, with a linear correlation coefficient greater than 0.9996 in both cases, indicating a good "fit" of the data to a straight line. In these equations, Y represents absorbance as determined with a Beckman DB spectrophotometer, and X is the calculated Cu(CN)\(_2^-\) plus Cu(CN)\(_3^{2-}\) concentration in moles/l. It should be emphasized that these same linear relationships apply to solutions with a CN to Cu molar ratio of 2 to 1, in which some CuCN(s) is calculated to be remaining at equilibrium, as well as to solutions in which this ratio
Figure 16. Ultraviolet absorption spectra for solutions of cuprocyanide formation at varying pH and total cyanide concentrations with CN to Cu molar ratios of 2, 2.5, and 3 to 1.
is 2.5 or 3 to 1 and the Cu(CN)\textsubscript{3}\textsuperscript{-2} ion concentration contributes significantly to the absorbance. This consistent relation between predicted and determined absorbance gives support to the validity of the proposed budget calculations presented in Appendices F through I. When absorbance values were greater than 1.222, the relationship of absorbance to calculated Cu(CN)\textsubscript{2}\textsuperscript{-} plus Cu(CN)\textsubscript{3}\textsuperscript{-2} molar concentrations at 210 nm shows a slight deviation from linearity, believed to be due to limitations of the accuracy of the Beckman DB spectrophotometer in determining high absorbance values. For corresponding solutions with lower absorbance values recorded at 234 nm, a linear relationship between absorbance and calculated Cu(CN)\textsubscript{2}\textsuperscript{-} plus Cu(CN)\textsubscript{3}\textsuperscript{-2} molar concentration was observed.

The absorption spectra of cuprocyanide complex solutions shown in Figure 16 vary with pH, particularly those for solutions containing 15 mg/l total cyanide or more. As the pH of these test solutions increases above approximately 7.7, the base-line absorbance at the upper wavelengths of the spectra increases, while the descending portion of the curves for wavelengths greater than about 250 nm shifts to the right. The shift in the absorbance spectra with increase in pH does not result from a change in Cu(CN)\textsubscript{2}\textsuperscript{-} and Cu(CN)\textsubscript{3}\textsuperscript{-2} ion concentrations, since the calculated change in concentration of these ions in solutions with 15 mg/l total cyanide and more was very small. The determined absorbance values at 210 and 234 nm for cuprocyanide
solutions containing 6 mg/I or less as total cyanide showed a slight pH dependence which could in this case be accounted for by a calculated change in the Cu(CN)$_2^-$ and Cu(CN)$_3^{2-}$ ion concentrations.

Changes in ultraviolet absorption spectra for the cuprocyanide solutions prepared by combining NaCN with CuCN and having CN to Cu molar ratios of 2, 2.5, and 3 to 1 (Table 12) were consistent with previously reported trends for experimentally determined HCN concentrations. Analysis of data presented in Tables 11 and 12 revealed that the initially determined HCN concentration in solutions with pH 6.5 and containing 0.20 mg/I total cyanide at the three CN to Cu ratios was less than the equivalent HCN concentration which the added NaCN alone could have produced at pH 6.5. Absorption data at 210 nm indicate that part of the CuCN combines with cyanide from NaCN to form cuprocyanide complex ions, presumed to be mainly Cu(CN)$_2^-$, by the time initial HCN determinations were performed. The absorbance values at this wavelength increase to a maximum that is attained in about 9,000 minutes. This increase in the cuprocyanide ion concentration is accompanied by an increase in HCN concentration, resulting from cyanide being liberated from CuCN. The absorbance then decreases slightly with a corresponding increase in the HCN concentration, suggesting a dissociation of the already formed Cu(CN)$_2^-$ ions. As the absorbance decreases to an apparent equilibrium level, the HCN concentration also tends to increase to an equilibrium value.
Table 12. Absorbance values at 210 nm and determined HCN concentrations of solutions in cupro-cyanide formation experiments after different reaction times, which were midpoints of the HCN analysis periods.

<table>
<thead>
<tr>
<th>Total cyanide as CN (mg/l)</th>
<th>CN to Cu molar ratio</th>
<th>Reaction time (min)</th>
<th>Average pH during determination</th>
<th>Determined HCN concentration (mg/l)</th>
<th>Absorbance at 210 nm</th>
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(Continued on next page)
Table 12. (Continued)

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<th>Total cyanide as CN (mg/l)</th>
<th>CN to Cu molar ratio</th>
<th>Reaction time (min)</th>
<th>Average pH during determination</th>
<th>Determined HCN concentration (mg/l)</th>
<th>Absorbance at 210 nm</th>
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| 0.50                      | 3                    | 2,910               | 7.691                           | 0.1844                             | 0.105                |
|                           | 4,560                | 7.691               | 0.1641                          |                                    | 0.131                |
|                           | 11,520               | 7.681               | 0.1484                          |                                    | 0.148                |
|                           | 20,190               | 7.699               | 0.1438                          |                                    | 0.134                |
|                           | 30,270               | 7.705               | 0.1386                          |                                    | 0.137                |
|                           | 53,535               | 7.686               | 0.1125                          |                                    | 0.149                |
|                           | 74,910               | 7.707               | 0.0998                          |                                    | 0.155                |
|                           | 90,750               | 7.683               | 0.0888                          |                                    | 0.137                |
|                           | 103,710              | 7.697               | 0.0853                          |                                    | 0.140                |
|                           | 112,350              | 7.692               | 0.0822                          |                                    | 0.143                |
|                           | 125,310              | 7.680               | 0.0747                          |                                    | 0.137                |
|                           | 142,590              | 7.696               | 0.0529                          |                                    | 0.131                |
|                           | 155,550              | 7.714               | 0.0542                          |                                    | 0.137                |
Since calculations in Appendix I show that no CuCN remains at equilibrium, the unstable copper (I) ion released into these solutions, other than that necessary to satisfy cuprocyanide ion equilibria, must (as explained above) be present in some form other than Cu (I) ion, possibly as Cu (II) and elemental copper. Because of the much lower absorptivity of the cuprocyanide complex ions at 234 nm, a similar comparison between absorbance and determined HCN concentrations for the various cuprocyanide solutions was not made since the accuracy of some low absorbance values at this wavelength was questionable.

The HCN concentration in the 0.25 mg/l total cyanide solution at pH 7.1 and with a CN to Cu molar ratio of 3 to 1 was initially slightly above the equilibrium level, and then rapidly decreased to a nearly constant level (Table 12). In this case, the ultraviolet absorbance at 210 nm is seen to be initially low (Table 12); a large percentage of the cyanide added as NaCN must be then present as HCN. Thereafter, the absorbance at 210 nm and therefore the total cupro-cyanide ion concentration, as well as the determined HCN concentration, proceeded rapidly to an equilibrium condition, which was maintained for at least 70,000 minutes. The constancy of these values after rapid attainment of equilibrium is decisive evidence that cyanide decomposition, even in solutions containing as little as 0.25 mg/l total cyanide, is negligible in test solutions prepared with distilled water.
The HCN concentrations in the 0.5 mg/l total cyanide solutions at pH 7.5 and 7.7 and with a CN to Cu molar ratio of 3 to 1 were initially about 0.18 mg/l; after a short more rapid decline at the outset, they then decreased in a nearly linear manner with progressing reaction time (Table 12 and Figure 15C). The initially determined ultraviolet absorbance values at 210 nm of 0.102 and 0.105, for the test solutions with pH 7.5 and pH 7.7, are shown in Table 12 to increase thereafter to apparent equilibrium values averaging 0.135 and 0.140, respectively. This stability of observed absorbance is evidence that the total concentration of absorbing cuprocyanide complex ionic species remains fairly constant while the HCN concentration is continuing to decline. One possible explanation for the observed results is that, with progressing reaction time, an increasing percentage of the total copper is complexed with cyanide to produce cuprocyanide complex ions with higher CN to Cu molar ratios, namely \( \text{Cu(CN)}_3^{2-} \) and \( \text{Cu(CN)}_4^{3-} \). This explanation receives support when one compares in Table 11 the initial and final \( \text{Cu(CN)}_3^{2-} \) molar concentrations calculated for these solutions. These calculated values indicate a considerable increase of the \( \text{Cu(CN)}_3^{2-} \) concentration accompanying the decrease of the determined HCN concentration. Since by the end of observations reported, equilibria were not attained in most of the cuprocyanide test solutions with a CN to Cu molar ratio of 2.5 or 3 to 1, it is not possible to determine the \( K_3 \) and \( K_4 \) formation constants.
from information obtained during this study. An in-depth investigation into this apparently slow equilibration is beyond the scope of this study and will not be pursued further.

The HCN concentration in the 1 mg/l total cyanide solution at pH 6.5 and with a CN to Cu molar ratio of 2.5 to 1 was initially about 0.22 mg/l and then rapidly declined to 0.19 mg/l, but this decline was followed by a gradual increase to values higher than the initial one (Table 12 and Figure 15D). For this solution, the ultraviolet absorbance at 210 nm was 0.264 after about 4,000 minutes and increased to 0.303 after about 10,000 minutes, when the minimum HCN concentration of 0.19 mg/l was observed. The absorbance then gradually decreased, while the HCN concentration increased. Because of the inverse relationship between HCN concentration and absorbance, it appears that, during the period when the HCN concentration was declining, NaCN was reacting with CuCN to form cuprocyanide complex ions. The subsequent increase of the HCN concentration and decline of the absorbance can be supposed to result from decomposition of some of the Cu(CN)$_2^-$ complex ions with liberation of CN$^-$ ions and disproportionation of liberated Cu (I) ions to produce Cu (II) and elemental copper.

The ultraviolet absorbance data at 210 nm do not contribute to understanding of the reactions occurring in the test solutions with CN to Cu molar ratios of 2.5 to 1 and containing 2.5 and 5 mg/l total.
cyanide. The determined absorbance value of each of these solutions was so high that no measurable change of absorbance with time after preparation of the test solutions could be expected to accompany the small observed decline in determined HCN concentration.

Toxicity of Cupro cyanide Complex Solutions

The results of all cupro cyanide bioassays are summarized in Table 13. In Figure 17, the median survival times of bluegills in solutions of varying total cyanide content and with a CN to Cu molar ratio of 2 to 1 are plotted against the determined molecular HCN concentrations, expressed in mg/l. When virtually no HCN was detected in cupro cyanide solutions with 5 mg/l of total cyanide, the median survival time was observed to be about 42 hours. Inasmuch as NaCN solutions containing less than about 0.11 mg/l HCN are not acutely toxic to bluegills, this is direct evidence that some constituent other than the HCN molecule contributes to the toxicity to bluegills of solutions with fairly low concentrations of the cupro cyanide complex. It is reasonable to conclude that the Cu(CN)$_2^-$ and Cu(CN)$_3^{2-}$ complex ions are themselves toxic, since the CN$^-$, Cu$^+$, and Cu(CN)$_4^{-3}$ ion concentrations are very low in these test solutions and undissolved CuCN could not readily penetrate fish gill filaments or other tissues.

At the high total cyanide concentrations of 25 and 50 mg/l, but not at lower concentrations (15 and 5 mg/l), the median survival time
Table 13. Properties and toxicity to bluegills of various NaCN solutions and of solutions of the cuprocyanide complex with cyanide to copper molar ratio of 2, 2.5, or 3 to 1.

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<th>Test No.</th>
<th>Total cyanide as CN (mg/l)</th>
<th>Mean pH</th>
<th>Determined HCN concentration (mg/l)</th>
<th>Calculated cuprocyanide ion concentration</th>
<th>Median survival time (min.)</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN to Cu - 2.5:1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>0.2</td>
<td>6.517</td>
<td>0.1342</td>
<td>0.1569</td>
<td>508</td>
</tr>
<tr>
<td>34</td>
<td>0.2</td>
<td>6.567</td>
<td>0.1590</td>
<td>0.1037</td>
<td>275</td>
</tr>
<tr>
<td>35</td>
<td>0.5</td>
<td>7.491</td>
<td>0.1702</td>
<td>0.7377</td>
<td>251</td>
</tr>
<tr>
<td>36</td>
<td>0.5</td>
<td>7.467</td>
<td>0.1411</td>
<td>0.6111</td>
<td>385</td>
</tr>
</tbody>
</table>
Figure 17. The relationships between median survival times and determined HCN concentrations for bluegills exposed to NaCN solutions and to cupro-cyanide solutions of varying pH and total cyanide concentration, in which the CN to Cu molar ratio was 2 to 1. ($CN_\text{T}$ = total cyanide as CN)
decreased with decreasing determined HCN concentration after attaining a maximum value. This reduction in survival time cannot be attributed to an accompanying change in the calculated $\text{Cu(CN)}_2^-$ complex ion concentrations since they are observed to decline (Table 13). If it is assumed that the $\text{Cu(CN)}_2^-$ and $\text{Cu(CN)}_3^{2-}$ complex ions are of approximate equal toxicity, then the small increase in the calculated $\text{Cu(CN)}_3^{2-}$ concentration could not account for the associated observed reduction in survival time. The pH of the test solutions with the two total cyanide concentrations at which a decrease in survival time was observed ranged from 8 to 9, whereas the pH of the water to which the fish had been acclimated was about 7.3. Several authors have stated that the rate of movement of solutes through plasma membranes is dependent on ion charge and relative size of the hydrated ions, but it is primarily dependent on the physiological state of the cells. The abrupt change in pH to which bluegills were subjected when they were transferred to the experimental solutions of high pH may have affected the gill filament cells, increasing their permeability to the cuprocyanide ions. The phenomenon under consideration is of little practical importance, since it is only observed in solutions at very high total cyanide concentrations.

The toxicity of the dicyanocuprate (I) ion was determined by bioassay of cuprocyanide formation solutions in which the CN to Cu
molar ratio was 2 to 1. In these solutions, the only potentially toxic ion present in considerable concentration, which is calculated by a procedure outlined in Appendix F, is the Cu(CN)$_2^-$ ion. The 48-hour TL$_m$ of Cu(CN)$_2^-$ for bluegills at 20°C was determined to be 9 mg/l as Cu(CN)$_2^-$ or 4 mg/l as CN. The toxicity of the Cu(CN)$_3^{2-}$ and Cu(CN)$_4^{3-}$ ions could not be determined by testing the 2 to 1 CN to Cu solutions with the various combinations of pH and total cyanide concentration and low HCN levels, because the concentrations of these ions were negligible. At CN to Cu molar ratios of 2, 5 and 3 to 1, a greater percentage of the total cyanide is present as Cu(CN)$_3^{2-}$ and Cu(CN)$_4^{3-}$, but even in these solutions with low HCN concentrations and realistic pH values, the Cu(CN)$_3^{2-}$ and Cu(CN)$_4^{3-}$ concentrations, as well as the Cu(CN)$_2^-$ concentrations, are quite small and non acutely toxic.

In tests nos. 31 through 36 of Table 13, the median survival times of bluegills in formation cuprocyanide solutions containing CN and Cu in the molar ratio of 2, 5 and 3 to 1 are presented. When these survival times are compared with those of bluegills exposed to NaCN solutions (tests nos. 1 through 4), the toxicity of the cuprocyanide solutions is found to be essentially determined by the HCN concentration.
Penetration of Hydrogen Cyanide and Metal-Cyanide Complexes

HCN and the Nickelocyanide Complex

The accumulation of cyanide or its metabolic by-products in various fish tissues was studied by exposing bluegills to solutions of $^{14}$C-labeled NaCN and to those in which NiSO$_4$ was combined with labeled NaCN to form the nickelocyanide complex, Ni(CN)$_4$$^{-2}$. The percentages of total cyanide present as different cyanide species in these various test solutions are presented in Table 14. Calculations used to determine these percentages are presented in Appendices J, K, and L. It should be pointed out that exchange of the cyanide radicals of the Ni(CN)$_4$$^{-2}$ complex ions with the simple cyanide ion or HCN in solution is immeasurably rapid, but at equilibrium, when the penetration experiments were performed, the labeled cyanide ions were still distributed among the different species in proportions corresponding to those of the percentages of all cyanide occurring in these different forms. The determined concentrations of accumulated $^{14}$C radioactivity (carbon from $^{14}$C-labeled cyanide), expressed as $\mu$g HCN per gram wet weight of tissue, in bluegills exposed to $^{14}$C-labeled NaCN and Ni(CN)$_4$$^{-2}$ experimental solutions at various pH values and total cyanide concentrations, are presented in Tables 15 and 16 and are shown plotted against exposure time in Figures 18 and 19.
Table 14. Calculated percentages of total cyanide present as different cyanide species in various $^{14}$C-labeled NaCN and nickelocyanide test solutions and the calculated concentrations of HCN and Ni(CN)$_4^{2-}$.

<table>
<thead>
<tr>
<th>Total cyanide as CN (mg/l)</th>
<th>Total nickel as Ni (mg/l)</th>
<th>pH</th>
<th>Calculated concentrations$^{1/}$ (mg/l)</th>
<th>Percentage of cyanide present as</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCN</td>
<td>Ni(CN)$_4^{2-}$</td>
</tr>
<tr>
<td>NaCN experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1928</td>
<td>-</td>
<td>6.50</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>0.1936</td>
<td>-</td>
<td>7.10</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>0.0484</td>
<td>-</td>
<td>7.10</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>0.1967</td>
<td>-</td>
<td>7.70</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Ni(CN)$_4^{2-}$ experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>0.28</td>
<td>6.50</td>
<td>0.2938</td>
<td>0.339</td>
</tr>
<tr>
<td>25</td>
<td>14</td>
<td>7.10</td>
<td>0.2580</td>
<td>38.7</td>
</tr>
<tr>
<td>500</td>
<td>282</td>
<td>7.70</td>
<td>0.1813</td>
<td>781.8</td>
</tr>
</tbody>
</table>

$^{1/}$ These concentrations were calculated by using the dissociation constant for HCN at 20°C of 4.365 x 10$^{-10}$ given by Izatt et al. (1962) and my apparent $K_D$ values for the Ni(CN)$_4^{2-}$ complex ion at 20°C, as determined for the different pH values and total cyanide concentrations (Table 4).
Table 15. Determined $^{14}$C radioactivity, expressed as µg HCN per gram wet weight, in various tissues of bluegills exposed to $^{14}$C-labeled NaCN solutions and their relationship to exposure time as defined by linear regression equations. ($CN_T = \text{total cyanide as CN}$)

<table>
<thead>
<tr>
<th>Time in test solution (min)</th>
<th>Tissue</th>
<th>$^{14}$C radioactivity expressed as µg HCN per gram wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 mg/l HCN test solution</td>
<td>pH 7.1-0.0484 mg/l $CN_T$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 6.5-0.1928 mg/l $CN_T$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 7.1-0.1936 mg/l $CN_T$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 7.7-0.1967 mg/l $CN_T$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>15</td>
<td>Blood</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.234</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.214</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.218</td>
</tr>
<tr>
<td>30</td>
<td></td>
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<td></td>
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<td>0.240</td>
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<tr>
<td></td>
<td></td>
<td>0.270</td>
</tr>
<tr>
<td>60</td>
<td></td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.294</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.312</td>
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<tr>
<td>120</td>
<td></td>
<td>0.162</td>
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<tr>
<td></td>
<td></td>
<td>0.362</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.454</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$t^b = 0.042$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$m = 1.06 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$r = 0.974$</td>
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<tr>
<td>15</td>
<td>Liver plus gall bladder</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.211</td>
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</tr>
<tr>
<td></td>
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<td>0.166</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.162</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>0.257</td>
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<td>0.301</td>
</tr>
<tr>
<td></td>
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<td>0.302</td>
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<td>120</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.466</td>
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<td></td>
<td></td>
<td>$b = 0.024$</td>
</tr>
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<td></td>
<td></td>
<td>$m = 3.42 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$r = 0.979$</td>
</tr>
<tr>
<td>15</td>
<td>Gills</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.403</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.597</td>
</tr>
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<td></td>
<td>0.617</td>
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<td></td>
<td>0.572</td>
</tr>
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<td>60</td>
<td></td>
<td>0.215</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.790</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.779</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td>0.335</td>
</tr>
<tr>
<td></td>
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<td>0.925</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$b = 0.056$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$m = 2.37 \times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$r = 0.995$</td>
</tr>
</tbody>
</table>

$^b$ Letters $b$, $m$, and $r$ respectively refer to the Y intercept, slope, and linear correlation coefficient for linear regression equations defining the relationship between exposure time (X) and determined accumulated tissue $^{14}$C radioactivity expressed as HCN (Y).

$^2$ Only the first three values are used to define the regression equation.
Table 16. Determined total \(^{14}\)C radioactivity and estimates of the expected radioactivity referable to penetration as HCN, both expressed as \(\mu g\) HCN per gram wet weight, in tissues of bluegills exposed to various \(^{14}\)C-labeled nickelocyanide solutions. The determined total \(^{14}\)C radioactivity and its relationship to exposure time, as defined by linear regression equations, is also presented. (\(C_{NT}\) = total cyanide as CN).

<table>
<thead>
<tr>
<th>Time in test solution (min)</th>
<th>Tissue</th>
<th>(^{14})C radioactivity expressed as (\mu g) HCN per gram wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Determined</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Blood</td>
<td>0.280</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.322</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.441</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td>0.535</td>
</tr>
<tr>
<td>b</td>
<td>0.257</td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>2.44 \times 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.977</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Liver</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.132</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.251</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td>0.398</td>
</tr>
<tr>
<td>b</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>2.93 \times 10^{-3}</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.995</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Gills</td>
<td>0.564</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.826</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.107</td>
</tr>
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<td>120</td>
<td></td>
<td>1.100</td>
</tr>
<tr>
<td>b</td>
<td>0.423</td>
<td></td>
</tr>
<tr>
<td>m</td>
<td>1.17 \times 10^{-2}</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>0.986</td>
<td></td>
</tr>
</tbody>
</table>

1/ Letters b, m, and r respectively refer to the Y intercept, slope, and linear correlation coefficient for linear regression equations defining the relationship between exposure time (X) and determined accumulated tissue \(^{14}\)C radioactivity expressed as HCN (Y).

2/ Only the first three values are used to define the regression equation.
Figure 18. The relationship between exposure time and determined $^{14}$C radioactivity, expressed as $\mu$g HCN per gram wet weight of blood, liver plus gall bladder, or gill tissue of bluegills exposed to NaCN solutions containing 0.05 or 0.20 mg/liter of $^{14}$C-labeled HCN.
Figure 19. The relationship between exposure time and determined $^{14}$C radioactivity, expressed as $\mu$g HCN per gram wet weight of blood, liver plus gall bladder, or gill tissue of bluegills exposed to $^{14}$C-labeled nickelocyanide solutions with various pH values and HCN and total cyanide concentrations. ($CN_T =$ total cyanide as CN)
GILLS  
LIVER AND GALL  
BLADDER  
BLOOD  

\[ \text{pH 6.5} \]
0.5 mg/liter \( \text{CN}_7 \)
0.294 mg/liter HCN

\[ \text{pH 7.1} \]
25 mg/liter \( \text{CN}_7 \)
0.258 mg/liter HCN

\[ \text{pH 7.7} \]
500 mg/liter \( \text{CN}_7 \)
0.181 mg/liter HCN

TIME IN TEST SOLUTION (min)
The procedures used to calculate these determined values are outlined in Appendix M.

From data presented in Table 15 for three NaCN solutions containing 0.20 mg/l HCN, it can be seen that the $^{14}$C radioactivity in various tissues, after different periods of exposure of the fish, do not show any consistent relationship to pH of the test solutions. Since $^{14}$C accumulation appears to be independent of pH within the range 6.5 to 7.7, an average of the accumulated carbon concentrations, expressed as HCN, for the three $^{14}$C-labeled NaCN experimental solutions at each of the exposure periods was determined for each tissue, and these average values are used to define the rates of $^{14}$C accumulation from NaCN solutions containing 0.20 mg/l HCN. It should be emphasized that these concentrations actually represent those of certain carbon atoms, expressed as HCN, and that this mode of expression is not meant to imply that they necessarily correspond to the actual HCN concentrations present. However, since cyanide in simple cyanide solutions with pH ranging between 6.5 and 7.7 exists essentially as HCN, the determined $^{14}$C radioactivity in the various tissues of bluegills exposed to NaCN solutions is assumed to have resulted from penetration of the fish by molecular HCN. Hydrocyanic acid can be carried unbound in the plasma and does not combine with hemoglobin because its Fe atom is divalent (ferrous). Instead, cyanide can combine with methemoglobin, a mildly oxidized form of
hemoglobin occurring in low plasma concentration in which the Fe atom is trivalent (ferric), forming cyanmethemoglobin. In general, the blood and skeletal muscle of vertebrates do not contain the enzyme rhodanese which converts free cyanide in the presence of thiosulfate to non-toxic thiocyanate (SCN\(^-\)), thereby accounting for the primary method of detoxification of cyanide (Williams, 1959). Therefore, thiosulfate in the bloodstream is not likely to have any action on HCN, but when the HCN reaches other tissues, especially the liver where rhodanese is abundant, it is converted to non-toxic thiocyanate. It is also possible that cyanide existing in the bloodstream as cyanmethemoglobin is liberated as HCN in other tissues, and is then detoxified by the reaction with thiosulfate catalyzed by rhodanese or metabolized in various other ways.

By expressing the accumulated carbon from \(^{14}\text{C}\)-labeled cyanide uniformly as HCN levels, and by plotting these values against exposure time, the rates at which \(^{14}\text{C}\) enters and accumulates in various tissues of bluegills exposed to NaCN and \(\text{Ni(CN)}_2\) solutions were determined. Slope values of linear regression equations presented in Tables 15 and 16 represent these accumulation rates, relating to exposure time the \(^{14}\text{C}\) radioactivity expressed as \(\mu\text{g}\) of HCN present per gram of tissue. The equations for the blood and liver plus gall bladder tissues apply to all four exposure times and corresponding observed \(^{14}\text{C}\) radioactivity values. In the case of the gill tissue, only
the first three values were used to define the regression equations, with the exception of the series of four values for fish exposed to the NaCN solution containing 0.05 mg/l HCN, all four of which were used.

Comparison of the rates of $^{14}$C uptake by various tissues of blue-gills exposed to $^{14}$C-labeled NaCN and nickelocyanide experimental solutions reveals that the relationships between exposure time and the amounts of $^{14}$C radioactivity accumulated in the blood and liver plus gall bladder were essentially linear. On the other hand, the rates of increase of $^{14}$C radioactivity in the gill tissue tended to decrease in all experiments as exposure time increased, except for the NaCN experiment at pH 7.1 and 0.05 mg/l HCN, in which the rate was constant. The effect of HCN concentration on the rate of $^{14}$C accumulation was also considered by comparing results of two NaCN experiments, one at 0.05 and another at 0.20 mg/l HCN (Figure 18). The rate of $^{14}$C accumulation by all tissues sampled in both experiments was nearly constant, with the exception of the progressively declining rate of accumulation by the gills at 0.20 mg/l HCN. The most apparent and probably the most significant difference between comparable curves is that between the two curves for liver plus gall bladder tissue; in the 0.05 mg/l HCN solution, the liver plus gall bladder accumulated $^{14}$C at a greater rate than it did for fish exposed to the 0.20 mg/l HCN solution. The slopes for the respective linear regression equations are $3.42 \times 10^{-3}$ and $2.89 \times 10^{-3}$. It appears
that, at the high HCN concentration, the ability of the liver to detoxify cyanide and store the detoxification by-products such as thiocyanate is decreased to the point where the amount of $^{14}\text{C}$ present approximates that found in the blood. The rate of $^{14}\text{C}$ accumulation in the blood and gills of bluegills exposed to the NaCN solutions increased by 2- and 4-fold, respectively, with the fourfold increase in HCN concentration from 0.05 to 0.20 mg/l.

It is probable that the decrease in rate of accumulation of $^{14}\text{C}$ in the gills with increasing exposure time was due to decreased irrigation of the gills of fish beginning to succumb to the toxicant. This decrease of water flow over the gill filaments would lower the rate of $^{14}\text{C}$ uptake. The most dramatic change in uptake rate occurred in a nickel-cyanide complex solution with pH 6.5 and 0.50 mg/l total cyanide, in which the calculated HCN concentration was at the very toxic level of 0.294 mg/l (Figure 19). In fact, the fish whose tissues were used to define the points after 120 minutes of exposure to this solution was on the verge of death.

In the Ni(CN)$_4$$^{2-}$ experiments, accumulation of $^{14}\text{C}$ in tissues was generally similar to that observed in the NaCN experiments at 0.20 mg/l HCN, with one notable difference. As the total cyanide concentration increased from 0.50 to 500 mg/l, the rates of $^{14}\text{C}$ accumulation in the liver plus gall bladder and gill tissues, represented by the slope values of the respective regression equations presented in
Table 16, increased by factors of approximately 51 and 52, respectively. The rate of accumulation of $^{14}$C by the blood was much lower, and there was only a 3.8-fold increase in slope of the regression equations with the increase of total cyanide concentration. As the total cyanide concentration of these solutions increased, the calculated HCN concentration was found to decrease from about 0.29 to 0.18 mg/l because of accompanying increases in pH, yet the rate of increase of $^{14}$C in all the tissues increased substantially. If it can be assumed that the permeability of the gill to HCN is not appreciably altered in the high tetracyanonickelate (II) ion concentrations, then the substantial unexpected increase in $^{14}$C accumulation can be considered as evidence that some cyanide species other than HCN enters bluegills when they are exposed to nickelocyanide solutions.

Doudoroff et al. (1966) performed acute toxicity bioassays with bluegills in which the total alkalinity and the free carbon dioxide content of test solutions containing the nickelocyanide complex varied widely. The toxicity of solutions with a low content of free carbon dioxide thus was compared with that of solutions having the same pH values and nickelocyanide content but a high concentration of free carbon dioxide. These authors found that the pronounced decrease of the pH at the gill surfaces (due to respiratory CO$_2$) of solutions with low levels of free carbon dioxide did not result in any material increase of the toxicity of the solutions to fish. Had a large increase
of the molecular HCN content of these solutions occurred at the gill surfaces because of increased dissociation of the Ni(CN)\textsubscript{4}\textsuperscript{2-} complex ion at the lower pH, the tests would have shown the toxicity of the nickelocyanide solutions with low levels of free CO\textsubscript{2} to be greater than that of the comparable solutions with high levels of free CO\textsubscript{2}. Therefore, it was concluded that the dissociation of the Ni(CN)\textsubscript{4}\textsuperscript{2-} complex ion is not rapid enough to materially increase the molecular HCN concentration at the gill surfaces. In my own experiments, solutions of the copper- and silver-cyanide complexes with levels of HCN that could not be acutely toxic but with Cu(CN)\textsubscript{2}\textsuperscript{-} or Ag(CN)\textsubscript{2}\textsuperscript{-} complex ions in relatively high concentrations, proved to be highly toxic. Because of relatively slow liberation of cyanide from metallo-cyanide complex ions following pH changes such as those that can occur at the gill surfaces, I believe that the observed toxicity to fish of solutions of metallo-cyanide complexes with low levels of HCN but high complex ion concentrations must be attributed to penetration of their gills by the complex ions themselves. The nature of intoxication symptoms supports the view that free HCN was not the effective toxic agent. The fate of the complexes after penetration was not examined by me, and whether they continue to exist in the fish tissues as intact complex ions or are soon decomposed and converted into other compounds is a matter upon which I shall not speculate. However, it should be noted that, during the relatively short exposure periods in
my tests, the complex ions must have remained intact or else free cyanide was rapidly detoxified upon decomposition of the complexes, since the amounts of free cyanide that can be tolerated or detoxified by fish subjected to dilute NaCN solutions evidently are relatively small.

On the basis of the above considerations, it can be concluded that cyanide penetrates into fish exposed to nickelocyanide solutions both as HCN and as Ni(CN)$_2^-$, with the penetration by the complex ions mainly accounting for the relatively high $^{14}$C radioactivity of the tissue samples from fish exposed to solutions with high total cyanide levels. Its uptake probably accounts for the fact that the nickel-cyanide complex solutions proved more toxic than NaCN solutions with the same molecular HCN concentrations.

By calculating the percentage of total cyanide present as HCN in the test solutions used in the three Ni(CN)$_4^2$ experiments (Appendices K and L), it is possible to estimate the amount of $^{14}$C radioactivity present per gram of tissue that is due to penetration of HCN, on the basis of results obtained with the NaCN solutions which contained 0.20 mg/l HCN and the assumption that permeability of the gill to HCN is not appreciably altered in the presence of the Ni(CN)$_4^2$ ion. Any large deviation of the determined values from the values expected must result from penetration and accumulation of the Ni(CN)$_4^2$ complex ion itself or a product of its decomposition or metabolism,
since the concentration of CN\textsuperscript{-} ion was very small or negligible in all
the nickelocyanide experimental solutions. A sample calculation for
the blood of a bluegill exposed for 15 minutes to the test solution at
pH 7.7 and 500 mg/l total cyanide is given in Appendix M. In calculat-
ing the expected amount of \textsuperscript{14}C radioactivity, expressed as \(\mu\)g of HCN
per gram of tissue, in tissues from fish exposed to the nickel-cyanide
complex solutions, it was first assumed that the amount of accumu-
lated \textsuperscript{14}C entering as HCN is equal to the average concentration of
\textsuperscript{14}C accumulated in various tissues by bluegills exposed for corre-
ponding time periods to 0.20 mg/l HCN in NaCN solutions times the
ratio of the calculated HCN concentration in the nickel-cyanide com-
plex solution to 0.20 mg/l HCN. The assumption is apparently valid
with respect to all tissues from fish exposed to nickelocyanide solu-
tions containing HCN within the concentration range of 0.18 and 0.29
mg/l HCN. Reference to the data presented in Table 15 reveals that
the same assumption is not valid for the blood and liver plus gall
bladder tissues over the wide HCN concentration range from 0.05 to
0.20 mg/l. However, the assumption does hold over this wide con-
centration range for gill tissues, since the amount of \textsuperscript{14}C accumu-
lated in the gills by bluegills in the NaCN solutions with 0.20 mg/l
HCN was about four times that observed in the solution with 0.05 mg/l
HCN. As explained earlier, the difference in liver plus gall bladder
values for low and high ambient HCN levels is thought to result from
poisoning of the detoxification mechanism at the high HCN concentrations, thus causing a reduction in accumulated $^{14}C$. Therefore, the assumption that the amount of expected carbon from $^{14}C$-labeled cyanide due to penetration of HCN alone is directly and linearly related to the ratios of HCN in the nickelocyanide solutions to 0.20 mg/l HCN may still be and is assumed to be correct when only solutions containing HCN concentrations not widely different from 0.20 mg/l HCN are involved.

The nickel-cyanide complex solution containing 0.50 mg/l total cyanide at pH 6.5 is calculated to contain 0.294 mg/l HCN and 0.339 mg/l $\text{Ni(CN)}_4^{-2}$. When one compares the values in Table 16 for the determined and expected $^{14}C$ radioactivity, expressed as HCN, in the blood and gill tissues, a ratio of determined to expected values near unity is obtained for all exposure periods. If the assumption that the $\text{Ni(CN)}_4^{-2}$ ion does not affect the penetration of HCN and the procedure of calculating expected $^{14}C$ radioactivity is applicable, then at the low $\text{Ni(CN)}_4^{-2}$ ion concentration of 0.339 mg/l a negligible amount of $^{14}C$ enters fish as $\text{Ni(CN)}_4^{-2}$ and appears as the $\text{Ni(CN)}_4^{-2}$ ion or in some other form in the blood or gill tissues, as compared with that which enters as the HCN molecule. The great discrepancy between the determined and much higher expected $^{14}C$ radioactivity values for the liver plus gall bladder tissue obtained in the same experiment is thought to be the result of inactivation of the detoxification mechanism
at the high HCN concentration of 0.294 mg/l, i.e., inactivation more pronounced than that observed at 0.20 mg/l HCN in the NaCN experiments that provided data on which the expected values were based.

The data in Table 16 for the 25 mg/l total cyanide solution with calculated Ni(CN)_2^-^2 concentration of 38.7 mg/l (Appendix L) are difficult to explain. The calculated HCN concentration of 0.258 mg/l for this nickelocyanide solution is approximately equal to the 0.20 mg/l HCN concentration in the NaCN solutions used in obtaining data from which the expected cyanide concentrations in tissues were calculated. Nevertheless, the blood and liver plus gall bladder tissues appear initially to contain considerably less ^14C than the amounts expected, while the gill tissue is found to contain about twice as much as the expected amounts resulting from penetration of HCN alone. It is possible that, in the presence of large Ni(CN)_2^-^2 ion concentrations, the HCN molecule cannot penetrate the gill tissues as readily as it does from NaCN solutions, because of damage to the gill filaments, this accounting for low initial ^14C radioactivity in the blood and liver plus gall bladder tissues.

The determined ^14C radioactivity levels in the various tissues of bluegills exposed to a nickelocyanide solution with 500 mg/l of total cyanide and calculated Ni(CN)_2^-^2 concentration of 782 mg/l (Appendix L) were much larger than values expected to result from penetration of HCN alone (Table 16). Errors in the estimation of expected tissue
$^{14}\text{C}$ radioactivity that could result from an effect of the large Ni(CN)$_4^{2-}$ ion concentration in the test solution on the permeability of the gill to HCN, are negligible in view of the large radioactivity levels observed. The change with increasing exposure time in the determined $^{14}\text{C}$ radioactivity and in the ratio of determined to expected values reveals ways in which $^{14}\text{C}$, presumably penetrating as HCN and Ni(CN)$_4^{2-}$, is distributed in various body tissues. The blood and gills contained about 4.6 and 75 times as much $^{14}\text{C}$, respectively, as was expected after each of the exposure periods. The accumulation rates thus remained quite constant as exposure time progressed. The liver plus gall bladder, however, showed a response quite different from that of the blood and gills. In Table 16 it can be seen that, as exposure time increased, the ratio of determined to expected $^{14}\text{C}$ radioactivity in the liver plus gall bladder did not remain approximately constant, as it did in the case of blood tissue or of gill tissue, but progressively increased from a value of 4.3 after 15 minutes to 38.2 after 120 minutes of exposure. It should also be pointed out that for a 2-fold increase in exposure time there was an approximately 2-fold increase in calculated ratio. These observations provide convincing evidence that the penetrating $^{14}\text{C}$ atoms, most likely those initially associated with the Ni(CN)$_4^{2-}$ ion, are concentrated in the liver or the gall bladder or in both either as the Ni(CN)$_4^{2-}$ ion itself or as a metabolized by-product. Therefore, the liver may be an important site for
detoxification of the Ni(CN)_4^{2-} ion. Since unexpectedly high \(^{14}\text{C}\) concentrations were observed in the gills, the data also suggest that the Ni(CN)_4^{2-} ion may have considerable difficulty penetrating the gill filaments and its concentration in this tissue may have been high for that reason. Of uncertain significance but perhaps pertinent to this matter is an interesting observation made by comparing the rates of accumulation of \(^{14}\text{C}\) by the various tissues of bluegills exposed to either the 25 or the 500 mg/l total cyanide solution. It has been calculated that with the 20-fold increase in the total cyanide or the Ni(CN)_4^{2-} concentration and a decrease of HCN concentration from 0.26 to 0.18 mg/l in the external medium, the rates of increase of \(^{14}\text{C}\) in the blood, liver plus gall bladder, and gills increased by factors of 1.1, 14, and 24, respectively.

**Copper- and Silver-Cyanide Complexes**

The accumulation of copper or silver in various tissues of bluegills exposed to a series of copper-or silver-cyanide complex solutions was studied by flame atomic absorption spectrophotometry. The calculated percentages of total cyanide present as different cyanide species in these various test solutions and the calculated HCN, metallrocyanide ion, and \(\text{Ag}^+\) ion concentrations are presented in Table 17. The calculated \(\text{Cu}^+\) ion concentration was only about \(10^{-13}\) molar in each of the copper-cyanide test solutions (Table 11). Therefore,
Table 17. Calculated percentages of total cyanide present as different cyanide species in test solutions of the copper- and silver-cyanide complexes, and the calculated concentrations of HCN and of Cu(CN)$_2^-$ or Ag(CN)$_2^-$ and Ag$^+$.

<table>
<thead>
<tr>
<th>Total metal as: Cu or Ag (mg/l)</th>
<th>Total cyanide as CN (mg/l)</th>
<th>pH</th>
<th>Calculated concentrations/1/ (mg/l)</th>
<th>Percentage of cyanide present as:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCN</td>
<td>Cu(CN)$_2^-$</td>
</tr>
<tr>
<td>Copper-cyanide complex experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.1</td>
<td>5.0</td>
<td>7.947</td>
<td>0.0132</td>
<td>10.956</td>
</tr>
<tr>
<td>18.3</td>
<td>15.0</td>
<td>7.715</td>
<td>0.0496</td>
<td>32.506</td>
</tr>
<tr>
<td>30.5</td>
<td>25.0</td>
<td>7.668</td>
<td>0.0598</td>
<td>54.193</td>
</tr>
<tr>
<td>Silver-cyanide complex experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>3.4</td>
<td>7.979</td>
<td>0.0035</td>
<td>-</td>
</tr>
<tr>
<td>22.2</td>
<td>10.7</td>
<td>8.027</td>
<td>0.0064</td>
<td>-</td>
</tr>
<tr>
<td>36.7</td>
<td>17.7</td>
<td>8.027</td>
<td>0.0075</td>
<td>-</td>
</tr>
</tbody>
</table>

/1/ These concentrations were calculated by using the dissociation constant for HCN at 20°C of $4.365 \times 10^{-10}$ given by Izatt et al. (1962) and by apparent $K_d$ values for the Cu(CN)$_2^-$ and Ag(CN)$_2^-$ complex ions at 20°C, as determined for the different pH values and total cyanide concentrations (Tables 6 and 11).
Cu⁺ ion is not believed to have contributed either to the toxicity of the solutions or to the determined copper concentrations in tissues of bluegills exposed to the solutions. The importance of the Ag⁺ ion with respect to toxicity or its contribution to silver concentrations in tissues of bluegills exposed to silver-cyanide complex solutions will be discussed later.

The determined copper and silver concentrations (total metal which enters, minus that excreted, plus that normally present), expressed in µg per gram of tissue, in bluegills exposed to copper- and silver-cyanide experimental solutions at various pH values and total cyanide concentrations are presented in Tables 18 and 19 and are shown plotted against exposure time in Figures 20 and 21, respectively. The metal concentrations were determined on a per gram dry weight basis for all tissues except the blood, for which they are expressed as µg per gram wet weight. By weighing tissues from a total of 19 bluegills, it was determined that the average percentages of water in the tissues of the gills, the liver together with the gall bladder, and the spleen together with the heart were 75.83, 72.16, and 73.31 percent, respectively, with corresponding standard deviations of 1.22, 2.93, and 6.12 percent.

It should be understood that the high metal concentrations determined in the tissues of bluegills exposed to the various metal-cyanide complex solutions actually represent those of certain metal
Table 18. Determined total and calculated accumulated copper concentrations, in µg per gram, in tissues of bluegills exposed to various cuprocyanide solutions at different pH values and calculated HCN concentrations. The accumulated copper concentrations, expressed as µg per gram Cu(CN)₂⁻, and their relationship to exposure time as defined by linear regression equations are also presented. (Cu_T = total copper as Cu)

<table>
<thead>
<tr>
<th>Time in test solution (min)</th>
<th>Blood</th>
<th>Gills</th>
<th>Spleen plus heart</th>
<th>Liver plus gall bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Accumulated copper</td>
<td>Total</td>
<td>Accumulated copper</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>expressed as Cu(CN)₂⁻</td>
<td>Cu</td>
<td>expressed as Cu(CN)₂⁻</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td>-</td>
<td>2.71</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.02</td>
<td>-</td>
<td>2.50</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.61</td>
<td>-</td>
<td>3.04</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.14²/</td>
<td>-</td>
<td>2.75</td>
<td>-</td>
</tr>
<tr>
<td><strong>Test solution pH 7.9--6.1 mg/l Cu_T and 0.013 mg/l HCN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>1.32</td>
<td>0.18</td>
<td>0.33</td>
<td>2.53</td>
</tr>
<tr>
<td>450</td>
<td>1.82</td>
<td>0.68</td>
<td>1.24</td>
<td>3.94</td>
</tr>
<tr>
<td>630</td>
<td>3.00</td>
<td>1.86</td>
<td>3.38</td>
<td>5.68</td>
</tr>
<tr>
<td>750</td>
<td>4.98</td>
<td>3.84</td>
<td>6.98</td>
<td>6.82</td>
</tr>
<tr>
<td></td>
<td>-3.40</td>
<td>b -3.87</td>
<td>b -6.87</td>
<td>m 1.23 x 10⁻²</td>
</tr>
<tr>
<td></td>
<td>0.927</td>
<td>r 0.992</td>
<td>r 0.966</td>
<td>r 1.000</td>
</tr>
<tr>
<td><strong>Test solution pH 7.7--18.3 mg/l Cu_T and 0.050 mg/l HCN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>7.14</td>
<td>6.00</td>
<td>10.91</td>
<td>7.87</td>
</tr>
<tr>
<td>300</td>
<td>11.17</td>
<td>10.03</td>
<td>18.24</td>
<td>13.02</td>
</tr>
<tr>
<td>420</td>
<td>18.03</td>
<td>16.89</td>
<td>30.72</td>
<td>17.30</td>
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<tr>
<td>480</td>
<td>27.38</td>
<td>28.24</td>
<td>47.73</td>
<td>30.33</td>
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<tr>
<td></td>
<td>-18.5</td>
<td>b -21.1</td>
<td>b -44.3</td>
<td>m 1.29 x 10⁻¹</td>
</tr>
<tr>
<td></td>
<td>0.966</td>
<td>r 0.927</td>
<td>r 0.991</td>
<td>r 0.987</td>
</tr>
</tbody>
</table>

(Continued on next page)
Table 18. (Continued)

<table>
<thead>
<tr>
<th>Time in test solution (min)</th>
<th>Blood $^{1/}$</th>
<th>Gills $^{1/}$</th>
<th>Spleen plus heart $^{1/}$</th>
<th>Liver plus gall bladder $^{1/}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Accumulated copper expressed as</td>
<td>Total</td>
<td>Accumulated copper expressed as</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>Cu</td>
<td>Cu(CN)$_2$</td>
<td>Cu</td>
</tr>
<tr>
<td>180</td>
<td>11.17</td>
<td>10.03</td>
<td>18.24</td>
<td>16.77</td>
</tr>
<tr>
<td>255</td>
<td>17.55</td>
<td>16.41</td>
<td>29.85</td>
<td>26.26</td>
</tr>
<tr>
<td>300</td>
<td>25.74</td>
<td>24.60</td>
<td>44.75</td>
<td>36.84</td>
</tr>
<tr>
<td>390</td>
<td>35.42</td>
<td>34.28</td>
<td>62.35</td>
<td>41.80</td>
</tr>
</tbody>
</table>

Test solution pH 7.7—30.5 mg/l Cu$_2^+$ and 0.060 mg/l HCN

<table>
<thead>
<tr>
<th></th>
<th>b</th>
<th>m</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>-21.9</td>
<td>2.16 x 10$^{-1}$</td>
<td>0.993</td>
</tr>
<tr>
<td>255</td>
<td>-12.7</td>
<td>2.24 x 10$^{-1}$</td>
<td>0.968</td>
</tr>
<tr>
<td>300</td>
<td>6.14 x 10$^{-1}$</td>
<td>0.984</td>
<td>0.999</td>
</tr>
<tr>
<td>390</td>
<td>1.12</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>

$^{1/}$ Concentrations expressed in μg per gram for the blood and other tissues are based on their wet weight and dry weight, respectively.

$^{2/}$ Average determined copper concentration in tissues of control bluegills.

$^{3/}$ Letters b, m, and r respectively refer to the Y intercept, slope, and linear correlation coefficient for linear regression equations defining the relationship between exposure time (X) and accumulated tissue copper concentrations expressed as μg/g Cu(CN)$_2^+$ (Y).

$^{4/}$ Only the last three values were used to define this regression equation.
Table 19. Determined total and calculated accumulated silver concentrations, in µg per gram, in tissues of bluegills exposed to a silver nitrate solution containing 0.05 mg/l silver and to various solutions of the silver-cyanide complex at different pH values and calculated HCN concentrations. The accumulated silver concentrations, expressed as µg per gram Ag or as Ag(CN)₂, and their relationship to exposure time as defined by linear regression equations are also presented. (Ag₅ = Total silver as Ag)

<table>
<thead>
<tr>
<th>Time in test solution (min)</th>
<th>Blood&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Gills&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Spleen plus heart&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Liver plus gall bladder&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Accumulated silver expressed as</td>
<td>Accumulated silver expressed as</td>
<td>Accumulated silver expressed as</td>
<td>Accumulated silver expressed as</td>
</tr>
<tr>
<td></td>
<td>Ag</td>
<td>Ag(CN)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Ag</td>
<td>Ag(CN)&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>0.193</td>
<td>0.182</td>
<td>0.171</td>
<td>0.182&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test solution pH 8.0--0.05 mg/l Ag&lt;sub&gt;5&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>0.161</td>
<td>0.204</td>
<td>0.433</td>
<td>1.082&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>420</td>
<td>0.161</td>
<td>0.204</td>
<td>0.433</td>
<td>1.082&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>600</td>
<td>0.161</td>
<td>0.204</td>
<td>0.433</td>
<td>1.082&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>780</td>
<td>0.161</td>
<td>0.204</td>
<td>0.433</td>
<td>1.082&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Test solution pH 8.0--7.0 mg/l Ag&lt;sub&gt;5&lt;/sub&gt; and 0.004 mg/l HCN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>0.198</td>
<td>0.186</td>
<td>0.204</td>
<td>0.219&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>450</td>
<td>0.334</td>
<td>0.152</td>
<td>0.225</td>
<td>0.251</td>
</tr>
<tr>
<td>630</td>
<td>0.498</td>
<td>0.316</td>
<td>0.468</td>
<td>0.692</td>
</tr>
<tr>
<td>810</td>
<td>0.649</td>
<td>0.467</td>
<td>0.692</td>
<td>0.922</td>
</tr>
</tbody>
</table>

(Continued on next page)
Table 19. (Continued)

<table>
<thead>
<tr>
<th>Time in test solution (min)</th>
<th>Blood(^1)</th>
<th>Gills(^1)</th>
<th>Spleen plus heart(^1)</th>
<th>Liver plus gall bladder(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Accumulated silver</td>
<td>Total</td>
<td>Accumulated silver</td>
</tr>
<tr>
<td></td>
<td>Ag</td>
<td>expressed as Ag</td>
<td>Ag</td>
<td>expressed as Ag</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ag(CN)(_2)</td>
<td></td>
<td>Ag(CN)(_2)</td>
</tr>
<tr>
<td></td>
<td>4.91</td>
<td>8.309</td>
<td>12.32</td>
<td>10.95</td>
</tr>
<tr>
<td>300</td>
<td>17.83</td>
<td>17.65</td>
<td>26.16</td>
<td>22.69</td>
</tr>
<tr>
<td>660</td>
<td>22.18</td>
<td>22.00</td>
<td>32.61</td>
<td>43.45</td>
</tr>
<tr>
<td></td>
<td>-2.15</td>
<td>-14.2</td>
<td>b</td>
<td>-19.1</td>
</tr>
<tr>
<td></td>
<td>m 5.42 x 10(^{-2})</td>
<td>m 1.11 x 10(^{-1})</td>
<td>m 1.31 x 10(^{-1})</td>
<td>m 2.50 x 10(^{-1})</td>
</tr>
<tr>
<td></td>
<td>r 0.989</td>
<td>r 0.989</td>
<td>r 0.989</td>
<td>r 0.989</td>
</tr>
</tbody>
</table>

Test solution pH 8.0--22.2 mg/l Ag\(_T\) and 0.006 mg/l HCN

|                             | 8.378       | 8.196         | 12.15       | 16.20           | 15.84       | 23.48         | 18.46       | 16.87           | 25.00       | 45.61       | 45.57       | 67.55       |
|                             | 15.66       | 15.48         | 22.95       | 27.43           | 27.07       | 40.13         | 31.76       | 30.17           | 44.72       | 64.04       | 64.00       | 94.87       |
| 570                         | 22.34       | 22.16         | 32.85       | 45.34           | 44.98       | 66.68         | 58.04       | 56.44           | 83.67       | 92.38       | 92.33       | 136.9       |
|                             | 0.441       | b -9.40       | b -18.6     | b 12.9          | b           | 12.9          | b           | 12.9           | b           | 12.9       | 12.9       | 12.9       |
|                             | m 5.57 x 10\(^{-2}\) | m 1.31 x 10\(^{-1}\) | m 1.73 x 10\(^{-1}\) | m 2.14 x 10\(^{-1}\) | m 0.996     | m 0.992       | m 0.996     | m 0.992       | m 0.996     | m 0.992   | m 0.992   | m 0.992   |
|                             | r 0.977     | r 0.996       | r 0.992     | r 0.998         | r 0.998     | r 0.998       | r 0.998     | r 0.998       | r 0.998     | r 0.998   | r 0.998   | r 0.998   |

Test solution pH 8.0--36.7 mg/l Ag\(_T\) and 0.008 mg/l HCN

---

1/ Concentrations expressed in µg per gram for the blood and other tissues are based on their wet weight and dry weight, respectively.

2/ Average determined silver concentration in tissues of control bluegills.

3/ Letters b, m, and r respectively refer to the Y intercept, slope, and linear correlation coefficient for linear regression equations defining the relationship between exposure time (X) and accumulated tissue silver concentrations expressed as µg/g Ag or Ag(CN)\(_2\) (Y).
Figure 20. The relationships between determined total copper concentrations in tissues and time for bluegills exposed to various cuprocyanide solutions at different pH values and total copper concentrations in which the molar ratio of CN to Cu was 2 to 1. ($Cu_T =$ total copper as Cu)
Figure 21. The relationships between determined total silver concentrations in tissues and time for bluegills exposed to a silver nitrate solution containing 0.05 mg/l silver (lowest figure) and to various silver-cyanide complex solutions at different pH values and total silver concentrations in which the molar ratio of CN to Ag was 2 to 1. \( \text{Ag}_T = \text{total silver as Ag} \)
pH 8.0
36.7 mg/liter $\text{Ag}_T$

- LIVER AND GALL BLADDER
- SPLEEN AND HEART
- GILLS
- BLOOD
- BLOOD (WET WEIGHT)

TIME IN TEST SOLUTION (min)

pH 8.0
22.2 mg/liter $\text{Ag}_T$

pH 8.0
7.0 mg/liter $\text{Ag}_T$

pH 8.0
0.05 mg/liter $\text{Ag}_T$
atoms, which may be expressed as equivalent weights of metallo-
cyanide ions per gram of tissue, and that this mode of expression is
not meant to imply that the reported values necessarily correspond to
the actual metallo-cyanide ion concentrations present. However, for
reasons already explained, it can be assumed that the metal atoms
enter as complex ions and that they remain in the complexed form or
else the cyanide liberated by their decomposition is rapidly detoxified.
Evidence for in vivo stability of the cuprocyanide complex ion will be
presented later in the Discussion section.

Slope values of linear regression equations presented in Tables
18 and 19 represent accumulation rates, relating to exposure time the
µg of copper, expressed as Cu(CN)\textsubscript{2}\textsuperscript{−}, or of silver, as Ag(CN)\textsubscript{2}\textsuperscript{−},
present per gram of tissue. The regression equations apply to all
four exposure times, with the exception of the equation obtained for
the combined liver and gall bladder tissues from fish exposed to the
6.1 mg/l total copper solution, for the derivation of which only the
last three values were used.

Figures 20 and 21 permit comparison of the amounts and rates
of copper and silver uptake by various bluegill tissues of fish exposed
to cuprocyanide and silver-cyanide complex solutions. In most
instances, after brief initial periods of increasing accumulation rate,
the relationships between exposure time and the amounts of copper or
silver accumulated in the tissues of fish exposed to the various test
solutions were essentially linear.
By examining the tissues of bluegills exposed to cuprocyanide solutions containing 6.1, 18.3, and 30.5 mg/l total copper and to solutions of the silver-cyanide complex containing 7.0, 22.2, and 36.7 mg/l total silver, with corresponding Cu(CN)$_2^-$ or Ag(CN)$_2^-$ ion concentrations, calculated to be about 11, 33, and 54 mg/l, estimates can be made of the rates at which copper or silver accumulate in the tissues. In each of the experimental solutions, the blood accumulated copper or silver (on a wet weight basis) at the slowest rate, while the rates of accumulation (on a dry weight basis) by the gills, spleen plus heart, and liver plus gall bladder increased in the order of mention. One exception was found in the 7.0 mg/l total silver solution, in which the gills accumulated slightly more silver per gram of tissue than did the spleen plus heart tissues. It is important to emphasize the fact that blood copper and silver concentrations were computed on a wet weight basis. Since the whole blood of various fresh-water fishes is about 80 percent water (Buhler, 1973), the observed copper and silver concentrations expressed as µg per gram of dry weight of blood would be about five times those reported on a wet weight basis. It can be seen in Figures 20 and 21, in which this correction has been made, that, in solutions of low total metal concentration, the liver plus gall bladder tissues accumulated more metal than did the blood. At higher total metal concentrations in the test solutions, the blood and the liver plus gall bladder tissues were found to have approximately
equal concentrations. Since the blood and liver plus gall bladder tissues accumulated copper or silver at approximately the same rates, it does not appear that copper or silver were appreciably concentrated from the blood by liver plus gall bladder tissues in bluegills exposed to test solutions containing these metallo cyanide complex ions at fairly high concentration. However, some such concentrating effect was noted when the solutions were more dilute.

As the total copper concentration of cuprocyanide solutions increased from 6.1 to 18.3 and from 18.3 to 30.5 mg/l, the rates of accumulation of copper (expressed as Cu(CN)$_2^-$) in all tissues, represented by the slope values of the corresponding regression equations presented in Table 18, increased by factors averaging 8.14 ± 2.77 and 1.77 ± 0.15, respectively. The factor that deviates most from the average value is that for the liver and gall bladder sample and for increase in total copper from 6.1 to 18.3 mg/l; this factor is only 4.12. Since on a dry weight basis, the blood accumulated copper at a rate approximately four to five times faster than did the gill tissue, it would appear that the Cu(CN)$_2^-$ ion passes through the gill membranes and into the bloodstream rather readily.

As the total silver concentration in solutions of the silver-cyanide complex increased from 7.0 to 22.2 and from 22.2 to 36.7 mg/l, the rates of accumulation of silver (expressed as Ag(CN)$_2^-$) in all tissues, represented by the slope values of the respective
regression equations in Table 19, increased by factors averaging 21.34 ± 16.96 and 1.10 ± 0.20, respectively. If we assume that the determined silver concentrations per gram of dry weight of blood are about five times those reported on a wet weight basis, then the blood accumulated considerably more silver than did the gill tissue. It appears that the Ag(CN)$_2$ ion, like the Cu(CN)$_2$ ion, passes through the gill membranes and into the bloodstream rather readily.

Since one can calculate the Cu(CN)$_2$ and Ag(CN)$_2$ ion concentrations in the test solutions of various total metal content, one can also compute normalized rates of accumulation of copper (normalized with respect to concentration in the external medium) expressed as μg Cu(CN)$_2$ per gram of tissue per minute per mg/l Cu(CN)$_2$ or Ag(CN)$_2$ in the test solution. Calculated values for the various tissue samples are presented in Tables 20 and 21.

At the high dicyanocuprate (I) ion concentrations of 32.5 and 54.2 mg/l calculated to be present in the test solutions, the actual rates of accumulation of copper in each kind of tissue, in μg Cu(CN)$_2$ per gram of tissue per minute, appear to be proportional to concentrations in the medium, since the reported normalized rates are approximately the same for the two concentrations. At the lower dicyanocuprate (I) ion concentration of 11.0 mg/l, the normalized rate of accumulation of copper is about one-third that determined at the higher
Table 20. Normalized rates of accumulation of copper by various tissues of bluegills exposed to cupro-cyanide solutions, expressed in μg Cu(CN)₂⁻ per gram of tissue per minute per mg/l Cu(CN)₂⁻ ion calculated to be present in the test solution. (R = normalized rate of accumulation)

<table>
<thead>
<tr>
<th>Test solution</th>
<th>Total copper as Cu (mg/l)</th>
<th>pH</th>
<th>Calculated Cu(CN)₂⁻ (mg/l)</th>
<th>Blood¹/ (R x 10³)</th>
<th>Gills¹/ (R x 10³)</th>
<th>Spleen plus heart¹/ (R x 10³)</th>
<th>Liver plus gall bladder¹/ (R x 10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.1</td>
<td>7.9</td>
<td>10.96</td>
<td>1.13</td>
<td>1.34</td>
<td>3.19</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5.65)²/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.3</td>
<td>7.7</td>
<td>32.51</td>
<td>3.96</td>
<td>4.12</td>
<td>9.54</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(19.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.5</td>
<td>7.7</td>
<td>54.19</td>
<td>3.98</td>
<td>4.14</td>
<td>11.3</td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(19.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹/ Rates for the blood and other tissues are based on their wet weight and dry weight, respectively.

²/ Since whole fish blood is about 80 percent water, the rate of copper accumulation by blood on a dry weight basis is about five times the calculated value on a wet weight basis.
Table 21. Normalized rates of accumulation of silver by various tissues of bluegills exposed to solutions of silver nitrate or of the silver-cyanide complex, expressed in µg Ag (I) for the silver nitrate solution or µg Ag(CN)₂⁻ for the silver-cyanide complex solutions, per gram of tissue per minute per mg/l Ag⁺ or Ag(CN)₂⁻ ion calculated to be present in the test solution. (R = normalized rate of accumulation)

<table>
<thead>
<tr>
<th>Test solution</th>
<th>Blood¹/²</th>
<th>Gills¹/²</th>
<th>Spleen plus heart¹/²</th>
<th>Liver plus gall bladder¹/²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total silver as Ag (mg/l)</td>
<td>pH</td>
<td>Calculated Ag(CN)₂⁻ (mg/l)</td>
<td>(R x 10³)</td>
<td>(R x 10³)</td>
</tr>
<tr>
<td>Silver Nitrate Solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>8.0</td>
<td>-</td>
<td>30.5 (152)³/</td>
<td>96.7</td>
</tr>
<tr>
<td>Silver-Cyanide Complex Solutions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>8.0</td>
<td>10.44</td>
<td>0.114 (0.570)</td>
<td>0.980</td>
</tr>
<tr>
<td>22.2</td>
<td>8.0</td>
<td>32.86</td>
<td>1.65 (8.25)</td>
<td>3.38</td>
</tr>
<tr>
<td>36.7</td>
<td>8.0</td>
<td>54.37</td>
<td>1.02 (5.10)</td>
<td>2.40</td>
</tr>
</tbody>
</table>

¹/ Rates for the blood and other tissues are based on their wet weight and dry weight, respectively.

²/ Since whole fish blood is about 80 percent water, the rate of silver accumulation by blood on a dry weight basis is about five times the calculated value on a wet weight basis.
concentrations. The liver plus gall bladder proved to be an exception, since the normalized rate of accumulation by these tissues at the low concentration was not much different from the rates at higher dicyano-cuprate (I) ion concentrations. A similar relationship was noted in the case of silver. In solutions containing high dicyanoargentate (I) ion concentrations of 32.9 and 54.4 mg/l, the actual rates of accumulation of silver in the various tissues, in μg Ag(CN)₂⁻ per gram of tissue per minute, also appear to be proportional to concentrations in the medium. At the lower dicyanoargentate (I) ion concentration of 10.4 mg/l, the normalized rate of accumulation of silver averages about 0.3 times that determined at the higher concentrations, except for the liver and gall bladder. Thus, the normalized rates at which the Cu(CN)⁻² and Ag(CN)₂⁻ ions penetrate into the bodies of bluegills and accumulate in various tissues as a metallocyanide complex ion, or in some other form, probably increase as the metal-cyanide complex ion concentrations in the test solutions increase up to some level between 11 and 33 mg/l, beyond which there is no further increase of the normalized rate. This suggests that when the metallocyanide concentration in the test solution is low, the permeability of external membranes of the fish to the complex ions also is relatively low. As the complex ion concentration in the test solution increases, the permeability of the membranes to the metallocyanide ion seems to increase to a constant, maximal value, the actual rate of penetration
by the ion then becoming proportional to its concentration in the medium.

Apparent rates of entry of the two metal-cyanide complex ions under consideration can be compared by looking at the normalized accumulation rates of copper and silver in tissues of bluegills exposed to solutions containing approximately equal metal-cyanide complex ion concentrations. The average ratios of the normalized rates of accumulation of copper, expressed as \( \text{Cu(CN)}_2^- \), to those of accumulation of silver, expressed as \( \text{Ag(CN)}_2^- \) at the metallo cyanide ion concentrations of approximately 11, 33, and 54 mg/l, were respectively, \( 5.40 \pm 3.50, 2.16 \pm 0.63, \) and \( 3.61 \pm 1.46 \) (Tables 20 and 21). The overall average for all four tissues and for the three metal-cyanide complex ion concentrations was \( 3.72 \pm 2.44 \). Therefore, at comparable concentrations, the \( \text{Cu(CN)}_2^- \) ion appears to enter the body of a bluegill about 3.7 times as fast, on the average, as does the \( \text{Ag(CN)}_2^- \) ion.

Previously described toxicity bioassays have shown that the 48-hour \( \text{TL}_{m} \) values for bluegills of the copper- and silver-cyanide complexes are approximately 9 mg/l \( \text{Cu(CN)}_2^- \) and 27 mg/l \( \text{Ag(CN)}_2^- \), or 78 \( \mu \text{M/l} \) \( \text{Cu(CN)}_2^- \) and 169 \( \mu \text{M/l} \) \( \text{Ag(CN)}_2^- \), respectively. Since, on a molar basis, about one-half as much \( \text{Cu(CN)}_2^- \) as \( \text{Ag(CN)}_2^- \) is required to produce the same response, and the \( \text{Cu(CN)}_2^- \) ion appears to have a penetration rate about four times that of the \( \text{Ag(CN)}_2^- \) ion, one may
conclude that the difference in toxicity of the copper- and silver-cyanide complex ions probably is due partly to a difference of permeability of external membranes of the fish to the two ions. After penetration, the Ag(CN)\textsubscript{2}\textsuperscript{-} ion actually may be more toxic than the Cu(CN)\textsubscript{2}\textsuperscript{+} ion.

By calculation, it can be shown that, at equilibrium, the Ag\textsuperscript{+} ion concentrations in the three silver-cyanide complex solutions were between 0.0073 and 0.0157 mg/l (Table 17). One experiment was therefore performed to estimate the rate at which silver is accumulated by various tissues of bluegills exposed to a silver nitrate solution containing 0.05 mg/l silver and at pH 8.0. Data plotted in Figure 21 show that the concentrations of silver, in µg per gram of dry tissue, found in the tissues of fish exposed to the silver nitrate solution are quite low as compared with those found in tissues from bluegills exposed to solutions of the silver-cyanide complex with total silver content of 22.2 and 36.7 mg/l, but are approximately equal to those in tissues from fish exposed to the solution of the complex with 7.0 mg/l of total silver. By calculation, the latter solution was found to contain only 0.0073 mg/l Ag\textsuperscript{+} ion at equilibrium, which is about one-seventh the concentration of silver assumed to be present in the silver nitrate solution. Therefore, even when bluegills were exposed to the solution of the complex with 7.0 mg/l total silver, the accumulation of silver in the tissues is believed to have resulted
mainly from penetration and accumulation of the Ag(CN)$_2^-$ ion or its metabolized by-product, and not the Ag$^+$ ion. There were some important differences in the accumulation of silver by tissues between bluegills exposed to the silver-cyanide and silver nitrate solutions. When the fish were exposed to the silver nitrate solution, the gill tissues and the combined tissues of the spleen and heart accumulated the most silver; on the other hand, when the fish were in solutions of the silver-cyanide complex, the liver together with the gall bladder accumulated the most silver, the gills containing considerably less. It can be seen in Table 21 that the normalized rates of accumulation in the four tissues of silver, in $\mu$g Ag (l) per gram of tissue per minute per mg/l Ag$^+$ ion in the test solution, were considerably greater when the fish were exposed to the silver nitrate solution than the largest corresponding, normalized rates of accumulation of silver from solutions of the silver-cyanide complex, in $\mu$g Ag(CN)$_2^-$ per gram of tissue per minute per mg/l Ag(CN)$_2^-$ ion in the external medium. It is therefore believed that the Ag$^+$ ion can penetrate the gills of these fish and accumulate in the tissues more readily than can the Ag(CN)$_2^-$ ion.

Fish dying in copper- and silver-cyanide complex solutions showed typical signs of acute poisoning with most heavy metals. Much coagulated mucus both on the gills and body surface was observed. The significance of this observation is not at all clear.
DISCUSSION

Analytical Method

The modification of the method of Schneider (1962) developed as a part of my research has yielded a procedure for the routine determination of HCN usable in both field and laboratory situations where only limited facilities are available. Throughout this research, my method proved to be an accurate, sensitive, reliable, easy, and inexpensive method for determining molecular HCN in solutions of sodium cyanide and various metal-cyanide complexes, at concentrations ranging from about 0.005 to 1.0 mg/l. Therefore, the utility of the analytical method in connection with problems related to the toxicity of simple cyanides and metal-cyanide complexes to fish was demonstrated.

The most important modification of Schneider's method was the use of a new concentration column of glass beads coated with NaOH, in which the displaced HCN in air that has been bubbled through cyanide solutions is effectively trapped and concentrated for measurement of cyanide by a conventional analytical method. The apparatus could easily be modified for use in mobile or temporary field laboratories by employing a smaller compressed gas cylinder, only a two-stage gas regulator for controlling the flow of carrier gas, a 10-liter wide-mouth carboy filled with test solution, a medium porosity fritted
glass disperser positioned near the bottom of the carboy rather than the more expensive circulating bubbling, and one concentration column and water displacement bottle. If the temperature of the test solutions will differ from that at which a calibration curve relating HCN in solution to the amount of HCN displaced per liter of dispersed air has been determined, the relationship between displacement rate of HCN and temperature of the test solution must also be defined.

To reduce as much as possible the time required to collect on the concentration column enough HCN from solutions with low HCN levels, the flow rate of carrier gas could be increased from 50 cc/min to about 100 cc/min. Such an increase in flow rate probably would not materially reduce the HCN displacement efficiency, and any small effect could be adjusted for by using a new calibration curve defined at the greater flow rate. The pH of the combined column washings before addition of the final color-developing reagent for determination of cyanide by the pyridine pyrazolone method was found to be most critical and should be between pH 6.0 and 7.0.

Rates of Equilibration

Experiments have been performed to define the rates at which equilibria are established when alkaline solutions containing already formed metal-cyanide complex ions are diluted, causing dissociation of the complex ions, and also when the metal and cyanide ions are
added to water separately to form the complexes. The time required for attainment of equilibrium in the dissociation experiments was observed to be quite variable, ranging from a few hundred minutes for silver-cyanide complexes to many months for iron-cyanide complexes kept in the dark. It is difficult to make a meaningful comparison of times required for attainment of equilibrium through dissociation of different metal-cyanide complexes and their instability constants, since the ligand numbers for the various complexes are not constant. It was observed that for metal-cyanide complex solutions in which only one predominant metal-cyanide complex ion exists, the time to equilibrium in comparable solutions, through dissociation of the complex, increased as the ligand number increased. Metal-cyanide complexes studied other than copper can be listed in order of increasing time required for attainment of equilibrium in corresponding dissociation experiments as follows: Ag(CN)$_2^-$, Ni(CN)$_4^{2-}$, and Fe(CN)$_6^{4-}$ or Fe(CN)$_6^{3-}$. The time to equilibrium through dissociation of complex ions in cupro-cyanide solutions in which the cyanide to copper molar ratio was 2 to 1 and both Cu(CN)$_2^-$ and Cu(CN)$_3^{2-}$ ions were present was considerably longer than that required for equilibration of corresponding Ag(CN)$_2^-$ solutions and more closely approximated that required for equilibration of Ni(CN)$_4^{2-}$ solutions. The involvement of the $K_3$ dissociation reaction of Cu(CN)$_3^{2-}$ to form the Cu(CN)$_2^-$ ion probably is the rate limiting step that causes the time required for
attainment of equilibrium in cuprocyanide solutions to be markedly longer than the time required for equilibration of silver-cyanide solutions in which $\text{Ag(CN)}_2$ is the only complex ion. In solutions in which CuCN and NaCN were combined in the cyanide to copper molar ratio of either 2.5 or 3 to 1, constancy of the HCN concentration usually was not attained even as many as 110 days after their preparation.

When the comparison could be made, the time to equilibrium through dissociation of the metal-cyanide complexes was found to increase with decrease of either pH or the total cyanide concentration. In the case of the nickelocyanide complex, the time to equilibrium through dissociation was observed to be directly related to the percentage of total cyanide that was present as HCN at equilibrium. This percentage increases with decrease of pH to a value near pH 6.5, at which pH the complex is more than half dissociated in dilute solutions in which the HCN concentration is not far above the limit of tolerance of fish such as the bluegill after equilibrium has been attained. In a solution with a total cyanide content of 0.50 mg/l and pH 6.5, the nickelocyanide complex was about 56 percent dissociated at equilibrium.

**Instability Constants**

After determination of HCN concentrations at equilibrium in metal-cyanide complex solutions with various total cyanide
concentrations and pH values, it was possible to calculate apparent cumulative dissociation and formation constants for the metal-cyanide complex ions studied. For the nickel- and silver-cyanide complexes, the calculated apparent dissociation constants showed a slight increase with increase in total cyanide concentration and pH. No definite explanation can be given for this apparent variation of the constants. Experimentation has shown that the change in the apparent $K_D$ value for nickelocyanide could not be reasonably ascribed in large degree to biological or chemical breakdown of cyanide during the prolonged period required for attainment of equilibrium in solutions with the low total cyanide concentrations. A small loss of cyanide from these very dilute solutions would have a greater effect on the equilibrium HCN concentration than would the same loss of cyanide from more concentrated solutions of the nickelocyanide complex. Some loss of cyanide, which would result in reduction of the apparent value of $K_D$, is suggested by the gradual decrease in HCN concentration observed after the attainment of a maximal HCN level in an experiment at pH 6.5 in which the total cyanide concentration was 0.50 mg/l (Figure 5). However, there was no evidence of any appreciable loss of cyanide in the tests with more concentrated solutions. Therefore, the approximately two-fold increase in apparent $K_D$ with an increase in pH by 1.2 in the experiments with each of the higher concentrations of total cyanide (5.0 and 500 mg/l) could not have been
a consequence of loss of cyanide. For the change of apparent $K_D$ values obtained with silver-cyanide complex solutions, in which equilibrium was always rapidly attained, gradual loss of cyanide also clearly is not an appropriate explanation. Some other factor or some reactions not considered in this study must account for the slight change in the apparent $K_D$ of the nickel- and silver-cyanide complexes with increase in pH and total cyanide concentration. Approximate calculations have shown that changes in the activity of ionic species, as a result of changes in ionic strength of the test solutions, may account, in part at least, for the observed variation in calculated dissociation constants.

There was not consistent agreement of instability constants calculated by me and the values recently reported in the literature. The mean apparent constant calculated for the tetracyanonickelate (II) ion of $1.00 \times 10^{-31}$ and that for the dicyanocuprate (I) ion of $3.94 \times 10^{-24}$, both at $20^\circ C$, are in close agreement with the values of $5.4 \times 10^{-31}$ or $7.94 \times 10^{-31}$ at $25^\circ C$ for the former complex and $1.9 \times 10^{-24}$ or $5 \times 10^{-24}$ at $25^\circ C$ for the latter complex that have been recently reported in the literature. However, my constants of $1.94 \times 10^{-19}$ for the dicyanoargentate (I) ion and of approximately $10^{-47}$ and $10^{-52}$ for the hexacyanoferrate (II) and (III) ions, respectively, all determined at $20^\circ C$, are considerably different from the respective presently accepted values of $3.67 \times 10^{-21}$, $10^{-36.8}$ and $10^{-43.9}$ at $25^\circ C$. 
Support for my determined instability constant for the dicyanoargentate (I) ion can be found, by appropriate calculations (Appendix D), in data presented by Doudoroff et al. (1966), since the value of $1.75 \times 10^{-19}$ based on their determined HCN concentrations is almost identical to the value of $1.94 \times 10^{-19}$ determined by me. It has already been noted that the presently accepted stepwise dissociation constants for the cuprocyanide complex ions may also be unreliable.

Because of thevariability of determined (apparent) equilibrium constants of metallocyanide complexes and the possible inaccuracy of presently accepted values, it can be concluded that calculation of HCN concentrations in metallocyanide solutions from equilibrium constants is not accurate enough in most cases for prediction of possible toxicity due to HCN of waters containing the complexes. It may also be difficult and in some cases impossible to define the actual concentrations of various cyanide complex species present and the degree of equilibration of the solutions. Instead, the direct analytical measurement of molecular HCN should be employed.

Acute Toxicity

Toxicity of Nickelocyanide Solutions in Relation to pH

The chemistry and toxicity of nickelocyanide solutions was studied specifically in an attempt to explain some of the toxicity
results reported by Doudoroff (1956) on the influence of pH on the toxicity to fathead minnows (*Pimephales promelas* Rafinesque) of the nickelocyanide complex. From information given by Christensen et al. (1963), one can calculate the $K_D$ for the tetracyanonickelate (II) ion at 20°C by the equation:

$$\log \frac{K_2}{K_1} = \frac{\Delta H^0(T_2 - T_1)}{2.303RT_1T_2}$$

where $K_1 = K_D$ at temperature $T_1$; $K_2 = K_D$ at temperature $T_2$; $\Delta H^0$ = enthalpy; and $R$ = ideal gas constant. This calculation gives a $K_D$ value of $2.28 \times 10^{-31}$, which is in good agreement with the average apparent $K_D$ value of $1.00 \pm 0.37 \times 10^{-31}$ determined by me. By using $K_D$ values, it is possible to calculate the theoretical relationships between pH and median tolerance limits ($T_L_m$) of the nickelocyanide complex, expressed in mg/l total cyanide as CN, assuming HCN to be the only toxic species in the solutions (Appendix K).

Figure 22 illustrates these relationships and shows that Doudoroff's sigmoid curve relating logarithms of $T_L_m$ values to pH does not agree fully with the theoretical curves relating pH and the logarithms of the total cyanide concentrations required to produce 0.16 mg/l HCN. The HCN concentration of 0.16 mg/l was selected for two reasons: First, it was chosen because I found by trial that curves obtained by using this value and my apparent $K_D$ values for the nickelocyanide complex or the $K_D$ of $2.28 \times 10^{-31}$ based on data of Christensen et al. (1963)
Figure 22. Comparison of curves relating pH and concentrations of cyanide initially complexed with nickel (total cyanide concentrations) corresponding to:

(A) 50% survival of fathead minnows for 48 hours (solid line);

(B) 0.16 mg/l molecular HCN by computation from $K_D = 2.28 \times 10^{-31}$ (broken line), and

(C) 0.16 mg/l molecular HCN by computation from apparent $K_D$ values determined experimentally in this study for solutions of varying concentration and pH (dotted line).

Curve A is from Doudoroff (1956).
most nearly approximate Doudoroff's (1956) 48-hour TL\textsubscript{m} curve obtained with fathead minnows. Secondly, this value was judged appropriate because Doudoroff et al. (1966) reported that the 48-hour TL\textsubscript{m} of HCN concentration for bluegills exposed to NaCN solutions that were continuously renewed was near 0.16 mg/l.

At a low pH, the toxicity of the complex observed by Doudoroff (1956) was much less than that which is theoretically predictable on the basis of the K\textsubscript{D} value of 2.28 x 10^{-31} and the assumptions that molecular HCN is the only toxic component of the solutions and that equilibrium has been attained. On the other hand, at a high pH, the toxicity is greater than that which is predictable on the same basis. The slope of the middle portion of the curve relating toxicity to pH does not differ materially from the theoretical slope. Much smaller differences between theoretical and observed toxicities are seen when one compares Doudoroff's sigmoid curve with the theoretical curve calculated from the various apparent K\textsubscript{D} values derived by computation from results of my dissociation experiments at various total cyanide concentrations and presented in Table 4. The curve calculated with the apparent K\textsubscript{D} values approximates Doudoroff's 48-hour TL\textsubscript{m} curve quite closely at pH values between 7.2 and 7.8; outside these limits the curves diverge considerably.

Because of the long time required for equilibrium to be attained in solutions of the nickelocyanide complex at pH values near 6.5 and
at low total cyanide concentrations in Doudoroff's experiments, it is now obvious that his test solutions at pH 6.5 were not at equilibrium when bioassays were begun, and that complete equilibrium had not been attained by the time of their conclusion. This accounts for the deviation of the lower portion of his curve from the theoretical curve calculated from the various apparent $K_D$ values. The theoretical curves are markedly above Doudoroff's 48-hour TL$_m$ curve at pH 8.0. Toxicity bioassays performed by me demonstrated that the Ni(CN)$_4^{2-}$ complex ion itself may be toxic at high concentrations. Therefore, the deviation of the upper portion of Doudoroff's 48-hour TL$_m$ curve from the theoretical curves can in part be attributed to the high concentration of the Ni(CN)$_4^{2-}$ complex ion in a solution with the high pH and the high total cyanide concentration necessary to produce an acutely toxic level of HCN. When the total cyanide concentration is not much greater than 10 mg/l, my results showed that the contribution of the Ni(CN)$_4^{2-}$ complex ion to the toxicity of an acutely toxic solution is negligible and the toxicity depends entirely on the molecular HCN concentrations determinable by chemical analysis. Comparison of Doudoroff's results and the theoretical relationships presented in Figure 22 suggest that the toxicity of the Ni(CN)$_4^{2-}$ ion is insignificant at levels below about 200 to 400 mg/l as CN. However, this indication of very low toxicity of the Ni(CN)$_4^{2-}$ ion probably is due to the somewhat arbitrary choice of 0.16 mg/l HCN as the 48-hour TL$_m$
value for fathead minnows used to calculate the theoretical relationship. Although the tolerance limit of about 0.24 mg/l HCN indicated by the results of Doudoroff's (1956) experiments with fathead minnows and NaCN alone in solutions that were not renewed probably is higher than the true value because of loss of cyanide during his tests, the true value evidently may be above the 0.16 mg/l concentration used to define the above theoretical relationship. As the estimate of the 48-hour TL$_m$ of HCN is increased, the theoretical curve is displaced upward with some change in shape of the lower portion. The observed divergence of the calculated curve from the experimental curve due to the toxicity of the Ni(CN)$_4$$^{2-}$ ion then occurs at a lower pH and lower total cyanide concentration.

**Toxicity of Other Complex Ions**

The acute toxicity of comparable solutions of the different metal-cyanide complexes studied was shown to vary widely, but generally was found to be a function of the measured molecular HCN level. However, the silver- and copper-cyanide complex ions and probably also the silver cation were the predominant toxic components of some solutions or at least contributed to their toxicity. The toxic metal ions are derived through dissociation or decomposition of metal-cyanide complexes.

The 48-hour TL$_m$ values for bluegills at 20°C were determined
to be approximately 27 and 9 mg/l as the Ag(CN)$_2^-$ and Cu(CN)$_2^-$ ions (9 and 4 mg/l as CN), respectively. On the basis of the available data, the metal-cyanide complex ions studied can be arranged in order of decreasing toxicity as follows: Cu(CN)$_2^-$, Ag(CN)$_2^-$, Ni(CN)$_4^{2-}$, and Fe(CN)$_6^{3-}$ or Fe(CN)$_6^{4-}$.

Doudoroff et al. (1966) demonstrated that HCN virtually alone determined the acute toxicity to bluegills of various solutions of simple and complex cyanides. The silver-cyanide complex proved exceptional; bluegills exposed to most of their silver-cyanide complex solutions showed signs of toxicity, such as superficial coagulation of mucus, strongly suggestive of heavy-metal poisoning and apparently referable to toxicity of the complex anions or of silver cations. They expressed the view that the Ag(CN)$_2^-$ ion itself probably has considerable toxicity to fish.

My own data support not only the view that the toxicity of nearly neutral and alkaline solutions of the silver-cyanide complex is not attributable to their molecular HCN content, but also the view that the principal toxic agent was the complex anion and not the silver cation. The median survival times of bluegills in solutions of the silver-cyanide complex with a total cyanide content of 10 mg/l as CN and with pH values of 7.5 and 8.5 were nearly identical, namely, 31 hours and 29 hours, respectively (Table 6). The determined HCN concentrations in these two solutions were 0.012 and 0.003 mg/l, respectively,
and the calculated Ag⁺ ion concentrations were 0.025 and 0.007 mg/l, respectively. The absence of any difference in toxicity of the two solutions with sublethal levels of HCN and with very different concentrations of Ag⁺ ion is evidence that the Ag⁺ ion contributed little or not at all to the toxicity of either solution. Additional supporting evidence is provided by some unpublished data of Doudoroff (personal communication). He determined in the year 1964 that the median survival time at 20°C of bluegills in a solution of the silver-cyanide complex in concentration equivalent to 10 mg/l CN, prepared with stream water and brought to pH 9.0 by the addition of NaOH to be about 512 minutes. The calculated HCN and Ag⁺ ion concentrations in this solution are only 0.001 and 0.004 mg/l, respectively, hardly sufficient to have contributed materially to the high toxicity of the solution. The median survival time of 512 minutes is decidedly less than those recorded by Doudoroff et al. (1966) in tests with bluegills of similar solutions prepared with the same stream water at pH 7.5 and 6.5 (acidified slightly with HCl), which were 833 and 789 minutes, respectively. The reduced survival time observed in the later test may have been due to an increase of the toxicity of the complex at the elevated pH, or to lower resistance of the test animals due to undetermined physiological differences and perhaps minor differences in composition of the stream water used. Some other data recorded by Doudoroff in 1964 indicate that the bluegills at that time were indeed
somewhat less resistant than those tested by Doudoroff et al. (1966) in 1961. The even more striking difference in median survival time at Ag(CN)$_2^-$ concentrations of 10 mg/l as CN between my bluegills (at pH 7.5 and 8.5) and those of the earlier investigators (at pH 6.5-9.0) apparently must be ascribed to differences in physiological state of the fish or differences in mineral content of the waters used.

Doudoroff et al. (1966) observed that bluegills dying in two solutions of the cuprocyanide complex formed by combining cupric sulfate with sodium cyanide showed typical signs of cyanide poisoning only. Evidently, the rapid toxic effect on the bluegills in these solutions, which contained much residual free cyanide for reasons explained by the authors and had determined molecular HCN concentrations of 0.30 and 1.20 mg/l, was due primarily to HCN. With the high molecular HCN concentrations tending to mask any toxic effect of the Cu(CN)$_2^-$ ion, it is not surprising that the toxicity to fish of this complex ion was not demonstrated. The possibility of material toxicity of this anion was mentioned by the authors. My results indicate that the toxicity of the complex can indeed be an important water pollutant in the absence of harmful levels of HCN.

Kariya et al. (1967) observed that the copper content of whole fish which were exposed to a copper-cyanide plating bath solution containing excess free cyanide and with a cyanide to copper molar ratio of about 3 to 1 was much less than the accumulated concentration in
fish exposed to a CuSO$_4$ solution of the same total copper content. In fact, the copper concentrations in fish killed by the copper-cyanide plating solutions containing up to a few hundred mg/l Cu were only moderately higher than the levels determined in control fish. Fish exposed to a plating solution at a given total copper concentration survived longer than did fish in a CuSO$_4$ solution with the same total copper concentration. Apparently, the excess free cyanide of the plating solution had somehow been removed and the copper was mainly in the form of copper-cyanide complex at the time of the bioassay, this accounting for the reduced toxicity. The sodium diethyl-dithiocarbamate method which Kariya used for determining copper in experimental fish is believed to be specific for free copper rather than copper complexed with cyanide. This may account for the low determined concentrations of copper in fish killed by various copper-cyanide plating solutions in which cyanide was apparently not the lethal factor. Since I have demonstrated that copper in the form of the dicyano-cuprate (I) ion must penetrate fish, it appears that the copper-cyanide ions that penetrate remain intact in the complex form and do not readily liberate free copper.

Injected copper has been shown to inactivate hydrocyanic acid in rabbits, presumably by complexation with cyanide, and has even been recommended as an antidote in cyanide poisoning (Grollman and Grollman, 1970). This is further evidence of internal persistence of the cuprocyanide complex.
Accumulation experiments were performed as a part of this research to see if there is a direct relationship between the toxicity of the different metal-cyanide complex ions and the amounts accumulated by various fish tissues. By extrapolation of Doudoroff's (1956) TL curve presented in Figure 22, the 48-hour TL value of the \( \text{Ni}(\text{CN})_4^{2-} \) ion alone for fathead minnows can be estimated to be above 2,000 mg/l as CN. The 48-hour TL values of \( \text{Ag}(\text{CN})_2^- \) and \( \text{Cu}(\text{CN})_2^- \) for bluegills were, respectively, 9.0 and 4.0 mg/l as CN.

The most notable difference between the accumulation of carbon from \(^{14}\text{C}\)-labeled cyanide in nickelocyanide solutions and the accumulation of copper or silver from solutions of their complexes is that, in fish exposed to the former solutions, the gills accumulated \(^{14}\text{C}\) in much greater amounts, per gram of tissue, than did the liver plus gall bladder tissues, whereas in fish exposed to the cuprocyanide and silver-cyanide complex solutions, the liver plus gall bladder accumulated much more of the metals than did the gills. Such a comparison between amounts accumulated per gram of either wet or dry weight of tissue can be made, since the percentages of water in the gill and liver plus gall bladder tissues were observed to be approximately the same.

Since it was observed that it was much more difficult for the \( \text{Ni}(\text{CN})_4^{2-} \) ion than for the \( \text{Cu}(\text{CN})_2^- \) and \( \text{Ag}(\text{CN})_2^- \) ions to penetrate the gill tissues, the toxicity of the three metal-cyanide complex ions is
directly related to their ability to penetrate the gills and not to the extent to which the complex ions concentrate in the gills. The rate of penetration is probably related to the charge on the anionic metal-cyanide complexes, but it may be more dependent on their physical structure. The \[\text{Ni(CN)}_{2}^{2-}\] ion is characteristically tetrahedral in shape and may for this reason penetrate more slowly than the \[\text{Ag(CN)}_{2}^{-}\] and \[\text{Cu(CN)}_{2}^{-}\] ions, which are smaller and linear in form.

Kariya et al. (1967; 1968) observed that copper and nickel are absorbed by the bodies of fish killed in solutions of the metal sulfates. The gills of the dead fish were reported to contain the most metal, the skin much less, and the viscera and muscle the smallest amounts. Likewise, Lloyd (1960) and Saiki and Mori (1955) observed that the concentration of zinc in the tissues of fish killed in solutions of a zinc salt was greatest in the gills. Its lethal action at acutely toxic concentrations appeared to be superficial, death probably resulting from respiratory failure following destruction of the gill epithelium (Lloyd, 1960). In general, it can be concluded that most heavy metal ions are not concentrated in internal organs of fresh water fish which have died at high concentrations of metal salts; rather, the metals concentrate in the gills.

The \[\text{Ni(CN)}_{4}^{2-}\] ion and ionic nickel are similar in that neither can readily penetrate into the body of a fish and both appear to be concentrated in the gills; perhaps only on the gill surface and not even in the
gill tissue itself. The signs of toxic action and amounts of penetration at acutely toxic levels of the Ni(CN)$_4^{2-}$ ion alone are unknown. The Ag(CN)$_2^-$ and Cu(CN)$_2^-$ ions are similar to their uncomplexed metal ionic form in that fish killed by all of the solutions produced large amounts of coagulated mucus, both on the gills and the body surface. They differ from the ionic metal form in that they penetrate as the complex itself and accumulate as the complex or as some other form in high concentration in the blood and liver and to a much lesser extent in the gills.

The low toxicity of the Ni(CN)$_4^{2-}$ ion is most likely due to its inability to penetrate the gill tissues or to destroy the gill epithelium. The Cu(CN)$_2^-$ and Ag(CN)$_2^-$ complex ions produce external signs suggestive of superficial toxic action, but since the ions readily penetrate a major contribution to their lethal action could be internal.

**Influence of Chloride on Complexation of Ag(CN)$_2^-$**

The toxicity to fish of numerous pollutants has been demonstrated to vary with the chemistry of the receiving water into which the toxicants are discharged. Through toxicity bioassays and analysis for HCN of NaCN solutions in fresh water and in various dilutions of full strength sea water, it was determined that HCN becomes more toxic to threespine sticklebacks with increases in chlorinity of the
The toxicity of silver-cyanide complex solutions in which the CN to Ag molar ratio was 2 to 1 also was greater in sea water than in similar solutions prepared with fresh water. This has been shown to result in part from increases of HCN concentration which accompanied increases of chlorinity. The increase of HCN is due to the fact that the two ligands CN\(^-\) and Cl\(^-\) are in competition for silver cations in aqueous solutions, forming silver-cyanide and silver-chloride complexes, respectively. Therefore, upon dilution with sea water of silver-cyanide complex solutions, the competition for the silver ion causes more extensive dissociation of the silver-cyanide complex than that observed in fresh water and a relatively high HCN concentration results. The approximate 24-hour TL\(_m\) values for threespine sticklebacks in silver-cyanide solutions prepared with full-strength sea water of pH 7.9 and with HCN levels less than 0.09 mg/l and in solutions in fresh water of pH 7.7 and with HCN levels less than 0.009 mg/l were determined to be 3.0 and 6.0 mg/l as total cyanide, respectively. The difference of toxicity of the fresh water and saline solutions could be due either to the higher concentrations and greater toxicity of HCN in the sea water solutions or to an increase of the toxicity of the Ag(CN)_2\(^-\) ion in sea water.

Solutions of any of the other metal-cyanide complexes which can form metal-chloride complexes can be expected to be more toxic in sea water than in fresh water. Copper has been shown to form
metal-chloride complexes (Kolthoff et al., 1969) at least to a slight extent; therefore, solutions of cupro-cyanide complexes prepared with sea water, like those of the silver-cyanide complex, may be more toxic than comparable solutions prepared with fresh water.

Defining the Potential Toxicity of Effluents Containing Metallo-cyanides

From available information on use and toxicity of cyanides, it is reasonable to conclude that, since the silver-cyanide complex is not likely to occur in effluents (for purely economic reasons), the cupro-cyanide complex ions are the only metallo-cyanide ions likely ever to occur persistently in toxic concentrations in waters receiving industrial wastes. Cupro-cyanide complexes occur in waters as a result of discharge of copper plating bath solutions (usually accidental) or of water used for rinsing of plated objects. Copper-cyanide plating solutions are prepared by combining CuCN and a simple cyanide, like NaCN, in a cyanide to copper molar ratio of about 3.4 to 3.6. Since the pH of these solutions is quite high (pH 10 to 12), it is generally believed that most of the copper exists in the form of the tricyano-cuprate complex, \( \text{Cu(CN)}_3^{-2} \), with a high proportion of free cyanide also present. Upon dilution of copper plating bath solutions with waters of normal pH, the \( \text{Cu(CN)}_3^{-2} \) ion dissociates almost completely to produce the predominant dicyanocuprate complex, \( \text{Cu(CN)}_2^{-2} \), with an
accompanying release of cyanide. Free cyanide present initially in a spent plating bath solution or rinse water, together with that produced through dissociation of complex ions, may have toxic effects masking any chronic effect of the cuprocyanide complex ions. Toxic concentrations of the cuprocyanide or copper ions may sometimes persist in waters from which the free cyanide has been eliminated by natural processes or by partial treatment of waste waters before discharge. However, the importance of metal-cyanide complexes in general as water pollutants derives mainly from their capacity to dissociate or decompose with the production of HCN, which can be either acutely or chronically toxic to fish in receiving waters. Only through a direct chemical analysis can the presence of HCN in concentration sufficient to produce a given known harmful effect on the test animals be definitely established.

Since cyanide is not highly persistent, especially in organically polluted waters, determination of molecular HCN in samples must be undertaken as soon as possible after collection. Doudoroff et al. (1966) have outlined a procedure by which the maximal concentration in a receiving water of molecular HCN deriving from an industrial effluent that contains complex cyanides and is discharged at a known rate can be readily estimated by testing suitably prepared dilutions of effluent samples. The following steps should be taken in arriving at such an estimate.
First, dilute some of the effluent with a suitable natural water to the highest concentration likely to occur in the receiving water. Next, adjust the pH of the dilution with a mineral acid such as $\text{H}_2\text{SO}_4$ (do not use $\text{HCl}$) to the lowest value that may possibly occur in the stream within a reasonable distance below the waste discharge point and determine the resulting level of molecular HCN. Carbon dioxide is removed from aqueous solution by aeration more rapidly than is hydrogen cyanide. Therefore, severe pH changes, which may affect the dissociation of metallo-cyanides, are likely to occur in an organically polluted test solution during HCN determinations. This potential problem can be partly overcome by reducing the bubbling time to the minimum required for collecting a reliably measurable amount of HCN. Readjustment of the test solution pH during the HCN determinations would also minimize the problem. Some metallo-cyanides such as ferro- and ferricyanides are photodecomposable. When presence of these complexes in industrial discharges is suspected, effluent dilutions should be exposed to bright sunlight until a maximal HCN level is obtained. Since equilibrium is attained quite slowly in some metal-cyanide complex solutions, sufficient time always must be allowed after preparation of a dilution until repeated testing shows that the highest molecular HCN level that can develop in the dilution has been attained. If it can be shown that the HCN concentration remains below a safe level when enough time is allowed for the maximum HCN
level to be attained, one can conclude that the safe level will not be exceeded in the receiving water.

The chemical reactions which cyanide may undergo when discharged into natural waters either as simple or complex cyanides are numerous, and in many instances actual HCN concentrations in receiving waters cannot be accurately predicted by merely studying the effluents. Therefore, the crucial test in defining the impact an effluent containing cyanide may have on aquatic life is to perform HCN determinations on samples taken directly from the receiving water and at various locations.


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APPENDIX A

Thermodynamic Data and Log Formation Constants
for Various Cyanide Reactions

Key to Appendix A

Method of Measurement - The method by which the stability constants were measured is shown in the second column of the table by the following abbreviations:

- Cal - Calorimetry
- Col - Colorimetry
- Gl - Glass electrode
- Hyp - From some hypothesis
- Ir - Infrared spectra
- Lit - From critical survey of literature data
- pHCN - Partial pressure of HCN
- Sol - Solubility
- Sp - Spectrophotometry
- Ag, Cu - e.m.f. with electrode of metal stated
- ? - Method not known
- Ther - Combination of thermodynamic data

Temperature - The third column of each table gives the temperature in °C. "rt" denotes room temperature, and "?" is used if the value was not found.

Medium - The nature of the medium to which the equilibrium constants refer is specified in the fourth column.

- ---0 - Constants extrapolated to zero ionic strength
- 0 Corr - Constants corrected to zero ionic strength by the application of some theoretical or empirical formula
- 0.01 - An ionic strength of 0.01 mole/liter
- Var - Ionic medium varied, and in some cases no special attempt was made to control the ionic strength.

Thermodynamic Properties

\[ \Delta F_f^O \] - Standard free energy of formation
\[ \Delta H_f^O \] - Standard heat of formation
\[ \Delta S_f^O \] - Standard entropy of formation

Results - The eighth column records the logarithm to base 10 (log K) of the equilibrium constants.
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<th>Species or reaction</th>
<th>Method</th>
<th>Temperature (°C)</th>
<th>Medium</th>
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<th>( \Delta H^o_f ) (Kcal/mole)</th>
<th>( \Delta S^o_f ) (cal/deg mole)</th>
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$\beta_4 = 30.2676$ (K a (HCN) 9.2076)

$k_s^\circ = -0.7212 ± 2.000$ (NaClO 4)

$k_s^\circ = -0.5544 ± 2.0969$ (NaClO 4)

$k_s^\circ = -1.0223 ± 1.8861$ (NaClO 4)
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$\beta_6 =$ Latimer, 1952

$\beta_6 =$ Stephenson and Morrow, 1956

$\beta_6 =$ Stephenson and Morrow, 1956
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### APPENDIX B

Spectrophotometric absorption values in the infrared, visible and ultraviolet spectrum region for various metal complex ion species

<table>
<thead>
<tr>
<th>Species</th>
<th>Molar extinction coefficient (ε) mole⁻¹ cm⁻¹</th>
<th>Infrared frequencies (ν) cm⁻¹</th>
<th>Visible wave lengths (nm)</th>
<th>Ultraviolet wave lengths (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN⁻</td>
<td>29±1</td>
<td>2080±2</td>
<td>430</td>
<td></td>
<td>McCullough et al., 1960</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>450</td>
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</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>470</td>
<td></td>
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</tr>
<tr>
<td>Ni(CN)₄⁻²</td>
<td>2135</td>
<td></td>
<td></td>
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<td>El-Sayed and Sheline, 1956</td>
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<tr>
<td></td>
<td>1625</td>
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<td>1603</td>
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<td></td>
<td>1,068±95</td>
<td>2124</td>
<td>430</td>
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<td>McCullough et al., 1960</td>
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<tr>
<td></td>
<td>1.811±0.005</td>
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<td></td>
<td>1.350±0.008</td>
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<td></td>
<td>0.967±0.009</td>
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<tr>
<td></td>
<td>1.175</td>
<td></td>
<td></td>
<td></td>
<td>Brigando, 1957</td>
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<td></td>
<td>436.5</td>
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<td>676.1</td>
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<td>3,981</td>
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<td>310</td>
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<td>Brummet and Hollweg, 1956</td>
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<td>286</td>
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<table>
<thead>
<tr>
<th>Species</th>
<th>Molar extinction coefficient (ε) mole(^{-1}) l cm(^{-1})</th>
<th>Infrared frequencies (V) cm(^{-1})</th>
<th>Visible wave lengths (nm)</th>
<th>Ultraviolet wave lengths (nm)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Ni(CN)(_4)(^{-2}) (cont'd)</td>
<td>11,680</td>
<td>267.5</td>
<td>267.5</td>
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<tr>
<td>Ni(CN)(_5)(^{-3})</td>
<td>1,730 ± 230</td>
<td>2102 ± 2</td>
<td>430</td>
<td>418</td>
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<td></td>
<td>263 ± 59</td>
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<td></td>
<td>213 ± 38</td>
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<tr>
<td></td>
<td>178 ± 25</td>
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<tr>
<td>Fe(CN)(_6)(^{-3})</td>
<td>977</td>
<td>420</td>
<td>322</td>
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<td>1,096</td>
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<td>1,585</td>
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<td>2,512</td>
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<td>1,202</td>
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<td>Fe(CN)(_6)(^{-4})</td>
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<td>Cu(CN)(_2)</td>
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<td>225</td>
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<td>9,162</td>
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<td>234</td>
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<td>Simpson and Waind, 1958</td>
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<td>10,590</td>
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<tr>
<td></td>
<td>(1,637)</td>
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<td>(260)(^{1/2})</td>
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<td>650,1</td>
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<td>274</td>
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(Continued on next page)
### Appendix B. (Continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Molar extinction coefficient ((\varepsilon)) mole(^{-1}) (1) cm(^{-1})</th>
<th>Infrared frequencies ((\vec{\nu})) cm(^{-1})</th>
<th>Visible wave lengths (nm)</th>
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<td>(1,499)</td>
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<td>(538.2)</td>
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<td>260</td>
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<td>159.9</td>
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<td>Cu(CN)(_4)(^{-3})</td>
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<td>Simpson and Waind, 1958</td>
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<td>(299.9)</td>
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<td>169.8</td>
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<td>199.5</td>
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<td>131.8</td>
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<td>Ag(CN)(_2)(^{-})</td>
<td>264±12</td>
<td>2135±1</td>
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<td>Jones and Penneman, 1954</td>
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<td>Ag(CN)(_3)(^{-2})</td>
<td>397±23</td>
<td>2105±1</td>
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<td>Ag(CN)(_4)(^{-3})</td>
<td>556±83</td>
<td>2092±1</td>
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</table>

1/ Parentheses indicate shoulders
APPENDIX C

Derivation of an equation used for determining the cumulative dissociation constant of the tetracyanonickelate (II) complex ion

<table>
<thead>
<tr>
<th>Formation Reactions</th>
<th>Formation Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni^{++} + 4CN⁻ ⇌ Ni(CN)_4⁻²</td>
<td>( \beta_4 = 10^{30.2676} = 1.852 \times 10^{30} ) Schneider and Freund, 1962</td>
</tr>
<tr>
<td>Ni(CN)_4⁻² + CN⁻ ⇌ Ni(CN)_5⁻³</td>
<td>( K_5 = 0.279 ) Penneman et al., 1963</td>
</tr>
<tr>
<td>Ni(CN)_5⁻³ + CN⁻ ⇌ Ni(CN)_6⁻⁴</td>
<td>( K_6 = 0.095 ) Penneman et al., 1963</td>
</tr>
</tbody>
</table>

With a CN⁻ molar concentration of about 10⁻⁷, it is apparent that the penta- and hexacyanonickelate ion concentrations are negligible when compared with that of the tetracyanonickelate (II) ion.

We can therefore assume:

Total Cyanide \( (\text{CN}_T ) = \text{CN}^- + \text{HCN} + 4\text{Ni(CN)}_4^-² \)

Total Nickel \( (\text{Ni}_T ) = \text{Ni}^{++} + \text{Ni(CN)}_4^-² \)

\( \text{Ni(CN)}_4^-² ⇌ \text{Ni}^{++} + 4\text{CN}^- \quad \frac{1}{\beta_4} = K_D = \frac{(\text{Ni}^{++})(\text{CN}^-)^4}{(\text{Ni(CN)}_4^-²)} \)

\( \text{HCN} ⇌ H^+ + \text{CN}^- \quad K_a = \frac{(H^+)(\text{CN}^-)}{(\text{HCN})} \)

According to Izatt et al. (1962), the \( K_a \) at 20°C is equal to \( 4.365 \times 10^{-10} \).
Let: \( X \) = Initial Total Cyanide  
\( Y \) = Initial Total Nickel  
\( Z \) = Equilibrium \( \text{Ni(CN)}_4^{-2} \)

\[
X = (\text{CN}^-) + (\text{HCN}) + 4(\text{Ni(CN)}_4^{-2})
\]

\[
(\text{CN}^-) = X - (\text{HCN}) - 4Z
\]

\[
(\text{Ni}^{++}) = Y - Z
\]

\[
K_D = \frac{(\text{Ni}^{++})(\text{CN}^-)^4}{(\text{Ni(CN)}_4^{-2})^4} \quad \text{and} \quad \frac{K_{\text{a}} (\text{HCN})}{(\text{H}^+)} = (\text{CN}^-)
\]

Therefore

\[
K_D = \frac{(\text{Ni}^{++})[\frac{K_{\text{a}} (\text{HCN})}{(\text{H}^+)}]^4}{(\text{Ni(CN)}_4^{-2})^4}
\]

We know that

\[
Z = \frac{X-(\text{HCN})-(\text{CN}^-)}{4} \quad \text{and} \quad (\text{Ni}^{++}) = Y - Z
\]

Therefore

\[
(\text{Ni}^{++}) = Y - \frac{(X-(\text{HCN})-(\text{CN}^-))}{4}
\]

\[
= (Y - \frac{X}{4}) + \frac{(\text{HCN})+(\text{CN}^-)}{4}
\]

Initially, \( \text{NiSO}_4 \) and \( \text{NaCN} \) were combined in the ratio of one gram atom of nickel plus four gram moles of cyanide to form the \( \text{Ni(CN)}_4^{-2} \) complex. Therefore, the total cyanide molar concentration is equal to four times the total nickel molar concentration.
With $X = 4Y$, then

$$(\text{Ni}^{\text{II}}) = (Y - \frac{4Y}{4}) + \frac{\text{(HCN)} + \text{(CN}^-)}{4}$$

$$(\text{Ni}^{\text{II}}) = \frac{\text{(HCN)} + \text{(CN}^-)}{4}$$

$$K_D = \frac{\frac{\text{(HCN)} + \text{(CN}^-)}{4} [ \frac{\text{K}^a (\text{HCN})}{(H^+)}]^4}{\frac{\text{(Ni}-(\text{CN})^2)}{4}} = \frac{\frac{\text{(HCN)} + \text{(CN}^-)}{4} [ \frac{\text{K}^a (\text{HCN})}{(H^+)}]^4}{\frac{\text{(Ni}-(\text{CN})^2)}{4}}$$

$$(\text{Ni}-(\text{CN})^2) = Z = \frac{X-(\text{HCN})-(\text{CN}^-)}{4} = X-(\text{HCN}) - \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} [ \frac{\text{K}^a (\text{HCN})}{(H^+)}]^4$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$

$$K_D = \frac{\text{K}^a (\text{HCN})}{(H^+)} \frac{\text{K}^a (\text{HCN})}{(H^+)} + \frac{\text{K}^a (\text{HCN})}{(H^+)}$$
The average $K_D$ value calculated during this research for nickel-cyanide complex solutions having a cyanide to nickel molar ratio of 4 to 1, and with pH and total cyanide concentrations ranging between:

- pH 6.5 - 0.5 mg/l Total Cyanide
- pH 7.7 - 500 mg/l Total Cyanide

is:

$$K_D = 1.004 \pm 0.375 \times 10^{-31}$$

$$\frac{1}{K_D} = \beta_4 = 9.960 \times 10^{30}$$
APPENDIX D

Derivation of an equation used for determining the cumulative dissociation constant of the dicyanoargentate (I) complex ion

**Formation Reactions**

\[
\text{Ag}^+ + 2\text{CN}^- \rightleftharpoons \text{Ag(CN)}_2^-
\]

**Formation Constants**

\[
\beta_2 = 10^{-21.0969} = 1.25 \times 10^{-21}
\]

Jones and Penneman, 1954

\[
\beta_2 = 10^{-20.4353} = 2.725 \times 10^{-20}
\]

Azzam and Shimi, 1963

\[
\text{Ag(CN)}_2^- + \text{CN}^- \rightleftharpoons \text{Ag(CN)}_3^-
\]

\[
K_3 = 10^{0.70} = 5.012
\]

Jones and Penneman, 1954

\[
\text{Ag(CN)}_3^- + \text{CN}^- \rightleftharpoons \text{Ag(CN)}_4^-
\]

\[
K_4 = 10^{-1.13} = 0.0741
\]

Jones and Penneman, 1954

With a CN\(^-\) molar concentration of about 10\(^{-7}\), it is apparent that the tri- and tetracyanoargentate (I) complex ion concentrations are negligible when compared with that of the dicyanoargentate (I) ion.

We can therefore assume:

Total Cyanide (\(\text{CN}_T\)) = \(\text{CN}^- + \text{HCN} + 2\text{Ag(CN)}_2^-\)

Total Silver (\(\text{Ag}_T\)) = \(\text{Ag}^+ + \text{Ag(CN)}_2^-\)

\[
\text{Ag(CN)}_2^- \rightleftharpoons \text{Ag}^+ + 2\text{CN}^- \quad \frac{1}{\beta_2} = K_D = \frac{(\text{Ag}^+(\text{CN})^-)^2}{(\text{Ag(CN)}_2^-)}
\]

\[
\text{HCN} \rightleftharpoons \text{H}^+ + \text{CN}^- \quad K_a = \frac{(\text{H}^+(\text{CN})^-)}{(\text{HCN})}
\]

According to Izatt et al. (1962), the \(K_a\) at 20°C is equal to 4.365 \(\times\) 10\(^{-10}\).
Let: $X = \text{Initial Total Cyanide}$

$Y = \text{Initial Total Silver}$

$Z = \text{Equilibrium Ag(CN)}_2^-$

$$X = (\text{CN}^-) + (\text{HCN}) + 2(\text{Ag(CN)}_2^-)$$

$(\text{CN}^-) = X - (\text{HCN}) - 2Z$

$(\text{Ag}^+) = Y - Z$

$$K_D = \frac{(\text{Ag}^+)(\text{CN}^-)^2}{(\text{Ag(CN)}_2^-)} \quad \text{and} \quad \frac{K_a (\text{HCN})}{(\text{H}^+)} = (\text{CN}^-)$$

Therefore

$$K_D = \frac{(\text{Ag}^+)[\frac{K_a (\text{HCN})}{(\text{H}^+)}]^2}{(\text{Ag(CN)}_2^-)}$$

We know that

$$Z = \frac{X-(\text{HCN})-(\text{CN}^-)}{2} \quad \text{and} \quad (\text{Ag}^+) = Y - Z$$

Therefore

$$(\text{Ag}^+) = Y - \frac{(X-(\text{HCN})-(\text{CN}^-))}{2}$$

$$= (Y - \frac{X}{2}) + \frac{(\text{HCN})+(\text{CN}^-)}{2}$$

 Initially, AgNO$_3$ and NaCN were combined in the ratio of one gram atom of silver plus two gram moles of cyanide to form the Ag(CN)$_2^-$ complex. Therefore, the total cyanide molar concentration is equal to two times the total silver molar concentration.
With $X = 2Y$, then

$$\text{(Ag}^+) = (Y - \frac{2Y}{2}) + \frac{(\text{HCN}) + (\text{CN}^-)}{2}$$

$$\text{(Ag}^+) = \frac{(\text{HCN}) + (\text{CN}^-)}{2}$$

$$K_D = \frac{\frac{(\text{HCN}) + (\text{CN}^-)}{2} \left[ \frac{K_a(\text{HCN})}{(H^+)} \right]^2}{\frac{(\text{Ag(CN)}_2^-)}{2}} = \frac{\frac{K_a(\text{HCN})}{(H^+)} \left[ \frac{K_a(\text{HCN})}{(H^+)} \right]^2}{\frac{(\text{Ag(CN)}_2^-)}{2}}$$

$$(\text{Ag(CN)}_2^-) = Z = \frac{X - (\text{HCN}) - (\text{CN}^-)}{2}$$

$$K_D = \frac{\frac{K_a(\text{HCN})}{(H^+)} \left[ \frac{K_a(\text{HCN})}{(H^+)} \right]^2}{\frac{X - (\text{HCN}) - (\text{CN}^-)}{2}}$$

$$K_D = \frac{\frac{K_a(\text{HCN})}{(H^+)} \left[ \frac{K_a(\text{HCN})}{(H^+)} \right]^2}{\frac{X - (\text{HCN}) - (\text{CN}^-)}{2}}$$

$$K_D = \frac{\frac{K_a(\text{HCN})}{(H^+)} \left[ \frac{K_a(\text{HCN})}{(H^+)} \right]^2}{\frac{X - (\text{HCN}) - (\text{CN}^-)}{2}}$$

$$K_D = \frac{\frac{K_a(\text{HCN})}{(H^+)} \left[ \frac{K_a(\text{HCN})}{(H^+)} \right]^2}{\frac{X - (\text{HCN}) - (\text{CN}^-)}{2}}$$
The average $K_D$ value calculated during this research for silver-cyanide complex solutions having a cyanide to silver molar ratio of 2 to 1, and with pH and total cyanide concentrations ranging between:

- pH 7.7 - 0.5 mg/l Total Cyanide
- pH 7.5 - 200 mg/l Total Cyanide

is:

$K_D = 1.943 \pm 2.819 \times 10^{-19}$

$\frac{1}{K_D} = \beta_2 = 5.148 \times 10^{18}$
Derivation of an equation used for determining the cumulative dissociation constant of the hexacyanoferrate (II) and hexacyanoferrate (III) complex ions

<table>
<thead>
<tr>
<th>Formation Reactions</th>
<th>Formation Constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Fe}^{++} + 6\text{CN}^- \rightleftharpoons \text{Fe(CN)}_6^{4-} )</td>
<td>( \beta_{6\text{II}}^{4-} = 10^{35.4} = 2.5119 \times 10^{35} )</td>
</tr>
<tr>
<td>( \text{Fe}^{+++} + 6\text{CN}^- \rightleftharpoons \text{Fe(CN)}_6^{3-} )</td>
<td>( \beta_{6\text{III}}^{3-} = 10^{36.8} = 6.3096 \times 10^{36} )</td>
</tr>
</tbody>
</table>

Iron mainly forms two complexes with cyanide, \( \text{Fe(CN)}_6^{4-} \) and \( \text{Fe(CN)}_6^{3-} \), and Adamson, Welker and Volpe (1950) have postulated that the concentrations of intermediates like \( \text{Fe(CN)}_5^{-2} \), \( \text{Fe(CN)}_4^{-1} \), and \( \text{Fe(CN)}_3^{-3} \) must be very small.

The following derivation is for the hexacyanoferrate (II) complex ion, but the same procedure and equations are applicable to solutions containing the hexacyanoferrate (III) complex ion.

We can therefore assume:

\[
\begin{align*}
\text{Total Cyanide (CN}_T\text{)} &= \text{CN}^- + \text{HCN} + 6\text{Fe(CN)}_6^{4-} \\
\text{Total Iron (Fe}_T\text{)} &= \text{Fe}^{++} + \text{Fe(CN)}_6^{4-}
\end{align*}
\]

\[
\text{Fe(CN)}_6^{4-} \rightleftharpoons \text{Fe}^{++} + 6\text{CN}^- \quad \frac{1}{\beta_{6\text{II}}} = K_D = \frac{(\text{Fe}^{++})(\text{CN}^-)^6}{(\text{Fe(CN)}_6^{4-})}
\]
With \( X = 6Y \), then

\[
(\text{Fe}^{\text{++}}) = (Y - \frac{6Y}{6}) + \frac{(\text{HCN})+(\text{CN}^-)}{6}
\]

\[
(\text{Fe}^{\text{++}}) = \frac{(\text{HCN})+(\text{CN}^-)}{6}
\]

\[
K_D = \frac{\left(\frac{\text{K}_a(\text{HCN})}{(\text{HCN})+\text{CN}^-}\right)^6}{\left(\frac{\text{K}_a(\text{HCN})}{(\text{HCN})+\text{CN}^-}\right)^6}
\]

\[
(\text{Fe}^{\text{++}}) = \frac{\text{K}_a(\text{HCN})}{(\text{HCN})+\text{CN}^-}
\]

\[
K_D = \frac{\text{K}_a(\text{HCN})}{(\text{HCN})+\text{CN}^-}
\]

\[
K_D = \frac{(\text{K}_a(\text{HCN})}{(\text{HCN})+\text{CN}^-}
\]

\[
(\text{Fe}^{\text{++}}) = \frac{\text{K}_a(\text{HCN})}{(\text{HCN})+\text{CN}^-}
\]

\[
K_D = \frac{(\text{K}_a(\text{HCN})}{(\text{HCN})+\text{CN}^-}
\]
The average $K_D$ values calculated during this research for hexacyanoferrate (II) and (III) complex solutions having a cyanide to iron molar ratio of 6 to 1, and with pH and total cyanide concentrations ranging between:

- pH 6.8 - 5.0 mg/l Total Cyanide
- pH 7.1 - 500 mg/l Total Cyanide

are:

$K_{D(II)}$ is approximately $10^{-47}$

$K_{D(III)}$ is approximately $10^{-52}$
APPENDIX F

Derivation of equations used for determining the formation constant of the dicyanocuprate (I) complex (Cu(CN)$_2^-$), and the concentration of various copper and cyanide species in solutions of cuprocyanide formation in which the cyanide to copper molar ratio is 2 to 1.

Values from the literature for the stepwise formation constants of cuprocyanide complex ionic species are as follows:

\[ \beta_2 = 10^{23.721} = 5.26 \times 10^{23} \]
Vladimirova and Kakovskii @ 25°C (1950)

\[ \beta_2 = 10^{23.301} = 2.00 \times 10^{23} \]
Penneman and Jones @ 25°C (1956)

\[ \beta_2 = 10^{21.7} = 5.0119 \times 10^{21} \]
Rothbaum @ 20°C (1957)

\[ K_3 = 10^{4.6} = 3.9811 \times 10^4 \]
Rothbaum @ 20°C (1957)

\[ K_3 = 10^{5.3424} = 2.2 \times 10^5 \]
Baxendale and Westcott @ 25°C (1959)

\[ K_4 = 10^{2.3} = 1.9953 \times 10^2 \]
Rothbaum @ 20°C (1957)

\[ K_4 = 10^{1.7404} = 5.5 \times 10^1 \]
Baxendale and Westcott @ 25°C (1959)
It should also be noted that:

\[
\frac{(H^+)(CN^-)}{(HCN)} = 4.365 \times 10^{-10} \quad \text{Izatt et al. @ 20°C (1962)}
\]

\[
K_{soCuCN} = (Cu^+)(CN^-) = 3.162 \times 10^{-20} \quad \text{Kolthoff et al. (1969)}
\]

Cuprocyanide complex test solutions were prepared by adding CuCN(s) and NaCN in the cyanide to copper molar ratio of 2 to 1.

It is assumed that:

Total Cyanide \( (\text{CN}_T) \) = \( \text{CN}^- + \text{HCN} + 2\text{Cu(CN)}_2^- + 3\text{Cu(CN)}_3^- + 4\text{Cu(CN)}_4^- + \text{CuCN(s)} \)

Total Copper \( (\text{Cu}_T) \) = \( \text{Cu}^+ + \text{Cu(CN)}_2^- + \text{Cu(CN)}_3^- + \text{Cu(CN)}_4^- + \text{CuCN(s)} \)

It should be noted that the proposed budgets consist of heterogeneous systems containing aqueous and undissolved copper species. It is not appropriate to think of CuCN(s) in mole per liter quantities but rather in total gram moles per solution. However, if we reduce our system to one liter and think of all species in terms of gram molar quantities the proposed derivation is still consistent.

We know that \( \frac{1}{2} \text{CN}_T = \text{Cu}_T \) and therefore:

\[
\frac{\text{CN}^- + \text{HCN} + 2\text{Cu(CN)}_2^- + 3\text{Cu(CN)}_3^- + 4\text{Cu(CN)}_4^- + \text{CuCN(s)}}{2} = \text{Cu}^+ + \text{Cu(CN)}_2^- + \text{Cu(CN)}_3^- + \text{Cu(CN)}_4^- + \text{CuCN(s)}
\]
\[
\frac{\text{CN}^- + \text{HCN}}{2} + \text{Cu(CN)}^- + 1.5\text{Cu(CN)}^2 + 2\text{Cu(CN)}^3 + 0.5\text{CuCN(s)}
\]

\[
= \text{Cu}^+ + \text{Cu(CN)}^{-2} + \text{Cu(CN)}^3 + \text{Cu(CN)}^4 + \text{CuCN(s)}
\]

\[
\frac{\text{CN}^- + \text{HCN}}{2} + 0.5\text{Cu(CN)}^{-2} + \text{Cu(CN)}^3 + 0.5\text{CuCN(s)} = \text{Cu}^+
\]

In Table 11, Section 2 to 1, it can be seen that the pertinent calculated \(\text{CN}^-\) molar concentrations range between \(9.50 \times 10^{-9}\) and \(1.98 \times 10^{-7}\). Therefore, from the solubility product for \(\text{CuCN}\) the maximum \(\text{Cu}^+\) molar concentration is calculated to range between \(3.33 \times 10^{-12}\) and \(1.60 \times 10^{-13}\). Because the \(\text{Cu}^+\) concentration is very small it can be neglected in the above equation. Therefore:

\[
\frac{\text{CN}^- + \text{HCN}}{2} = 0.5\text{CuCN(s)} - 0.5\text{Cu(CN)}^{-2} - \text{Cu(CN)}^{-3}
\]

By using the presently accepted stepwise formation constants for the cuprocyanide complexes and assuming a \(\text{CN}^-\) molar concentration ranging between \(9.50 \times 10^{-9}\) and \(1.98 \times 10^{-7}\), it can be calculated that as a first approximation the \(\text{Cu(CN)}^{-3}\) molar concentration is negligible when compared with the \(\text{Cu(CN)}^2\) and \(\text{Cu(CN)}^3\) molar concentrations. Therefore:

\[
\text{CN}^- + \text{HCN} = \text{CuCN(s)} - \text{Cu(CN)}^{-2}
\]

Assuming:

\[
\frac{(\text{Cu(CN)}^{-2})}{(\text{Cu(CN)}^{-2})(\text{CN}^-)} = 2.2 \times 10^5
\]
The denominator of the expression used to calculate the Cu(CN)$_2^-$ concentration is approximately equal to 1 for all calculated CN$^-$ concentrations ranging between $9.50 \times 10^{-9}$ and $1.98 \times 10^{-7}$. Therefore, even a significant error in the $K_3$ formation constant would have a nearly negligible effect on calculated Cu(CN)$_2^-$ concentrations.

Derivation of the equation used to calculate $K_{DCu(CN)_2^-}$ values is as follows:

$$\frac{1}{\beta_{2Cu(CN)_2^-}} = K_{DCu(CN)_2^-} \frac{(Cu^+)(CN^-)^2}{(Cu(CN)_2^-)^2} = \frac{(Cu^+)(CN^-)^2}{CN_T - Cu_T - CN^- - HCN}$$

$$= \frac{CN_T - Cu_T - CN^- - HCN}{1 + 4.4 \times 10^5(CN^-)}$$

Then:

$$CN_T = CN^- + HCN + 2Cu(CN)_2^- + 3[(2.2 \times 10^5)(Cu(CN)_2^-)(CN^-)] + CuCN(s)$$

$$Cu_T = Cu(CN)_2^- + (2.2 \times 10^5)(Cu(CN)_2^-)(CN^-) + CuCN(s)$$

$$CN_T - Cu_T = CN^- + HCN + Cu(CN)_2^- + 2[(2.2 \times 10^5)(Cu(CN)_2^-)(CN^-)]$$

$$Cu(CN)_2^- = \frac{CN_T - Cu_T - CN^- - HCN}{1 + 4.4 \times 10^5(CN^-)}$$

$$Cu(CN)_3^- = (Cu(CN)_2^-)(CN^-)2.2 \times 10^5$$

$$CuCN(s) = Cu_T - Cu(CN)_2^- - Cu(CN)_3^-$$

$$CuCN(s) = CN^- + HCN + Cu(CN)_3^-$$
In the presence of \( \text{CuCN(s)} \):

\[
(Cu^+)(CN^-) = 3.162 \times 10^{-20}
\]

Therefore

\[
K_{\text{DCu(CN)}_2}^- = \frac{[(3.162 \times 10^{-20})^2][1 + 4.4 \times 10^5(CN^-)]}{CN_T - Cu_T - CN^- - HCN}
\]

\[
= \frac{(3.162 \times 10^{-20})(CN^-) + (1.3913 \times 10^{-14})(CN^-)^2}{CN_T - Cu_T - CN^- - HCN}
\]

In NaCN - CuCN formation test solutions where the CN to Cu molar ratio is 2 to 1, \( CN_T = 2Cu_T \) and therefore the term \( CN_T - Cu_T \) is equal to \( 2Cu_T - Cu_T = Cu_T \).

\[
K_{\text{DCu(CN)}_2}^- = \frac{CN^-\left(3.162 \times 10^{-20} + 1.3913 \times 10^{-14}(CN^-)\right)}{Cu_T - CN^- - HCN}
\]

The average calculated \( K_{\text{DCu(CN)}_2}^- \) value for cuprocyanide complex test solutions in which the CN to Cu molar ratio was 2 to 1, and the total cyanide and pH ranged between 4.5 to 100 mg/l and 6.5 to 7.7, respectively, was:

\[
K_{\text{DCu(CN)}_2}^- = 3.943 \pm 1.752 \times 10^{-24}
\]

\[
\beta_2Cu(CN)^-_2 = 2.536 \times 10^{23}
\]
APPENDIX G

Derivation of equations used for determining the $K_3$ formation constant, and the concentration of various copper and cyanide species in solutions of cuprocyanide formation in which the cyanide to copper molar ratio is 2.5 to 1.

Values from the literature for the stepwise formation constants of cuprocyanide complex ionic species, the dissociation constant for HCN, and the solubility product for CuCN are presented in the first page of Appendix F.

Cuprocyanide complex test solutions were prepared by adding CuCN(s) and NaCN in the cyanide to copper molar ratio of 2.5 to 1.

By reference to Table 11, Section 2.5 to 1, it can be seen that the calculated CN$^-$ molar concentration ranges between $9.30 \times 10^{-9}$ and $4.23 \times 10^{-7}$. Therefore, from the solubility product for CuCN the maximum Cu$^+$ molar concentration is calculated to range between $3.40 \times 10^{-12}$ and $7.47 \times 10^{-14}$.

Previous results and calculations presented in Table 11 and Appendix F show that the $1/\beta_2$ dissociation constant for Cu(CN)$_2^-$ determined during this research is equal to $3.94 \times 10^{-24}$. Calculations using this dissociation constant, and maximum Cu$^+$ and corresponding CN$^-$ molar concentrations show that the maximum Cu(CN)$_2^-$ concentration must range between $7.46 \times 10^{-5}$ and $3.40 \times 10^{-3}$ mole per liter.

Reference to Table 11 shows that the calculated Cu(CN)$_2^-$ maximum molar concentrations are greater than the initial total copper concentrations in corresponding test solutions with the two respective minimum and maximum CN$^-$ concentrations.

Since the Cu(CN)$_2^-$ ion concentrations must be less than the above
calculated maximum values, the Cu$^+$ ion concentrations are also less than the values determined from the solubility product for CuCN and the appropriate calculated CN$^-$ concentrations. Therefore, because the solubility product for CuCN is not exceeded in cuprocyanide test solutions where the CN to Cu molar ratio is 2.5 to 1, no undissolved CuCN(s) can be remaining at the time of the various HCN determinations.

It is assumed that:

$$\text{Total Cyanide (CN}_T\text{)} = \text{CN}^- + \text{HCN} + 2\text{Cu(CN)}_2^- + 3\text{Cu(CN)}_3^- + 4\text{Cu(CN)}_4^-$$

$$\text{Total Copper (Cu}_T\text{)} = \text{Cu}^+ + \text{Cu(CN)}_2^- + \text{Cu(CN)}_3^- + \text{Cu(CN)}_4^-$$

We know that \( \frac{1}{2.5} \text{CN}_T = \text{Cu}_T \) and therefore:

$$\frac{\text{CN}^- + \text{HCN} + 2\text{Cu(CN)}_2^- + 3\text{Cu(CN)}_3^- + 4\text{Cu(CN)}_4^-}{2.5} = \text{Cu}^+ + \text{Cu(CN)}_2^- + \text{Cu(CN)}_3^- + \text{Cu(CN)}_4^-$$

$$\frac{\text{CN}^- + \text{HCN}}{2.5} + 0.8\text{Cu(CN)}_2^- + 1.2\text{Cu(CN)}_3^- + 1.6\text{Cu(CN)}_4^- = \text{Cu}^+ + \text{Cu(CN)}_2^- + \text{Cu(CN)}_3^- + \text{Cu(CN)}_4^-$$

$$\frac{\text{CN}^- + \text{HCN}}{2.5} - 0.2\text{Cu(CN)}_2^- + 0.2\text{Cu(CN)}_3^- + 0.6\text{Cu(CN)}_4^- = \text{Cu}^+$$

Because the Cu$^+$ concentration is less than 3.40 x 10$^{-12}$ in all cases, it can be neglected in the above equation. Therefore:

$$\frac{\text{CN}^- + \text{HCN}}{2.5} = 0.2\text{Cu(CN)}_2^- - 0.2\text{Cu(CN)}_3^- - 0.6\text{Cu(CN)}_4^-$$
\[
\text{CN}^- + \text{HCN} = 0.5\text{Cu(CN)}_2^- - 0.5\text{Cu(CN)}_3^- - 1.5\text{Cu(CN)}_4^-
\]

By using the presently accepted stepwise formation constants for the cuprocyanide complexes and assuming a \(\text{CN}^-\) molar concentration ranging between \(9.30 \times 10^{-9}\) and \(4.23 \times 10^{-7}\), it can be calculated that as a first approximation the \(\text{Cu(CN)}_4^-\) molar concentration is negligible when compared with the \(\text{Cu(CN)}_2^-\) and \(\text{Cu(CN)}_3^-\) molar concentrations. Therefore:

\[
\text{CN}^- + \text{HCN} = 0.5\text{Cu(CN)}_2^- - 0.5\text{Cu(CN)}_3^-
\]

Assuming that \(\text{Cu}^+\) and \(\text{Cu(CN)}_4^-\) are negligible with \(\text{CuCN(s)}\) not present, it is proposed that:

Total Cyanide \((\text{CNT})\) = \(\text{CN}^- + \text{HCN} + 2\text{Cu(CN)}_2^- + 3\text{Cu(CN)}_3^-\)

Total Copper \((\text{CuT})\) = \(\text{Cu(CN)}_2^- + \text{Cu(CN)}_3^-\)

\(2\text{Cu(CN)}_2^- = \text{CNT} - \text{CN}^- - \text{HCN} - 3\text{Cu(CN)}_3^-\)

\(\text{Cu(CN)}_3^- = \text{CuT} - \text{Cu(CN)}_2^-\)

\(2\text{Cu(CN)}_2^- = \text{CNT} - \text{CN}^- - \text{HCN} - 3(\text{CuT} - \text{Cu(CN)}_2^-)\)

\(2\text{Cu(CN)}_2^- = \text{CNT} - \text{CN}^- - \text{HCN} - 3\text{CuT} + 3\text{Cu(CN)}_2^-\)

\(\text{Cu(CN)}_2^- = 3\text{CuT} + \text{CN}^- + \text{HCN} - \text{CNT}\)

We have defined the system so that \(\text{CNT} = 2.5\text{CuT}\).

Therefore:

\(\text{Cu(CN)}_2^- = 3\text{CuT} + \text{CN}^- + \text{HCN} - 2.5\text{CuT} = 0.5\text{CuT} + \text{CN}^- + \text{HCN}\)
In summary it can be stated that:

\[
\begin{align*}
\text{Cu}(CN)_2^- &= 0.5\text{Cu}_T + \text{CN}^- + \text{HCN} \\
\text{Cu}(CN)_3^{2-} &= \text{Cu}_T - \text{Cu}(CN)_2^- \\
\text{Cu}^+ &= \frac{(3.94 \times 10^{-24})(\text{Cu}(CN)_2^-)}{\text{CN}^-} \\
\end{align*}
\]

Another formula that can be used to calculate the \(\text{Cu}^+\) molar concentration is derived as follows:

It is assumed that:

Total Copper \(\text{Cu}_T\) = \(\text{Cu}^+ + \text{Cu}(CN)_2^- + \text{Cu}(CN)_3^{2-} + \text{Cu}(CN)_4^{3-}\)

Total Cyanide \(\text{CN}_T\) = \(\text{CN}^- + \text{HCN} + 2\text{Cu}(CN)_2^- + 3\text{Cu}(CN)_3^{2-} + 4\text{Cu}(CN)_4^{3-}\)

Therefore:

\[
\begin{align*}
\text{Cu}_T &= \text{Cu}^+ + (\text{Cu}^+)(\text{CN}^-)^2\beta_2 + (\text{Cu}(CN)_2^-)(\text{CN}^-)K_3 + (\text{Cu}(CN)_3^{2-})(\text{CN}^-)K_4 \\
\text{Cu}_T &= \text{Cu}^+ + (\text{Cu}^+)(\text{CN}^-)^2\beta_2 + (\text{Cu}^+)(\text{CN}^-)^2(\text{CN}^-)\beta_2K_3 \\
&\quad + (\text{Cu}(CN)_2^-)(\text{CN}^-)(\text{CN}^-)K_3K_4 \\
\text{Cu}_T &= \text{Cu}^+ + (\text{Cu}^+)(\text{CN}^-)^2\beta_2 + (\text{Cu}^+)(\text{CN}^-)^3\beta_2K_3 + (\text{Cu}^+)(\text{CN}^-)^4\beta_2K_3K_4 \\
\text{Cu}_T &= [1 + (\text{CN}^-)^2\beta_2 + (\text{CN}^-)^3\beta_2K_3 + (\text{CN}^-)^4\beta_2K_3K_4]\text{Cu}^+ \\
\text{Cu}^+ &= \frac{\text{Cu}_T}{1 + (\text{CN}^-)^2\beta_2 + (\text{CN}^-)^3\beta_2K_3 + (\text{CN}^-)^4\beta_2K_3K_4}
\end{align*}
\]
It is assumed that:

\[ \beta_2 = \frac{1}{3.943 \times 10^{-24}} = 2.536 \times 10^{23} \quad \text{(calculated in Appendix F)} \]

\[ K_3 = 2.2 \times 10^5 \quad \text{Baxendale and Westcott @ 25°C (1959)} \]

\[ K_4 = 5.5 \times 10^1 \quad \text{Baxendale and Westcott @ 25°C (1959)} \]

The Cu⁺ molar concentrations calculated by this method are approximately equal to the values obtained when the previous formula for Cu⁺ determination is used.

\[
\frac{1}{K_3} = \frac{(\text{Cu(CN)}_2^-\text{(CN)}^-)}{(\text{Cu(CN)}_3^-)} = \frac{(0.5\text{Cu}_T^-\text{CN}^-+\text{HCN})(\text{CN}^-)}{(\text{Cu}_T^-\text{Cu(CN)}_2^-)}
\]

\[
\frac{1}{K_3} = \frac{(0.5\text{Cu}_T^-\text{CN}^-+\text{HCN})(\text{CN}^-)}{(\text{Cu}_T^-0.5\text{Cu}_T^-\text{CN}^-\text{-HCN})}
\]

\[
\frac{1}{K_3} = \frac{(0.5\text{Cu}_T^-\text{CN}^-+\text{HCN})(\text{CN}^-)}{(0.5\text{Cu}_T^-\text{CN}^-\text{-HCN})}
\]
APPENDIX H

Derivation of equations used for determining the $K_3$ formation constant, and the concentration of various copper and cyanide species in solutions of cuprocyanide formation in which the cyanide to copper molar ratio is 3 to 1.

Values from the literature for the stepwise formation constants of cuprocyanide complex ionic species, the dissociation constant for HCN, and the solubility product for CuCN are presented in the first page of Appendix F.

Cuprocyanide complex test solutions were prepared by adding CuCN(s) and NaCN in the cyanide to copper molar ratio of 3 to 1.

By reference to Table 11, Section 3 to 1, it can be seen that the calculated CN$^-$ molar concentration ranges between $4.06 \times 10^{-8}$ and $1.30 \times 10^{-7}$. Therefore, from the solubility product for CuCN the maximum Cu$^+$ molar concentration is calculated to range between $7.79 \times 10^{-13}$ and $2.43 \times 10^{-13}$.

Previous results and calculations presented in Table 11 and Appendix F show that the $1/\beta_2$ dissociation constant for Cu(CN)$_2^-$ determined during this research is equal to $3.94 \times 10^{-24}$. Calculations using this dissociation constant, and maximum Cu$^+$ and corresponding CN$^-$ molar concentrations shown that the maximum Cu(CN)$_2^-$ concentration must range between $3.25 \times 10^{-4}$ and $1.04 \times 10^{-3}$ mole per liter.

Reference to Table 11 shows that the calculated Cu(CN)$_2^-$ maximum molar concentrations are greater than the initial total copper concentrations in corresponding test solutions with the two respective minimum and maximum CN$^-$ concentrations.

Since the Cu(CN)$_2^-$ ion concentrations must be less than the
above calculated maximum values, the Cu\(^+\) ion concentrations are also less than the values determined from the solubility product for CuCN and the appropriate calculated CN\(^-\) concentrations. Therefore, because the solubility product for CuCN is not exceeded in cupro-cyanide test solutions where the CN to Cu molar ratio is 3 to 1, no undissolved CuCN(s) can be remaining at the time of the various HCN determinations.

It is assumed that:

Total Cyanide (\(\text{CNT}_T\)) = CN\(^-\) + HCN + 2Cu(CN)\(^-\)_2 + 3Cu(CN)\(^-\)_3 + 4Cu(CN)\(^-\)_4

Total Copper (\(\text{CUT}_T\)) = Cu\(^+\) + Cu(CN)\(^-\)_2 + Cu(CN)\(^-\)_3 + Cu(CN)\(^-\)_4

We know that \(\frac{1}{3}\) \(\text{CNT}_T\) = \(\text{CUT}_T\) and therefore:

\[
\frac{\text{CN}^- + \text{HCN} + 2\text{Cu(CN)}^-_2 + 3\text{Cu(CN)}^-_3 + 4\text{Cu(CN)}^-_4}{3} = \text{Cu}^+ + \text{Cu(CN)}^-_2 + \text{Cu(CN)}^-_3 + \text{Cu(CN)}^-_4
\]

Because the Cu\(^+\) concentration is less than \(7.79 \times 10^{-13}\) in all cases, it can be neglected in the above equation. Therefore:

\[
\frac{\text{CN}^- + \text{HCN}}{3} = \frac{\text{Cu(CN)}^-_2}{3} - \frac{(\text{Cu(CN)}^-_3)}{3}
\]
CN\(^-\) + HCN = Cu(CN)\(^2-\) - Cu(CN)\(^4-\)

By using the presently accepted stepwise formation constants for the cuprocyanide complexes and assuming a CN\(^-\) molar concentration ranging between 4.06 \times 10^{-8} and 1.30 \times 10^{-7}, it can be calculated that as a first approximation the Cu(CN)\(^4-\) molar concentration is negligible when compared with the Cu(CN)\(^2-\) and Cu(CN)\(^3-\) molar concentrations. Therefore:

\[ CN^- + HCN = Cu(CN)_2^- \]

Assuming that Cu\(^+\) and Cu(CN)\(^4-\) are negligible with CuCN(s) not present, it is proposed that:

Total Cyanide \( (CN_T) \) = CN\(^-\) + HCN + 2Cu(CN)\(^2-\) + 3Cu(CN)\(^3-\)

Total Copper \( (Cu_T) \) = Cu(CN)\(^2-\) + Cu(CN)\(^3-\)

In summary it can be stated that:

\[
\begin{align*}
Cu(CN)_2^- &= CN^- + HCN \\
Cu(CN)_3^- &= Cu_T - Cu(CN)_2^- = Cu_T - CN^- - HCN \\
Cu^+ &= \frac{(3.9432 \times 10^{-24})(Cu(CN)_2^-)}{(CN^-)^2} \\
1 &= \frac{(Cu(CN)_2^-)(CN^-)}{(Cu(CN)_3^-)} = \frac{(CN^- + HCN)(CN^-)}{Cu_T - (CN^- + HCN)}
\end{align*}
\]
In Appendix G, a derivation is presented of another formula that can be used to calculate the Cu⁺ molar concentration. The formula is:

\[
\text{Cu}^+ = \frac{\text{Cu}_T}{1 + (\text{CN}^-)^2 \beta_2 + (\text{CN}^-)^3 \beta_2 K_3 + (\text{CN}^-)^4 \beta_2 K_3 K_4}
\]
APPENDIX I

Derivation of equations used for determining the concentration of various copper and cyanide species in solutions of cuprocyanide formation, in which the cyanide to copper molar ratio is 2, 2.5, or 3 to 1 and cupric (Cu++) and elemental copper (Cu°) are assumed to be present.

Values from the literature for the stepwise formation constants of cuprocyanide complex ionic species, the dissociation constant for HCN, and the solubility product for CuCN are presented in the first page of Appendix F.

Cuprocyanide complex test solutions were prepared by adding CuCN(s) and NaCN in the cyanide to copper molar ratio of 2, 2.5, or 3 to 1.

By reference to Table 11, Sections 2, 2.5, and 3 to 1, it can be seen that the calculated CN⁻ molar concentration, for test solutions assumed to contain cupric and elemental copper, ranges between 7.35 x 10⁻⁹ and 2.36 x 10⁻⁸. Therefore, from the solubility product for CuCN the maximum Cu⁺ molar concentration is calculated to range between 4.30 x 10⁻¹² and 1.34 x 10⁻¹².

Previous results and calculations presented in Table 11 and Appendix F show that the 1/β₂ dissociation constant for Cu(CN)₂⁻ determined during this research is equal to 3.94 x 10⁻²⁴. Calculations using this dissociation constant, and maximum Cu⁺ and corresponding CN⁻ molar concentrations show that the maximum Cu(CN)₂⁻ concentration must range between 5.90 x 10⁻⁵ and 1.90 x 10⁻⁴ mole per liter.

Reference to Table 11 shows that the calculated Cu(CN)₂⁻ maximum molar concentrations are greater than the initial total copper concentrations in corresponding test solutions with the two respective
minimum and maximum CN\(^-\) concentrations.

Since the Cu(CN)\(_2\)\(^-\) ion concentrations must be less than the above calculated maximum values, the Cu\(^+\) ion concentrations are also less than the values determined from the solubility product for CuCN and the appropriate calculated CN\(^-\) concentrations. Therefore, because the solubility product for CuCN is not exceeded in certain cuprocyanide test solutions where the CN to Cu molar ratio is 2, 2.5, or 3 to 1, no undissolved CuCN(s) can be remaining at the time of the various HCN determinations.

If we assume the presently accepted stepwise formation constants for Cu(CN)\(_2\)\(^-\) + CN\(^-\) = Cu(CN)\(_3\)\(^-\) \(K_3 = 2.2 \times 10^5\) and for Cu(CN)\(_3\)\(^-\) + CN\(^-\) = Cu(CN)\(_4\)\(^-\) \(K_4 = 5.5 \times 10^1\), then by calculation it can be shown that within the CN\(^-\) molar concentration range of 2.36 x 10\(^{-8}\) to 7.35 x 10\(^{-9}\) the Cu(CN)\(_2\)\(^-\) molar concentration varies from 192 to 618 and from 1.48 x 10\(^8\) to 1.53 x 10\(^9\) times as great as the Cu(CN)\(_3\)\(^-\) and Cu(CN)\(_4\)\(^-\) molar concentrations, respectively.

Because the Cu\(^+\) molar concentration was calculated to be less than 4.30 x 10\(^{-12}\) and since the Cu(CN)\(_2\)\(^-\) molar concentration is assumed to be considerably larger than that of the Cu(CN)\(_3\)\(^-\) and Cu(CN)\(_4\)\(^-\) ions, the Cu\(^+\), Cu(CN)\(_3\)\(^-\), and Cu(CN)\(_4\)\(^-\) ions are neglected from the total cyanide and total copper budgets.

Reference to Table 11 will show that at the determined HCN equilibrium concentrations, the difference between the total cyanide and CN\(^-\) plus HCN molar concentrations \((CT - (CN^- + HCN))\) is, on a liter basis, less than the initial gram moles of CuCN(s). Therefore, since there is no CuCN(s) remaining at HCN equilibrium a significant amount of Cu\(^+\) is released into the solution with the remaining CuCN(s) reacting with cyanide to form Cu(CN)\(_2\)\(^-\).

The excess unbound copper ion released into these solutions must be present in some form other than the Cu\(^+\) ion. Copper (I) is
not stable in aqueous solution and it is likely that some of the Cu\(^+\) ions transfer electrons to other Cu\(^+\) ions. Disproportionation (self oxidation-reduction) is known to occur, with the formation of elemental copper and cupric ion (Sienko and Plane, 1966).

The cell reaction is given by:

\[
\begin{align*}
\text{Reaction} & \quad \text{Standard Electrode Potentials} \\
\text{Cu}^+ + e^- = \text{Cu}^0 & \quad \text{at } 25^\circ \text{C} \\
\text{Cu}^{++} + e^- = \text{Cu}^+ & \\
\hline
\text{Cu}^+ + \text{Cu}^{++} & = \text{Cu}^0 + \text{Cu}^{+} \\
\hline
\end{align*}
\]

\[
E_{\text{cell}} = E_{\text{reduction}} - E_{\text{oxidation}}
\]

\[
E_{\text{cell}} = 0.521 - 0.153 \quad \text{E}_{\text{cell}} = 0.368
\]

\[
\Delta G = -nFE = -(1 \text{ g-equiv.})(96,484 \text{ coulomb/g-equiv.)}(0.368 \text{ volt})/4.184 \text{ Joules/cal.}
\]

\[
\Delta G = 8.4862 \times 10^3 \text{ calories}
\]

\[
E = \frac{RT}{nF} \ln K = \frac{0.0591}{n} \log K
\]

\[
\log K = \frac{E_n}{0.0591} = \frac{0.368(1)}{0.0591} = 6.2267
\]

\[
K = \frac{(\text{Cu}^{++})}{(\text{Cu}^+)^2} = 1.6854 \times 10^6
\]
Because the $\text{Cu}^+$ molar concentration must be less than $4.30 \times 10^{-12}$, the $\text{Cu}^{++}$ molar concentration, calculated from the above equilibrium constant for the cell reaction, is less than $3.12 \times 10^{-17}$. Therefore, the excess unbound $\text{Cu}^+$ ion released into solutions in which the above derivation is applicable must be present as elemental copper or some other copper species.

It is assumed that:

Total Cyanide ($\text{CN}_T^-$) = $\text{CN}^- + \text{HCN} + 2\text{Cu(CN)}_2^-$

Total Copper ($\text{Cu}_T^-$) = $\text{Cu} + \text{Cu(CN)}_2^-$

Therefore:

$$\text{Cu} = \frac{\text{CN}_T^--\text{CN}^-\text{HCN}}{2}$$

$$\text{Cu} = \frac{\text{CN}_T^-}{2} + \frac{(\text{CN}^-+\text{HCN})}{2}$$

2 to 1 Ratio: $\frac{\text{CN}_T^-}{2} = \text{Cu}_T^-$

$$\frac{\text{CN}^-+\text{HCN}+2\text{Cu(CN)}_2^-}{2} = \text{Cu} + \text{Cu(CN)}_2^-$$

or

$$\text{Cu} = \frac{\text{CN}^-+\text{HCN}}{2}$$

2.5 to 1 Ratio: $\frac{\text{CN}_T^-}{2.5} = \text{Cu}_T^-$

$$\frac{\text{CN}^-+\text{HCN}+2\text{Cu(CN)}_2^-}{2.5} = \text{Cu} + \text{Cu(CN)}_2^-$$

$$\frac{\text{CN}^-+\text{HCN}}{2.5} + 0.8\text{Cu(CN)}_2^- = \text{Cu} + \text{Cu(CN)}_2^-$$
\[ \text{Cu} = \frac{\text{CN}^- + \text{HCN}}{2.5} - 0.2\text{Cu(CN)}_2^- \]

3 to 1 Ratio: \( \frac{\text{CN}_T}{3} = \text{Cu}_T \)

\[ \frac{\text{CN}^- + \text{HCN} + 2\text{Cu(CN)}_2^-}{3} = \text{Cu} + \text{Cu(CN)}_2^- \]

\[ \frac{\text{CN}^- + \text{HCN}}{3} + \frac{2}{3} \text{Cu(CN)}_2^- = \text{Cu} + \text{Cu(CN)}_2^- \]

\[ \text{Cu} = \frac{\text{CN}^- + \text{HCN} - \text{Cu(CN)}_2^-}{3} \]
APPENDIX J

Procedure for determining the total free cyanide required to produce the desired HCN concentrations for NaCN experiments

\[
K_a = \frac{(H^+)(CN^-)}{(HCN)}
\]

Let \( (H^+) = A \)
\( (CN^-) = B \)
\( (HCN) = C \)

\[
\frac{A \cdot B}{C} = K_a; \quad C = \frac{A \cdot B}{K_a}; \quad C = \frac{B}{K_a / A}
\]

Let \( Y = K_a / A \). Then \( C = \frac{B}{Y} \) or \( B = CY \); therefore

\[
C + B = C + CY
\]

\[
= C(1+Y)
\]

\[
C = \frac{C+B}{1+Y} = \frac{C+B}{1+K_a / A}
\]

Substituting we see:

\[
(HCN) = \frac{(HCN) + (CN^-)}{1+K_a / (H^+)}
\]

But \( (HCN) + (CN^-) = \) total free cyanide

Therefore

\[
(HCN) + (CN^-) = (HCN) + \frac{(HCN)K_a}{(H^+)}
\]

According to Izatt et al. (1962), the \( K_a @ 20^\circ C = 4.365 \times 10^{-10} \)

0.20 mg/l HCN = \( 7.40 \times 10^{-6} \) M  Mol. Wt. HCN = 27.0259

0.05 mg/l HCN = \( 1.85 \times 10^{-6} \) M  CN = 26.0179
<table>
<thead>
<tr>
<th>pH</th>
<th>$\text{H}^+$</th>
<th>HCN</th>
<th>(HCN)$K_a$</th>
<th>Molarity of total free cyanide</th>
<th>Total cyanide (mg/l CN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>31.62</td>
<td>7.40</td>
<td>1.022</td>
<td>7.410</td>
<td>0.1928</td>
</tr>
<tr>
<td>7.1</td>
<td>7.943</td>
<td>7.40</td>
<td>4.067</td>
<td>7.441</td>
<td>0.1936</td>
</tr>
<tr>
<td>7.1</td>
<td>7.943</td>
<td>1.85</td>
<td>1.017</td>
<td>1.860</td>
<td>0.0484</td>
</tr>
<tr>
<td>7.7</td>
<td>1.995</td>
<td>7.40</td>
<td>16.19</td>
<td>7.562</td>
<td>0.1967</td>
</tr>
</tbody>
</table>
APPENDIX K

Procedure for calculating the total cyanide concentration required to produce the desired HCN concentration for nickel-cyanide complex solutions at a defined pH value and where the cyanide to nickel molar ratio is 4 to 1

I. Assume: HCN concentration is 0.2938 mg/l or $1.0872 \times 10^{-5}$ M
pH = 6.5 or $(H^+) = 3.162 \times 10^{-7}$ M

$$\frac{(Ni^{++})(CN^-)^4}{(Ni(CN)_4^{2-})} = 6.6303 \times 10^{-32}$$

(Table 4, pH 6.5; $0.50 - 0.551$ mg/l CN$_T$)

$$\frac{(H^+)(CN^-)}{(HCN)} = 4.365 \times 10^{-10}$$

(Izatt et al., 1962)

If $(HCN) = 1.0872 \times 10^{-5}$ M, then:

$$(CN^-) = \frac{(4.365 \times 10^{-10})(1.0872 \times 10^{-5})}{(3.162 \times 10^{-7})}$$

$$(CN^-) = 1.5008 \times 10^{-8}$$ M

$$(CN^-) + (HCN) = 1.5008 \times 10^{-8} + 1.0872 \times 10^{-5}$$

$$(CN^-) + (HCN) = 1.0887 \times 10^{-5}$$

From Appendix C we observe that:

$$\frac{(Ni^{++})}{4} = \frac{(CN^-)+(HCN)}{4} = \frac{1.0887 \times 10^{-5}}{4} = 2.7217 \times 10^{-6}$$ M

$$(CN^-)^4 = (1.5008 \times 10^{-8})^4 = 5.0736 \times 10^{-32}$$

Let $X = \text{equilibrium } (Ni(CN)_4^{2-})$
Therefore:

\[
\frac{(2.7217 \times 10^{-6})(5.0736 \times 10^{-32})}{x} = 6.6303 \times 10^{-32}
\]

\[
x = \frac{13.8089 \times 10^{-38}}{6.6303 \times 10^{-32}} = 2.0827 \times 10^{-6}
\]

Cyanide can be present as:
1. \( \text{Ni(CN)}_4^- \)
2. HCN
3. \( \text{CN}^- \)

Total Cyanide \( (CN_T) = (CN^-) + (HCN) + 4(\text{Ni(CN)}_4^-) \)

1. CN present as \( \text{Ni(CN)}_4^- \)

\[
(\text{Ni(CN)}_4^-) \rightarrow (\text{Ni}^{++}) + 4(\text{CN}^-)
\]

\[
2.0827 \times 10^{-6} \quad 8.3308 \times 10^{-6}
\]

\[
(8.3308 \times 10^{-6})(26.0179) = 0.21675 \times 10^{-3} \text{ g/l}
\]

\[
= 0.21675 \text{ mg/l CN}
\]

2. CN present as HCN

\[
(1.0872 \times 10^{-5})(26.0179) = 0.28286 \times 10^{-3} \text{ g/l}
\]

\[
= 0.28286 \text{ mg/l CN}
\]

3. CN present as \( \text{CN}^- \)

\[
(1.5008 \times 10^{-8})(26.0179) = 0.00039 \times 10^{-3} \text{ g/l}
\]

\[
= 0.00039 \text{ mg/l CN}
\]

Total Cyanide = 0.21675

0.28286
0.00039
0.50000 mg/l
The percentages of the total cyanide present as the respective cyanide species are as follows:

\[ \frac{0.21675}{0.5} \times 100 = 43.349\% \text{ present as } \text{Ni(CN)}_4^{-2} \]

\[ \frac{0.28286}{0.5} \times 100 = 56.573\% \text{ present as HCN} \]

\[ \frac{0.00039}{0.5} \times 100 = 0.078\% \text{ present as } \text{CN}^- \]

II. Assume: HCN concentration is 0.2580 mg/l or \(9.5474 \times 10^{-6}\) M

\( pH = 7.1 \text{ or } (H^+) = 7.943 \times 10^{-8}\) M

\[ \frac{(\text{Ni}^{++})(\text{CN}^-)^4}{(\text{Ni(CN)}_4^{-2})} = 7.6471 \times 10^{-32} \quad \text{(Table 4, pH 7.1; 50 mg/l CNT)} \]

\[ \frac{(H^+)(\text{CN}^-)}{(HCN)} = 4.365 \times 10^{-10} \quad \text{(Izatt et al., 1962)} \]

If \((\text{HCN}) = 9.5474 \times 10^{-6}\), then:

\[ (\text{CN}^-) = \frac{(4.365 \times 10^{-10})(9.5474 \times 10^{-6})}{(7.943 \times 10^{-8})} \]

\[ (\text{CN}^-) = 5.2467 \times 10^{-8}\) M

\[ (\text{CN}^-) + (\text{HCN}) = 5.2467 \times 10^{-8} + 9.5474 \times 10^{-6} \]

\[ (\text{CN}^-) + (\text{HCN}) = 9.5999 \times 10^{-6} \]

From Appendix C we observe that:

\[ \frac{(\text{Ni}^{++})}{4} = \frac{(\text{CN}^-) + (\text{HCN})}{4} = \frac{9.5999 \times 10^{-6}}{4} = 2.4000 \times 10^{-6}\) M
\[(\text{CN}^-)^4 = (5.2467 \times 10^{-8})^4 = 7.5777 \times 10^{30}\]

Let \(X\) = equilibrium \((\text{Ni(CN)}_4^-)^2\)

Therefore:

\[
\frac{(2.4000 \times 10^{-6})(7.5777 \times 10^{-30})}{X} = 7.6471 \times 10^{-32}
\]

\[X = \frac{1.8186 \times 10^{-35}}{7.6471 \times 10^{-32}} = 2.3782 \times 10^{-4}\]

Cyanide can be present as:
1. \(\text{Ni(CN)}_4^-\)
2. \(\text{HCN}\)
3. \(\text{CN}^-\)

Total Cyanide \((\text{CN}_T)\) = \((\text{CN}^-) + (\text{HCN}) + 4(\text{Ni(CN)}_4^-)^2\)

1. \(\text{CN present as \text{Ni(CN)}_4^-}\)

\[
(\text{Ni(CN)}_4^-)^2 \rightarrow \text{Ni}^{++} + 4(\text{CN}^-)
\]

\[2.3782 \times 10^{-4} \quad 9.5128 \times 10^{-4}\]

\[
(9.5128 \times 10^{-4})(26.0179) = 24.7502 \times 10^{-3} \text{ g/l}
\]

\[= 24.7502 \text{ mg/l CN}\]

2. \(\text{CN present as \text{HCN}}\)

\[
(9.5474 \times 10^{-6})(26.0179) = 0.2484 \times 10^{-3} \text{ g/l}
\]

\[= 0.2484 \text{ mg/l CN}\]
3. CN present as \( \text{CN}^- \)

\[
(5.2467 \times 10^{-8})(26.0179) = 0.0014 \times 10^{-3} \text{ g/l}
\]

\[
= 0.0014 \text{ mg/l CN}
\]

Total Cyanide = 24.7502

\[
\begin{array}{c}
0.2484 \\
0.0014 \\
25.0000 \text{ mg/l}
\end{array}
\]

The percentages of the total cyanide present as the respective cyanide species are as follows:

\[
\frac{24.7502}{25} \times 100 = 99.001\% \text{ present as } \text{Ni(CN)}_4^-^2
\]

\[
\frac{0.2484}{25} \times 100 = 0.994\% \text{ present as } \text{HCN}
\]

\[
\frac{0.0014}{25} \times 100 = 0.005\% \text{ present as } \text{CN}^-^3
\]

III. Assume: HCN concentration is 0.1813 mg/l or \( 6.7085 \times 10^{-6} \) M

\[\text{pH} = 7.7 \text{ or } (\text{H}^+) = 1.995 \times 10^{-8} \text{ M}\]

\[
\frac{(\text{Ni}^{++})(\text{CN}^-)_4^4}{(\text{Ni(CN)}^-_4^2)} = 1.6563 \times 10^{-31} \quad \text{(Table 4, pH 7.7; 500 mg/l CNT)}
\]

\[
\frac{(\text{H}^+)(\text{CN}^-)}{\text{HCN}} = 4.365 \times 10^{-10} \quad \text{(Izatt et al., 1962)}
\]

If \( (\text{HCN}) = 6.7085 \times 10^{-6} \) M, then:

\[
(\text{CN}^-) = \frac{(4.365 \times 10^{-10})(6.7085 \times 10^{-6})}{(1.995 \times 10^{-8})}
\]
(CN\(^{-}\)) = 1.4678 \times 10^{-7} \text{ M}

(CN\(^{-}\)) + (HCN) = 1.4678 \times 10^{-7} + 6.7085 \times 10^{-6}

(CN\(^{-}\)) + (HCN) = 6.8553 \times 10^{-6} \text{ M}

From Appendix C we observe that:

\[
(Ni^{++}) = \frac{(CN^{-}) + (HCN)}{4} = \frac{6.8553 \times 10^{-6}}{4} = 1.7138 \times 10^{-6} \text{ M}
\]

\[
(CN^{-})^4 = (1.4678 \times 10^{-7})^4 = 4.6416 \times 10^{-28}
\]

Let \( X = \text{equilibrium} \ (Ni(CN)_{4}^{-2}) \)

Therefore:

\[
\frac{(1.7138 \times 10^{-6})(4.6416 \times 10^{-28})}{X} = 1.6563 \times 10^{-31}
\]

\[
X = \frac{7.9549 \times 10^{-34}}{1.6563 \times 10^{-31}} = 4.8028 \times 10^{-3}
\]

Cyanide can be present as:
1. Ni(CN)\(_{4}^{-2}\)
2. HCN
3. CN\(^{-}\)

Total Cyanide (\(CN_{T}\)) = (CN\(^{-}\)) + (HCN) + 4(Ni(CN)\(_{4}^{-2}\))

1. CN present as Ni(CN)\(_{4}^{-2}\)

\[
(Ni(CN)_{4}^{-2}) \rightarrow (Ni^{++}) + 4(CN^{-}) = 4.8028 \times 10^{-3} \quad 1.9211 \times 10^{-2}
\]
(1.9211 \times 10^{-2})(26.0179) = 499.8312 \times 10^{-3} \text{ g/l}
= 499.8312 \text{ mg/l CN}

2. CN present as HCN

(6.7085 \times 10^{-6})(26.0179) = 0.1745 \times 10^{-3} \text{ g/l}
= 0.1745 \text{ mg/l CN}

3. CN present as CN$^-$

(1.4678 \times 10^{-7})(26.0179) = 0.0038 \times 10^{-3} \text{ g/l}
= 0.0038 \text{ mg/l CN}

Total Cyanide = 499.8312

\[
\begin{array}{c}
0.1745 \\
0.0038 \\
\hline
500.0095 \text{ mg/l}
\end{array}
\]

The percentages of the total cyanide present as the respective cyanide species are as follows:

\[
\frac{499.8312}{500} \times 100 = 99.964\% \text{ present as N}_4\text{Ni(CN)}^2
\]

\[
\frac{0.1745}{500} \times 100 = 0.035\% \text{ present as HCN}
\]

\[
\frac{0.0038}{500} \times 100 = 0.00\% \text{ present as CN}^-(7.64 \times 10^{-4})
\]
APPENDIX L

Procedure for calculating the HCN concentration produced for a given total cyanide concentration in nickel-cyanide complex solutions at a defined pH value and where the cyanide to nickel molar ratio is 4 to 1

I. Assume: Total cyanide concentration is 0.50 mg/l or
\[ 1.9218 \times 10^{-5} \text{ M, pH} = 6.5 \text{ or } (H^+) = 3.162 \times 10^{-7} \text{ M} \]

\[
\frac{(Ni^{++})(CN^-)^4}{(Ni(CN)_4)^2} = 6.6303 \times 10^{-32} \quad \text{(Table 4, pH 6.5; 0.50 - 0.551 mg/l CN_T)}
\]

\[
\frac{(H^+)(CN^-)}{(HCN)} = 4.365 \times 10^{-10} \quad \text{(Izatt et al., 1962)}
\]

\[
\frac{(CN^-)}{(HCN)} = \frac{4.365 \times 10^{-10}}{3.162 \times 10^{-7}} = 1.3805 \times 10^{-3}
\]

If \((HCN) = X\), then \((CN^-) = 1.3805 \times 10^{-3})X\)

\[
(CN^-) + (HCN) = (1.3805 \times 10^{-3})(X) + (X) = X(1.3805 \times 10^{-3} + 1) = X(1.0013805)
\]

\[
(Ni^{++}) = \frac{(CN^-) + (HCN)}{4} = \frac{X(1.0013805)}{4}
\]

Total cyanide is equal to: 1. CN as \(Ni(CN)_4^2\) 
2. CN as HCN 
3. CN as \(CN^-\)

Total Cyanide \((CN_T) = (CN^-) + (HCN) + 4(Ni(CN)_4)\)
\[(\text{Ni(CN)}_4^{2-}) = \frac{\text{CN}^-((\text{CN}^-) + (\text{HCN}))}{4} \]

\[= 1.9218 \times 10^{-5} - 1.0013805X \]

Therefore:

\[\frac{[(X)(1.0013805)][(1.3805 \times 10^{-3})(X)]^4}{4} = 6.6303 \times 10^{-32} \]

\[\frac{1.9218 \times 10^{-5} - 1.0013805X}{4} \]

\[\frac{[(X)(1.0013805)][(3.6316 \times 10^{-12})(X^4)]}{4} = 6.6303 \times 10^{-32} \]

\[\frac{1.9218 \times 10^{-5} - 1.0013805X}{4} \]

\[\frac{(X^5)(3.6366 \times 10^{-12})}{4} = 6.6303 \times 10^{-32} \]

\[\frac{1.9218 \times 10^{-5} - 1.0013805X}{4} \]

\[(X^5)(3.6366 \times 10^{-12}) = 1.2742 \times 10^{-36} - (6.6394 \times 10^{-32})X \]

\[(X^5)(3.6366 \times 10^{-12}) + (6.6394 \times 10^{-32})X = 1.2742 \times 10^{-36} \]

\[X = 1.0872 \times 10^{-5} \text{ M HCN = } 0.2938 \text{ mg/l HCN} \]

1. **CN present as CN⁻**

\[(1.3805 \times 10^{-3})(1.0872 \times 10^{-5}) = 1.5008 \times 10^{-8} \text{ M} \]

\[(1.5008 \times 10^{-8})(26.0179) = 0.00039 \times 10^{-3} \text{ g/l} \]

\[= 0.00039 \text{ mg/l CN} \]
2. CN present as HCN

\[(1.0872 \times 10^{-5})(26.0179) = 0.2829 \times 10^{-3} \text{ g/l} \]
\[= 0.2829 \text{ mg/l CN} \]

3. CN present as Ni(CN)\(_4\)^-^2^ \n
\[
\frac{(1.9218 \times 10^{-5}) - (1.0013805)(1.0872 \times 10^{-5})}{4} \\
= \frac{1.9218 \times 10^{-5} - 1.0887 \times 10^{-5}}{4} = \frac{8.3306 \times 10^{-6}}{4} \\
= 2.0826 \times 10^{-6} \text{ M Ni(CN)}_4^{-2} = 0.3390 \text{ mg/l Ni(CN)}_4^{-2} \\
\text{or } 8.3306 \times 10^{-6} \text{ M CN as Ni(CN)}_4^{-2} \\
(8.3306 \times 10^{-6})(26.0179) = 0.2167 \times 10^{-3} \text{ g/l} \\
= 0.2167 \text{ mg/l CN} \]

Total Cyanide = 0.00039
0.2829
0.2167
0.49999 mg/l CN

The percentages of the total cyanide present as the respective cyanide species are as follows:

\[
\frac{0.00039}{0.5} \times 100 = 0.078\% \text{ present as CN}^- \\
\frac{0.2829}{0.5} \times 100 = 56.573\% \text{ present as HCN} \\
\frac{0.2167}{0.5} \times 100 = 43.349\% \text{ present as Ni(CN)}_4^{-2} \\
\]
II. Assume: Total cyanide concentration is 25 mg/l or 
\[ 9.6088 \times 10^{-4} \text{ M}, \text{ pH} = 7.1 \text{ or } (H^+) = 7.943 \times 10^{-8} \text{ M} \]

\[
\frac{(Ni^{++})(CN^-)^4}{(Ni(CN)_2^2)} = 7.6471 \times 10^{-32} \quad \text{(Table 4, pH 7.1; 50 mg/l CN_T)}
\]

\[
\frac{(H^+)(CN^-)}{(HCN)} = 4.365 \times 10^{-10} \quad \text{[Izatt et al., 1962]}
\]

\[
\frac{(CN^-)}{(HCN)} = 4.365 \times 10^{-10} \div 7.943 \times 10^{-8} = 5.4954 \times 10^{-3}
\]

If \((HCN) = X\), then \((CN^-) = (5.4954 \times 10^{-3})X\)

\[
(CN^-) + (HCN) = (5.4954 \times 10^{-3})(X) + (X) = X(5.4954 \times 10^{-3} + 1) = X(1.0054954)
\]

\[
(Ni^{++}) = \frac{(CN^-)+(HCN)}{4} = X\frac{(1.0054954)}{4}
\]

Total cyanide is equal to: 1. CN as Ni(CN)_2^2
2. CN as HCN
3. CN as CN^-

Total Cyanide (CN_T) = (CN^-) + (HCN) + 4(Ni(CN)_2^2)

\[
(Ni(CN)_2^2) = \frac{CN_T - ((CN^-)+(HCN))}{4}
\]

\[
= \frac{9.6088 \times 10^{-4} - 1.0054954X}{4}
\]
Therefore:

\[
\frac{[X](1.0054954)\left[\frac{(5.4954 \times 10^{-3})(X)^4}{4}\right]}{9.6088 \times 10^{-4} - 1.0054954X} = 7.6471 \times 10^{-32}
\]

\[
\frac{[X](1.0054954)\left[\frac{(9.12005 \times 10^{-10})(X^4)}{4}\right]}{9.6088 \times 10^{-4} - 1.0054954X} = 7.6471 \times 10^{-32}
\]

\[
\frac{(X^5)(9.1702 \times 10^{-10})}{4} = 7.6471 \times 10^{-32}
\]

\[
(X^5)(9.1702 \times 10^{-10}) = 7.3479 \times 10^{-35} - (7.6891 \times 10^{-32})X
\]

\[
(X^5)(9.1702 \times 10^{-10}) + (7.6891 \times 10^{-32})X = 7.3479 \times 10^{-35}
\]

\[X = 9.5474 \times 10^{-6} \text{ M HCN} = 0.2580 \text{ mg/l HCN}\]

1. CN present as CN\(^{-}\):

\[
(5.4954 \times 10^{-3})(9.5474 \times 10^{-6}) = 5.2467 \times 10^{-8}
\]

\[
(5.2467 \times 10^{-8})(26.0179) = 0.00137 \times 10^{-3} \text{ g/l} = 0.00137 \text{ mg/l CN}
\]

2. CN present as HCN:

\[
(9.5474 \times 10^{-6})(26.0179) = 0.2484 \times 10^{-3} \text{ g/l} = 0.2484 \text{ mg/l CN}
\]
3. CN present as Ni(CN)$_4^{-2}$

\[
\frac{9.6088 \times 10^{-4} - (1.0054954)(9.5474 \times 10^{-6})}{4} = \frac{9.6088 \times 10^{-4} - 9.5999 \times 10^{-6}}{4} = \frac{9.5128 \times 10^{-4}}{4}
\]

2.3782 $\times 10^{-4}$ M Ni(CN)$_4^{-2}$ = 38.713 mg/l Ni(CN)$_4^{-2}$

or 9.5128 $\times 10^{-4}$ M CN as Ni(CN)$_4^{-2}$

\[
(9.5128 \times 10^{-4})(26.0179) = 24.7502 \times 10^{-3} \text{ g/l}
\]

= 24.7502 mg/l CN

Total Cyanide = 0.00137

0.2484

24.7502

24.99997 mg/l CN

The percentages of the total cyanide present as the respective cyanide species are as follows:

\[
\frac{0.00137}{25} \times 100 = 0.005\% \text{ present as } \text{CN}^{-}
\]

\[
\frac{0.2484}{25} \times 100 = 0.994\% \text{ present as } \text{HCN}
\]

\[
\frac{24.7502}{25} \times 100 = 99.001\% \text{ present as } \text{Ni(CN)}_{4}^{-2}
\]

III. Assume: Total cyanide concentration is 500 mg/l or

1.9218 $\times 10^{-2}$ M, pH = 7.7 or (H$^+$) = 1.995 $\times 10^{-8}$ M
\[
\frac{(\text{Ni}^{\text{++}})(\text{CN}^-)^4}{(\text{Ni}(\text{CN})_4^{\text{-2}})} = 1.6563 \times 10^{-31} \quad \text{(Table 4, pH 7.7; 500 mg/l } \text{CN}_T \text{)}
\]

\[
\frac{(\text{H}^+)(\text{CN}^-)}{(\text{HCN})} = 4.365 \times 10^{-10} \quad \text{(Izatt et al., 1962)}
\]

\[
\frac{(\text{CN}^-)}{(\text{HCN})} = \frac{4.365 \times 10^{-10}}{1.995 \times 10^{-8}} = 2.1880 \times 10^{-2}
\]

If \((\text{HCN}) = X\), then \((\text{CN}^-) = (2.1880 \times 10^{-2})X\)

\[
(\text{CN}^-) + (\text{HCN}) = (2.1880 \times 10^{-2})(X) + (X)
\]

\[
= X(2.1880 \times 10^{-2} + 1)
\]

\[
= X(1.021880)
\]

\[
(\text{Ni}^{\text{++}}) = \frac{(\text{CN}^-) + (\text{HCN})}{4} = \frac{X(1.021880)}{4}
\]

Total cyanide is equal to: 1. CN as \(\text{Ni}(\text{CN})_4^{\text{-2}}\)  
2. CN as HCN  
3. CN as \(\text{CN}^-\)

Total Cyanide \((\text{CN}_T) = (\text{CN}^-) + (\text{HCN}) + 4(\text{Ni}(\text{CN})_4^{\text{-2}})\)

\[
(\text{Ni}(\text{CN})_4^{\text{-2}}) = \frac{\text{CN}_T - ((\text{CN}^-) + (\text{HCN}))}{4}
\]

\[
= \frac{1.9218 \times 10^{-2}}{4} - 1.021880X
\]

Therefore:
\[
\left( \frac{(X)(1.021880)}{4} \right) \left( (2.1880 \times 10^{-2})(X)^4 \right) = \frac{1.6563 \times 10^{-31}}{1.9218 \times 10^{-2} - 1.021880X}
\]

\[
\left( \frac{(X)(1.021880)}{4} \right) \left( (22.9174 \times 10^{-8})(X^4) \right) = \frac{1.6563 \times 10^{-31}}{1.9218 \times 10^{-2} - 1.021880X}
\]

\[
\left( \frac{(X^5)(23.4188 \times 10^{-8})}{4} \right) = \frac{1.6563 \times 10^{-31}}{1.9218 \times 10^{-2} - 1.021880X}
\]

\[
(X^5)(2.3419 \times 10^{-7}) = 3.1830 \times 10^{-33} - (1.6926 \times 10^{-31})X
\]

\[
(X^5)(2.3419 \times 10^{-7}) + (1.6926 \times 10^{-31})X = 3.1830 \times 10^{-33}
\]

\[
X = 6.7085 \times 10^{-6} \text{ M HCN} = 0.1813 \text{ mg/l HCN}
\]

1. CN present as CN\(^{-}\)

\[
(2.1880 \times 10^{-2})(6.7085 \times 10^{-6}) = 1.4678 \times 10^{-7}
\]

\[
(1.4678 \times 10^{-7})(26.0179) = 0.00382 \times 10^{-3} \text{ g/l} = 0.00382 \text{ mg/l CN}
\]

2. CN present as HCN

\[
(6.7085 \times 10^{-6})(26.0179) = 0.1745 \times 10^{-3} \text{ g/l} = 0.1745 \text{ mg/l CN}
\]
3. CN present as $\text{Ni(CN)}_4^{-2}$

$$\frac{1.9218 \times 10^{-2} - (1.021880)(6.7085 \times 10^{-6})}{4}$$

$$= \frac{1.9218 \times 10^{-2} - 6.8553 \times 10^{-6}}{4} = \frac{1.92106 \times 10^{-2}}{4}$$

$$4.8027 \times 10^{-3} \text{M Ni(CN)}_4^{-2} = 781.8 \text{mg/l Ni(CN)}_4^{-2}$$

or $1.92106 \times 10^{-2} \text{M CN as Ni(CN)}_4^{-2}$

$$(1.92106 \times 10^{-2})(26.0179) = 499.8205 \times 10^{-3} \text{g/l}$$

$$= 499.8205 \text{mg/l CN}$$

Total Cyanide = 0.00382
0.1745
$\frac{499.8205}{500} = 499.99882 \text{mg/l CN}$

The percentages of the total cyanide present as the respective cyanide species are as follows:

$$\frac{0.00382}{500} \times 100 = 7.64 \times 10^{-4} \% \text{ present as CN}^{-1}$$

$$\frac{0.1745}{500} \times 100 = 0.035 \% \text{ present as HCN}$$

$$\frac{499.8205}{500} \times 100 = 99.964 \% \text{ present as Ni(CN)}_4^{-2}$$
APPENDIX M

Procedures for calculating the determined concentrations of accumulated $^{14}$C radioactivity (carbon from $^{14}$C-labeled cyanide), expressed as \( \mu g \) HCN per gram wet weight of tissue, in bluegills exposed to $^{14}$C-labeled NaCN and Ni(CN)$_2^-$ experimental solutions at various pH values and total cyanide concentrations.

**NaCN Solutions**

Example: pH 6.5 and 0.1928 mg/l total cyanide solution

Gill tissue of bluegill after 120 minutes of exposure (Table 15)

Test solution had 4,924.1 DPM/ml and 0.20 \( \mu g \) HCN per ml.

Therefore:

\[
\frac{4,924.1 \text{ DPM/ml}}{0.20 \mu g \text{ HCN/ml}} = 24,620 \text{ DPM/\( \mu g \) HCN}
\]

Because 99.86% of the total cyanide was calculated to be present as HCN (Table 14), the "HCN Specific Activity" of the test solution was:

\[
\frac{24,620 \text{ DPM}}{\mu g \text{ HCN}} \times \frac{99.86}{100} = 24,586 \text{ DPM/\( \mu g \) HCN}
\]

The gill tissue was determined to have 21,036 DPM/g.

Assuming that all the cyanide enters as HCN, then:

\[
\frac{21,036 \text{ DPM/g gill}}{24,586 \text{ DPM/\( \mu g \) HCN}} = 0.8556 \mu g \text{ HCN/g gill}
\]
Ni(CN)$_4^{2-}$ Solutions

Example: pH 7.7; 500 mg/l total cyanide and 0.1813 mg/l HCN

Blood of bluegill after 15 minutes of exposure (Table 16)

Test solution had 9,700.3 DPM/ml and 0.1813 µg HCN per ml. Therefore:

\[
\frac{9,700.3 \text{ DPM/ml}}{0.1813 \mu \text{g HCN/ml}} = 53,504.14 \text{ DPM/µg HCN}
\]

Because 0.0349% of the total cyanide was calculated to be present as HCN (Table 14), the "HCN Specific Activity" of the test solution was:

\[
\frac{53,504.14 \text{ DPM}}{\mu \text{g HCN}} \times \frac{0.0349}{100} = 18.677 \text{ DPM/µg HCN}
\]

The blood was determined to have 16.89 DPM/g. Assuming that all the cyanide enters as HCN, then:

\[
\frac{16.89 \text{ DPM/g blood}}{18.677 \text{ DPM/µg HCN}} = 0.9043 \mu \text{g HCN/g blood}
\]

From NaCN solutions containing 0.20 µg HCN/ml, it was determined that a blood sample from a bluegill after 15 minutes of exposure contained 0.2178 µg HCN/g (Table 15).

It is assumed that the amount of accumulated HCN is equal to the average concentration of HCN accumulated in various tissues by bluegills exposed for corresponding time periods to the 0.20 mg/l HCN NaCN solutions times the ratio of the calculated HCN concentration in the nickel-cyanide complex solution to 0.20 mg/l HCN.

Therefore, the expected amount of accumulated HCN is equal to:
\[
\frac{(0.2178)(0.1813)}{(0.20)} = 0.1975 \, \mu g \, HCN/g \, blood
\]

Observed $0.9043$

Expected $0.1975$

$0.7068 \, \mu g \, HCN/g \, blood$ that did not enter as HCN

\[
\frac{7.068 \times 10^{-7} \, g \, HCN/g \, blood}{27.0259 \, g \, HCN/mole \, HCN} = 2.6153 \times 10^{-8} \, \frac{mole \, HCN \, or \, CN}{g \, blood}
\]

Assuming that the $2.6153 \times 10^{-8}$ mole excess cyanide enters as $\text{Ni(CN)}_4^{2-}$, then:

\[
\frac{2.6153 \times 10^{-8}}{4} = 6.538 \times 10^{-9} \, \text{mole} \, \text{Ni(CN)}_4^{2-}/g \, \text{blood}
\]

\[
(6.538 \times 10^{-9} \frac{\text{mole} \, \text{Ni(CN)}_4^{2-}}{g \, \text{blood}})(162.7816 \frac{g \, \text{Ni(CN)}_4^{2-}}{\text{mole} \, \text{Ni(CN)}_4^{2-}}) = 1.064 \frac{\mu g \, \text{Ni(CN)}_4^{2-}}{g \, \text{blood}}
\]

\[
(6.538 \times 10^{-9} \frac{\text{mole} \, \text{Ni(II)}}{g \, \text{blood}})(58.71 \frac{g \, \text{Ni(II)}}{\text{mole} \, \text{Ni(II)}}) = 3.8385 \times 10^{-7} \frac{g \, \text{Ni(II)}}{g \, \text{blood}}
\]

\[= 0.3839 \frac{\mu g \, \text{Ni(II)}}{g \, \text{blood}}\]