

SOME EFFECTS OF CHRONIC COMBINED ARSENIC AND CYANIDE
POISONING ON THE PHYSIOLOGY OF RAINBOW TROUT

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ABSTRACT

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SOME EFFECTS OF COMBINED CHRONIC ARSENIC AND CYANIDE POISONING ON THE PHYSIOLOGY OF RAINBOW TROUT

This study was performed to examine the effects of arsenic, both alone and with cyanide, on the growth, respiration and swimming stamina of rainbow trout Salmo gairdneri, under laboratory conditions.

Three 21-day experiments were performed on fish kept in flow-through aquaria containing approximately 120 l of water at 11.0°C. The fish were exposed to arsenic trioxide at 1.0, 2.0, 3.0 and 6.0 mg/l arsenic, 3.0 mg/l As plus 0.02 mg/l HCN, 6.0 mg/l As plus 0.02 mg/l HCN and 0.02 mg/l HCN. Concentrations of 6.0 mg/l of arsenic and 0.02 mg/l of cyanide are approximately 25 percent of the LC50 value for rainbow trout.

The results indicate that only 6.0 mg/l As reduced the growth when the fish were exposed to arsenic alone, however, when exposed to 6.0 mg/l As plus 0.02 mg/l HCN a greater reduction was observed than that elicited by either of the toxicants acting singly. Swimming stamina of the test fish was only decreased by previous exposure to cyanide. Hemoglobin contents were 10-20 percent lower in fish toxified with arsenic at all concentrations.

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INTRODUCTION

This laboratory project was undertaken to measure some effects of chronic arsenic poisoning on rainbow trout, at levels encountered at mining sites. In view of the presence of cyanide which is also prevalent in mine tailing effluents, it was considered pertinent to study the combined effects of the two pollutants.

The movement of arsenic in the environment is not totally a natural phenomenon and man contributes significantly to the pollution of surface waters with arsenic. The main sources of arsenic pollution are the mining industries and the use of pesticides. Smaller industries such as leather tanning and glass staining add to this contamination but, in a lesser way.

Mines are the major contributors to the arsenic load of the waterways. Bérubé (1971) and Roy (1973), investigated four mining sites near Yellowknife in the Northwest Territories, and found a wide range of arsenic levels in the water. Roy found levels of arsenic in the tailing water to be 0.76 ppm at one site and 3.7 ppm at the other; Bérubé examined the concentrations of arsenic in the water entering the Great Slave Lake from the mining sites and discovered levels of 2.6 and 8.4 ppm of arsenic.

Bérubé (1971) proposes three stages in the evolution of arsenic in water: dissolution, precipitation and redissolution. At the mines studied, the main arsenic bearing mineral found was arsenopyrite (FeAsS). Generally arsenopyrite is insoluble, but in the cyanide leach of gold, in an alkaline medium, it does dissolve to some degree (Bérubé, 1971). Another possible means of increasing the solubility of arsenic involves the alkaline oxidation by air of arsenopyrite in concentrated lime solution producing an alkaline arsenite:



A third way that arsenopyrite may be converted to a soluble form is through bacterial oxidation. Ehrlich (1964) showed that members of the Thiobacillus-Ferrobacillus group accelerate the oxidation of arsenopyrite to arsenite and arsenate.

In nature arsenic is generally associated with gold, iron and copper ores as sulfides and arsenopyrite and it rarely occurs in its elemental form. Industries mining for gold and other metals, release large amounts of arsenic into the aquatic environment. The refining operations of the smelting process used in the iron ore industry also produce large quantities of arsenic trioxide as a by-product (Holland et al., 1960, p. 188). Another method by which the mining industry introduces arsenic into the surface waters is through the run-off waters from slag piles that are

created with the processed ore, and which contain arsenopyrite and sulfides of arsenic. Wind action on the slag piles can also spread dust containing arsenic to the surrounding countryside.

The use of arsenical compounds as insecticides and weedkillers is another source of arsenic contamination of the environment even though there has been a decline in their use. Penite 6X (Penn. Salt Mfg. Co.), a sodium arsenite preparation, has been used in teredo (Bankia sp.) control tests in British Columbia (Alderdice & Brett, 1957). Large rafts were sprayed with a preparation of this product before they were sent to the mills to prevent infestation of the logs by teredo worms. Sodium arsenite and arsenic trioxide have been and are still being used as aquatic weedkillers (Wiebe, 1930) (Gilderhus 1966).

There are several forms of arsenic found in fresh water. The most common of these forms are the arsenic and arsenious acids, the oxides of arsenic (As_2O_3), and some sulfur compounds (realgar and orpiment). The forms in which one finds arsenic in fresh water is largely dependent upon the eH and pH values of the water (Ferguson & Gavis, 1972). Arsenic can also be found in water, in a variety of salt forms, such as sodium arsenite and sodium arsenate. Under eH and pH conditions occurring in aquatic systems, there are four oxidation states of arsenic in which it is stable: 5,

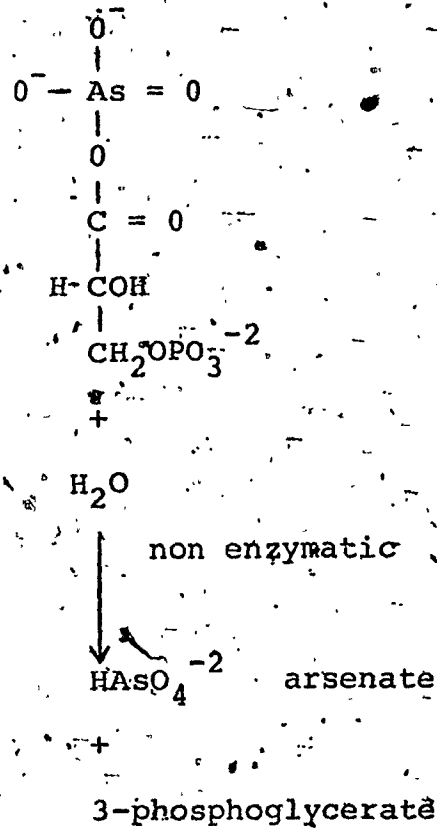
3, 0, -3 (Ferguson & Gavis, 1972). As arsenic in the 0 form is quite rare and the -3 form occurs only at extremely low pH values, the two most common forms are the +3 and +5 types, of which the +3 is the most toxic (Ferguson & Gavis, 1972).

An important feature of arsenic is that it will readily form covalent bonds with carbon and sulfur. This bonding occurs only with the trivalent form of arsenic, however, the pentavalent form can readily be reduced through oxidation to the trivalent form to facilitate this bonding.

Biologically, the importance of this covalent bonding is that arsenic can react with sulfhydryl groups of cysteines and proteins (Ferguson & Gavis, 1972). Arsenic, in the form of arsenate, uncouples oxidative phosphorylation in the triose oxidation step. Normally, the acyl group is transferred from the sulfhydryl group of the acyl enzyme to inorganic phosphate to form 1, 3-diphosphoglycerate, the oxidation product (Lehninger, p. 324). However, the enzyme can also utilize arsenate instead of phosphate, forming 1-arseno-3 phosphoglycerate. This highly unstable compound spontaneously decomposes into 3-phosphoglycerate and arsenate (Figure 1). Due to the arsenate, no high energy phosphate bond is produced by the dehydrogenase, even though overall oxidoreduction takes place (Lehninger, p. 324).

Therefore the presence of arsenic may create a shortage of available energy for physiological processes to continue operating at normal levels of efficiency.

Figure 1. Hydrolysis of 1-arseno-3 phosphoglycerate



(Lehninger, p. 324

1972)

In a stratified lake, depending upon the pH of the various strata, there exists more than one species of arsenic. In the sediments where the pH is fairly low, bacteria such as Ferrobacillus ferrooxidans, can oxidize

arsenopyrite to arsenite and/or arsenate (Ehrlich, 1964). Other microorganisms form trimethylarsine from inorganic arsenic compounds (Ferguson & Gavis, 1972). Trimethylarsine under some circumstances is highly toxic and has been identified as an important reservoir of arsenic in certain organisms, and has also caused human poisoning through exposure to air. Arsenic can also reach the bottom sediments through absorption to free iron ions.

Generally the range of natural arsenic levels in rivers is between 0 - 10 $\mu\text{g}/\text{l}$, establishing an average of 1 $\mu\text{g}/\text{l}$ for rivers free of arsenic pollution, whereas in the oceans the concentration of arsenic has been calculated to be 2 $\mu\text{g}/\text{l}$, although the levels vary considerably (Ferguson & Gavis, 1972).

The safe permissible level for public drinking water supply is 0.05 mg/l as recommended by the World Health Organization in 1963 and the United States Public Health Service in 1962 (Ferguson & Gavis, 1972).

The presence of arsenic in the aquatic environment has been proven, in some cases, to have deleterious effects on the organisms living there (Gilderhus, 1966; Lawrence, 1958). The toxicity of arsenic differs with the organisms involved and the conditions under which they are tested. Some workers have used sodium arsenite, (Gilderhus, 1966), while others have used arsenic trioxide (Holland, 1960).

Both of these arsenic compounds are used as aquatic herbicides and it has been found that 2 mg/l As_2O_3 yields the same amount of arsenic as does 2.5 mg/l $NaAs_2O_3$.

The effect of arsenic, in the form of sodium arsenite, on the aquatic environment, has been studied by few researchers, but not enough to fully understand the impact of arsenic upon the aquatic flora and fauna. The physiological effects are understood for the human body (Grollman & Grollman, p. 894), but unfortunately little is known about its effects on invertebrates and fish physiology.

Sodium arsenite is mainly used as a herbicide, but it may also be used as a pesticide. The British Columbia research council considered using it as a deterrent for teredo infestation of freshly cut logs, waiting to be transported to the mills. Toxicity tests of sodium arsenite to chum salmon fry (Oncorhynchus keta) were carried out with fish ranging in weight from 0.2 to 1.4 grams with a mean of 0.77 grams (Alderdice & Brett, 1957), and at a salinity of 18 ppt. From these bioassays Alderdice and Brett (1957) found the 48 hr TL_M to be approximately 11.0 ppm sodium arsenite.

Work done on the pink skeena salmon (O. gorbuscha) shows that even short term exposure to 16.0 ppm arsenic, in the form of arsenic trioxide, will cause a total kill (Holland et al., 1960, p. 188). Exposure of 40-day old pink skeena salmon

to an initial concentration of 5.3 ppm arsenic, which was not detectable 10 days later by the analytical methods used, resulted in a 22% mortality, with the survivors being transferred to pure running sea water and twenty days later a total of 48% mortality was recorded for this batch of fish (Holland, et al p. 188). When this experiment was repeated, only 12% mortality occurred with the only difference being the age of the fish; these were 31 days older. This suggests an increase in tolerance with age. Also, after 7 days, an initial concentration of 9.5 ppm had reduced to 6.0 ppm resulting in a total kill (Holland, et al, 1960, p. 188).

Lawrence (1958) investigated the use of sodium arsenite as an algicide and its effects on fish production. He stocked six one-quarter acre ponds with bluegills (Lepomis macrochirus) and used two randomly selected ponds as controls. Two of these ponds received two applications of 4.0 ppm As_2O_3 and the remaining two ponds received two doses of 8.0 ppm arsenic trioxide. In the ponds treated with the arsenic trioxide there was a 34% reduction of bottom organisms in the former and a similar reduction of 45% in the latter as compared to the controls. The average bluegill production in the control ponds was 144 pounds per acre; for the ponds treated with two applications of 4.0 ppm As_2O_3 the production was 84 pounds; and, for the ponds treated with two applications of 8.0 ppm arsenic trioxide

the production was 52 pounds. This decrease was accounted for by the reduction in the number of small fish.

Gilderhus (1966) studied the effects of sublethal concentrations of sodium arsenite on bluegills with exposure periods of 16 weeks. All his experiments were done in large concrete pools, containing bottom sediments, vegetation and invertebrates. He assessed chronic toxicity on the basis of survival, growth, hematocrit measurements, ratio of gonad weight to body weight, and pathological changes in the tissues. A considerable amount of the sodium arsenite applied to the pools reached the bottom sediments; levels of arsenic in the sediments accumulated as high as 394 ppm. Accumulation occurred during a two-year period where a total of 28.8 ppm was added to the pool. Fish, from a pool treated once with arsenic at 4.0 ppm concentration, had the following residue levels: flesh, 1.3 ppm; skin, 2.4 ppm; gills and digestive tract, 17.6 ppm; liver, 11.6 ppm; kidney, 5.9 ppm and ovaries, 8.4 ppm. It was also found that the duration of exposure and the amount of arsenic accumulated in the fish were directly related.

Gilderhus (1966) showed that as the concentration of arsenic was increased, the growth rate decreased. There was a weight loss in the adult fish that were exposed to the higher concentrations and a smaller weight gain in the immature fish.

Several pathological conditions were noted upon exposure of the fish to the herbicide treatment. The gills were found to have more hemorrhagic globes than the gills of the control fish. In some, the livers had been infiltrated by fat, vacuolation of hepatic cells and areas of necrosis (Gilderhus, 1966). He also noted acute damage to heart tissue in the exposed fish and also histopathological conditions in the ovaries. A marked reduction of bottom organisms and the elimination of Cladocerans can account for the reduced growth of fish.

As mentioned previously, because of the presence of cyanide in the tailing water, the effects of this highly toxic substance was studied in conjunction with arsenic. Cyanide is a known respiratory depressant whose mode of action is to inhibit the cytochrome oxidase and succinic dehydrogenase activity. Lethal levels, for cyanide, have been established for many species of fish. Brown (1968) found the 48 hour LC_{50} to rainbow trout to be 0.07 mg/l as HCN at a pH of 7.6 and a temperature of 15°C. Neil (1957) noted that prolonged exposure to cyanide at 0.01 to 0.05 mg/l reduced the swimming stamina of brook trout (Salvelinus fontinalis). Leduc (1966) using the following concentrations of cyanide, 0.01, 0.02, 0.04 and 0.08 mg/l, found a reduction in growth in coho salmon (Oncorhynchus kisutch) and in cichlids. Chan (1971) showed that cyanide concentrations.

of 0.01 to 0.37 mg/l, affected the ion balance in the blood and upset the osmoregulation in rainbow trout. Dixon (personal communication) demonstrated that chronic cyanide poisoning, at 0.01, 0.02 and 0.03 mg/l HCN, retarded growth, seriously affected respiration and created histopathological conditions in the liver.

There are presently various points of view over the problem of multiple toxicity. Generally the potency of each individual toxicant must be known to be able to determine whether the joint effect is additive, more-than-additive or less-than-additive. Sprague (1970), defines these terms as follows: if fish are exposed to half the concentration of toxicant A necessary to produce a given magnitude of response, usually the LC_{50} , and half the concentration of toxicant B necessary for the same response, then if this combination just causes the response, the actions of A and B are exactly additive. If it causes more than the given response, they are more-than-additive, and if it does not produce the response the toxicants are either less-than-additive, show no interaction or are antagonistic.

Anderson (1973) presents another method of examining the problem of multiple toxicity by predicting joint toxicity on the basis of response curves. Rather than using the toxic unit mechanism, where the effect of the mixture is predicted by adding the concentration of the constituents, independent joint action is predictable through summing of

each magnitude of response respectively evoked by the individual constituents of a mixture. Anderson (1973) proposed four types of addition: concentration addition, response addition, supra-addition and infra-addition. Concentration addition is the phenomenon where each toxicant contributes to a common effect in relation to relative potency, even though any one concentration of the toxicants may be below toxic levels. Response addition is where by each toxicant, in a mixture, contributes to a common effect in exact proportion to its effect studied discretely, or where the toxicants demonstrate no interaction. A supra-addition produces a response, through interaction, greater than either concentration addition or response addition. Infra-addition elicits a response, through interaction of the toxicants, which is less than one action produced by either concentration addition or response addition.

However, it must be noted that both Sprague (1970) and Anderson (1973), dealt with a single response, death. In this study the concentrations of arsenic and cyanide tested were well below the lethal levels and therefore aimed at different responses, these being the growth, swimming stamina and respiration of rainbow trout held in flow-through systems.

MATERIALS, APPARATUS AND METHODS

Materials

The fish used for this study were rainbow trout, Salmo gairdneri, which were purchased from La Pisciculture du Lac a L'eau Claire, St. Alexis-des-Monts, Maskinonge County, Quebec. Separate batches of 400 fish were ordered for each of the three experiments and the average weights of the fish obtained were 20.0, 27.0 and 24.3 grams.

Upon arrival at Sir George Williams Campus the fish were kept in two of the test tanks supplied with flowing water maintained at $11 \pm 0.5^{\circ}\text{C}$. These fish were allowed to acclimatize to these conditions for a period of two weeks, during which they were fed approximately 2% of their body weight daily with Purina Trout Chow. The tanks were cleaned each day, to remove feces and left over food, by means of a siphon tube. During the holding period there was less than 1% mortality.

Apparatus

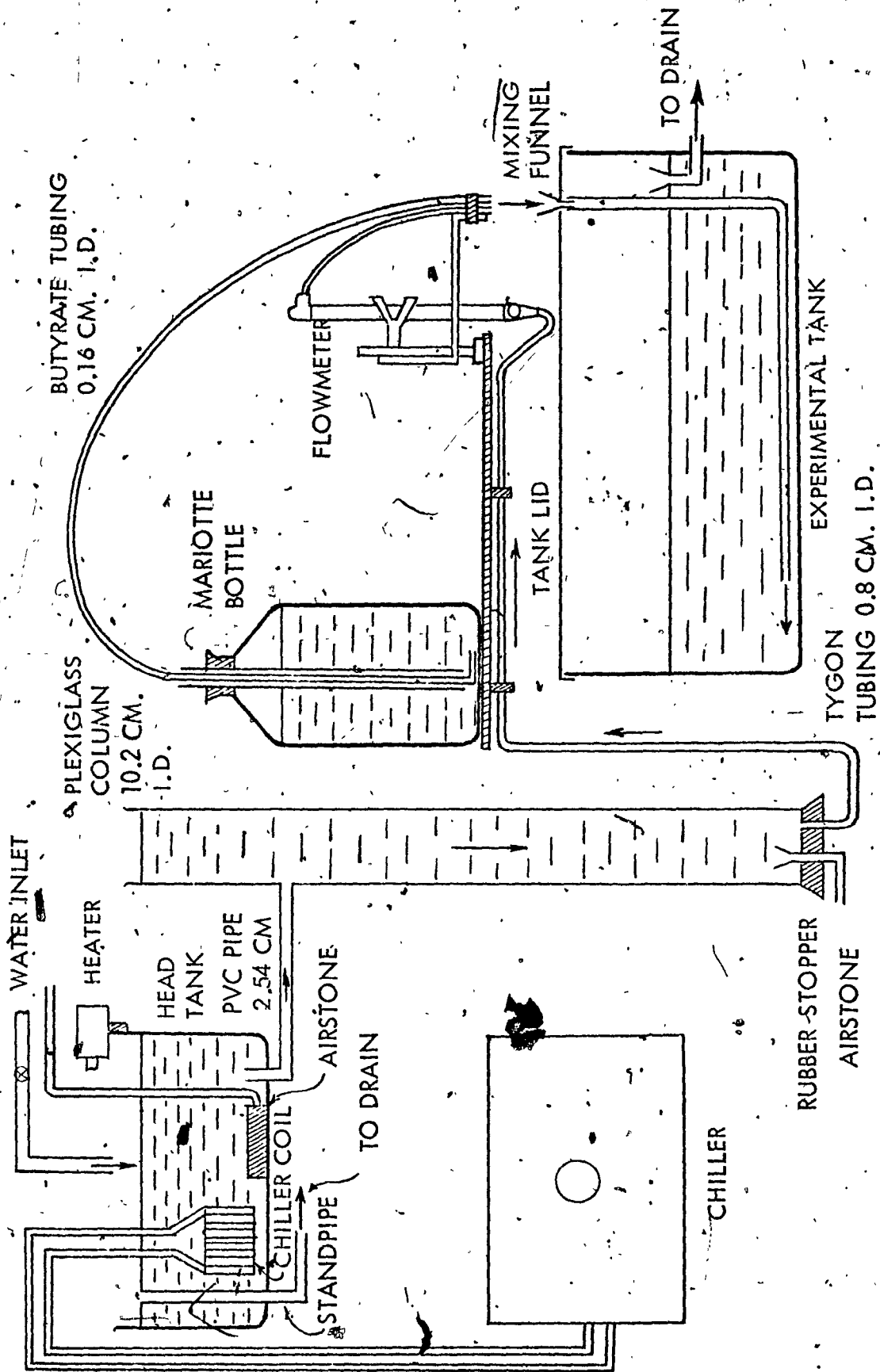
The water supply to the test tanks was dechlorinated city water (City of Montreal), delivered through PVC plumbing. The chemical characteristics of the water, for the period through which the experiments were performed are presented in Table 1.

Table 1. Chemical analysis of treated water for the City of Montreal (data provided by City of Montreal waterworks). October 1973 to June 1974 (Mean values)

| Alkalinity CaCO ₃ (mg/l) | Total Hardness (mg/l) | CO ₂ (mg/l) | pH |
|---|-----------------------------|---------------------------|-----|
| 87 | 127 | 0.3 | 7.9 |

The temperature control system and flow diagram is illustrated in Figure 1, a schematic drawing, and in Figure 2, a ~~photograph~~ showing the whole experimental apparatus in operation. Temperature control of the water was achieved with a stainless steel refrigeration unit (Blue-M Electric Co., Model PCC-1355A-2), which cooled the water down to 11.5°C in the summer. In the winter the incoming water, which entered the laboratory at 2.5°C, was heated to 9°C in the head tank by three eleven hundred watt stainless steel immersion heaters (Precision Scientific Co. Ltd., Portatemp No. 14-X-6), where air supersaturation was overcome by agitating the water with air stones and by passing the heated water through a plexiglass column (10.16 cm I.D.) supplied with compressed air. From there, the water was delivered to the test tanks at a flow of 1.0 l/min, controlled with a flow meter (Manostat Corp., New York, N.Y.). The

Figure 1. Schematic diagram of the flow-through system showing the head tank, temperature control devices, gas exchanger, flowmeter, Mariotte bottle and one experimental tank.



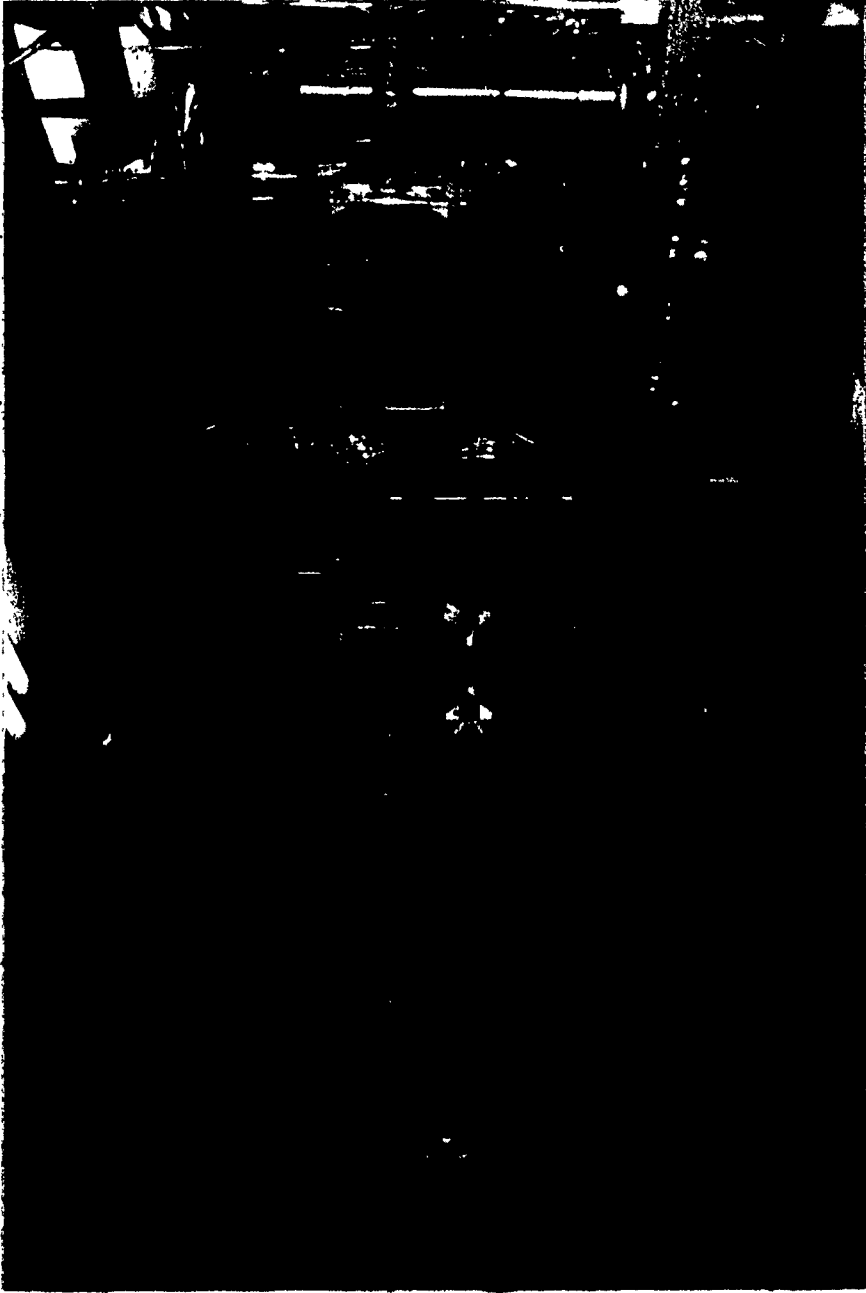


Figure 2. Photograph of the experimental tanks and the Mariotte bottles.

dissolved oxygen levels in the test oxygen levels in the test tanks for the first experiment were approximately 7.0 mg/l, while those of the last two experiments were between 8.0 and 10.0 mg/l.

The whole experimental apparatus consisted of a series of five test tanks (Rosedale Plastics, Montreal) made of white, translucent polyethylene and measuring 57.15 cm wide, 114.30 cm long, and 41.65 cm deep. Each of these tanks were covered by lids of the same material and had small hinged hatches to allow feeding and cleaning without removing the whole cover. The tank toxicants were metered in by Mariotte bottles (Leduc, 1966). Illumination was provided by fluorescent lighting which was evenly distributed over the test tanks and the photoperiod, automatically controlled by a time switch, was set for 12 hours daily.

The respiration studies were performed with a series of thirty respirometer flow through tubes. Each respirometer tube consisted of a plexiglass tube measuring 4.76 cm I.D. and 22.86 cm long. All the tubes were painted black on the exterior to minimize disturbance of the fish and ends stoppered with a No. 10 perforated stopper. The diagram of the flow from the head tank to the respirometer tubes is illustrated in Figure 3. The amount of oxygen consumed by the fish in the respirometer tube was calculated from the difference of dissolved oxygen content between the

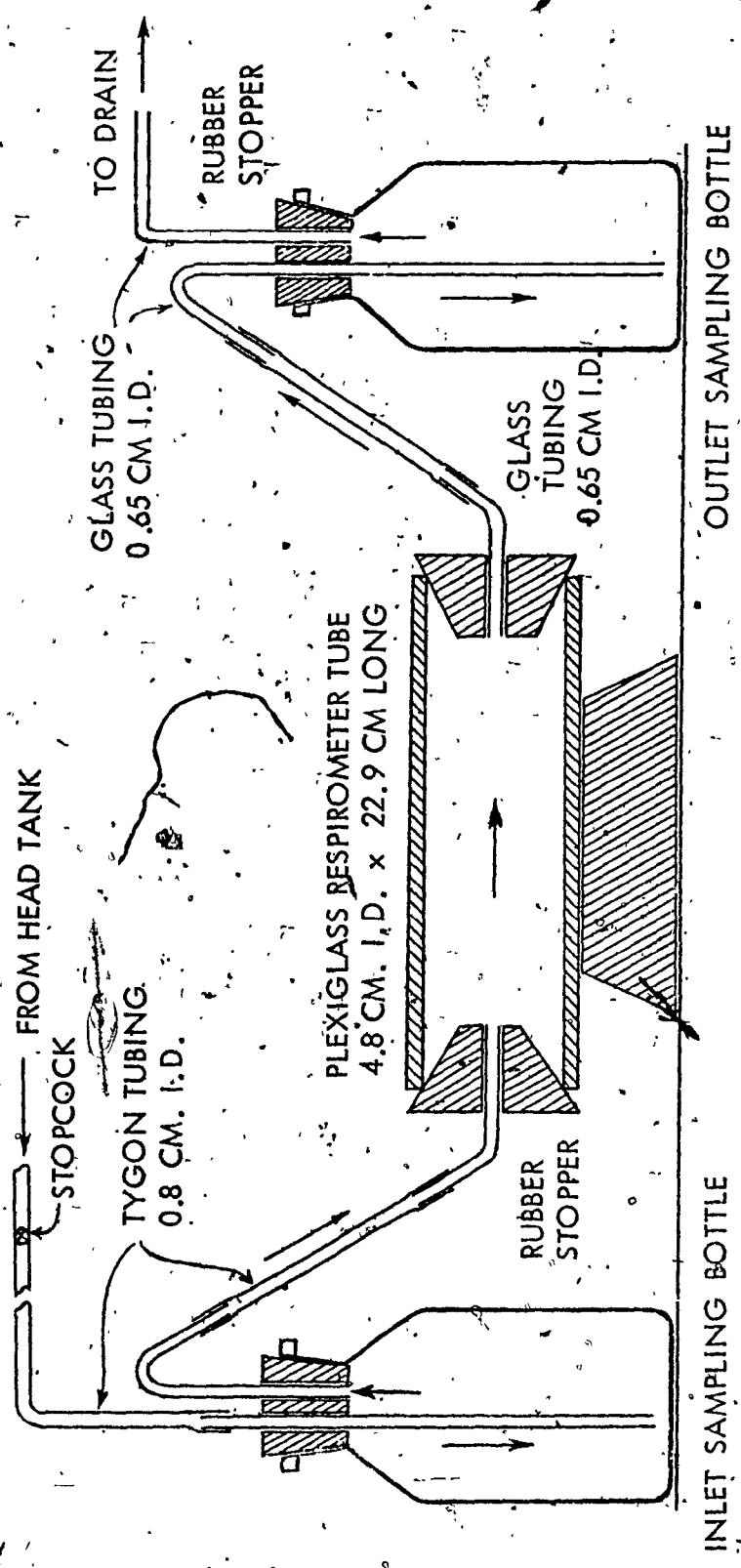


Figure 3: Schematic diagram of one flow-through respirometer showing the pattern of water flow through the system.

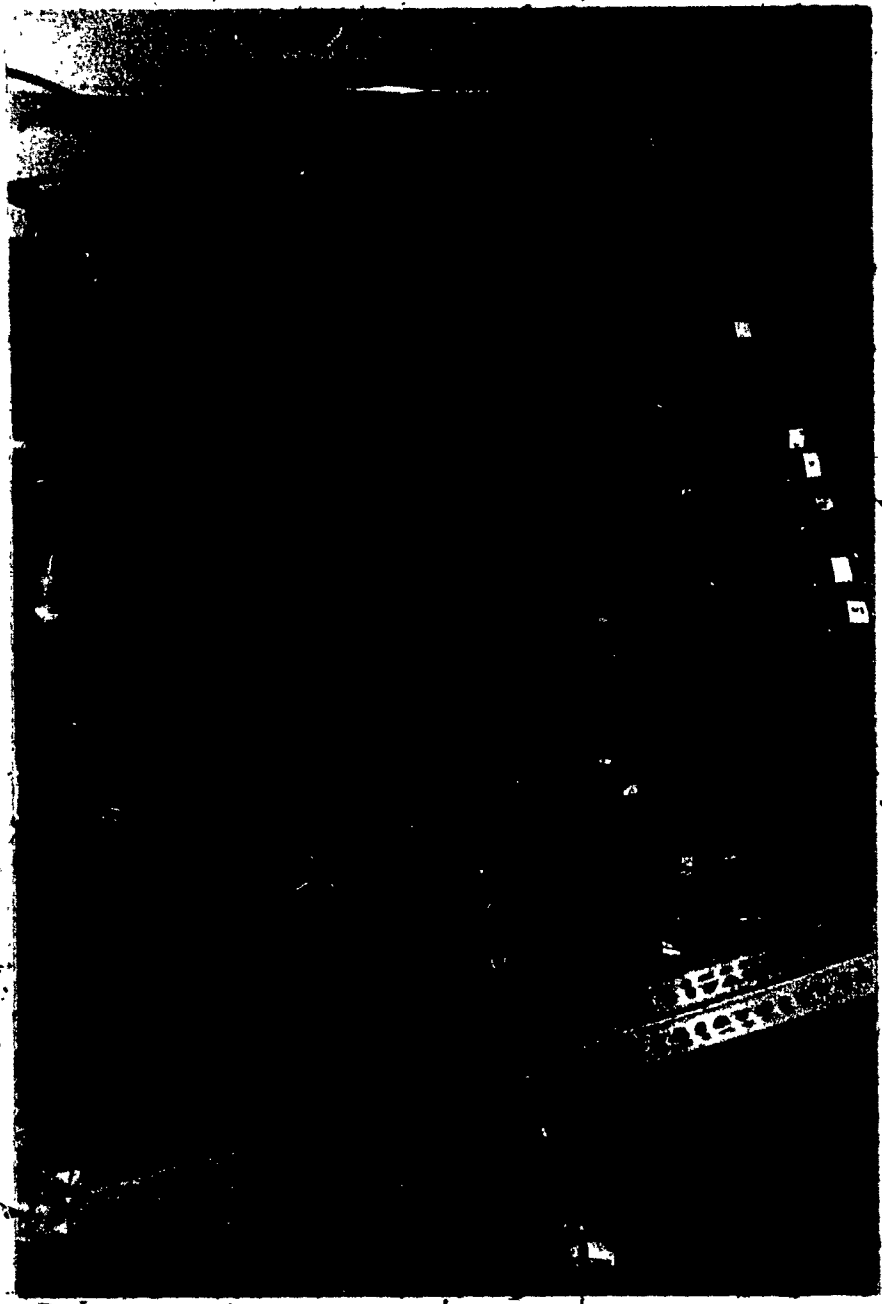


Figure 4. Photograph of the flow-through respirometers with the stand and head tank.

incoming and outgoing water.

The swimming stamina tests were performed in an activity chamber modified by Kruzynski (1972) after Smith and Newcomb (1970). It consists of two concentric, plexiglass tubes through which water is circulated by means of an impeller driven by a variable speed motor. The inner tube, which is the swimming chamber, measures 15.2 cm O.D. and 61.0 cm long. There is a plastic grid at both ends of the inner tube to prevent the fish from escaping. The water supply for the chamber came from the same head tank which supplied the other test tanks, so that the water temperature for all the swimming tests was similar to what the fish had been previously exposed to. The chamber is capable of achieving a range of water velocities from 0 to 61.0 cm/sec.

Methods

In this study three experiments were performed. In experiment 1 four concentrations of arsenic were tested, whereas in experiments 2 and 3, the fish were exposed to arsenic and/or cyanide. The number of fish per test tank in experiments 1 and 3 was 45, while in experiment 2 only 30 fish per test tank were used. With a 90% water replacement rate every 4 hours the fish loads per tank (approx. 2 l/g/day) meet the minimum requirements recommended by Sprague (1973).

All three experiments lasted 21 days and at the end, the fish were taken from the tanks and used for the following measurements: respiration, swimming stamina, hemoglobin counts, wet and dry weights, and fat and arsenic content. At the beginning of experiments 2 and 3, a group of fifteen fish were taken for dry weight measurement.

In experiment 1, the fish were exposed to arsenic concentrations of 1.0, 2.0 and 3.0 mg/l as arsenic. The fish in experiments 2 and 3 were exposed to arsenic and/or cyanide at the following concentrations: 0.02 mg/l HCN, 6.0 mg/l As, 0.02 mg/l HCN plus 3.0 mg/l As, and 0.02 mg/l HCN plus 6.0 mg/l As. In all three experiments there was a control group kept under similar conditions except for the toxicants.

Growth Experiments:

When the fish arrived at the laboratory they were held in two test tanks for one day and on the next day, they

were weighed and divided in five groups with approximately even weight distribution. Prior to the weighing, the fish were lightly anaesthetized with 30 ppm M.S. 222 (Tricaine methane sulphonate) and blotted on moist paper towels to remove the excess water. All the weighing was done under red light (Sylvania F40 Red) illumination to further minimize disturbance to the fish.

The day after the weighing, the fish were all individually marked with liquid nitrogen (Mighell, 1969). At the end of the two week acclimatization period the fish were starved for one day and were then individually weighed to the nearest one-hundredth of a gram. Upon return of the fish into the five experimental tanks, the flow of toxicants from the Mariotte bottles was started.

In all three experiments arsenic and/or cyanide levels were monitored during the exposure period every third day, along with the dissolved oxygen concentrations.

Arsenic levels in the water were monitored in two ways. For the first experiment arsenic determinations were performed by the silver diethyldithiocarbamate colorimetric test (Leblanc and Jackson, 1973). For the other two experiments arsenic concentrations were determined with an atomic absorption spectrophotometer, a Perkin-Elmer (Model 503), equipped with both an arsenic lamp and a graphite furnace.

Cyanide was determined by the Epstein's colorimetric method (Standard Methods, 1971), and the flow of cyanide stock solution, was adjusted accordingly. However, it was impossible to maintain exactly the desired concentrations throughout, but losses never exceeded 15 percent.

During the 21-day exposure period the fish were fed daily, 2% of their body weight, with Purina Trout Chow (see Appendix I for details). The fish were starved one day prior to their weighing on days 10 and 21 of exposure; the same weighing procedure was followed as mentioned previously.

Respiration:

Five fish of approximately the same size were selected from each of the five test tanks at the end of the 21-day growth experiment and placed in a respirometer for six days. During this period their oxygen consumption was measured each day at the same time. Prior to each measurement, the flow rate from each respirometer was recorded. The outlet bottle for each tube was then removed, stoppered, and replaced; the same procedure was repeated with the inlet bottle. In each experiment three blank respirometers were run as controls, to determine whether there was any significant difference in dissolved oxygen between the incoming and outgoing water, due to factors other than oxygen consumption by the fish.

Oxygen determinations were made following the Winkler Method (Azide Modification, Standard Methods, 1971) and colorimetrically read at 450 m μ with a Bausch & Lomb, Spectronic 20 spectrometer. This method was developed by Oulman and Baumann (1956) and Elliot (1963), modified by Dixon (pers. comm. 1974). Oxygen consumption was determined by calculating the differences in dissolved oxygen between the inlet and outlet bottles and expressed in mg/g of fish/hour.

Swimming:

The fish that were selected for the swimming stamina tests were all approximately the same weight and length. The fish in experiment 1 were tested on two different days, separated by an interval of three days due to a breakdown of the apparatus. However, the fish in experiments 2 and 3, were all tested in one day immediately following the end of the growth experiments.

The procedure used is as follows: one fish was placed inside the activity chamber while it was filling with water. After the chamber was sealed the electric motor was set to produce a velocity of 1 ft/sec (30.5 cm/sec), which was maintained for five minutes, to allow the fish to become accustomed to swimming inside the chamber. At the end of this training period, the speed of the motor was increased to produce a water velocity of 1.54 ft/sec (47.0 cm/sec) inside the chamber. The swimming stamina was determined as

the time spent at this velocity; the time was measured with a stopwatch to the nearest second. When the fish became tired, it was pressed up against the retaining grid. At this point, the motor was turned off for two seconds to allow the fish to free itself. The power was then turned back on and if the fish was immediately pressed against the grid again, the time was recorded. If the fish was able to swim for another extended period of time, this time was measured until it tired completely, and then the fish was removed from the chamber.

Hemoglobin:

Hemoglobin determinations were done by the cyanmethemoglobin method (Wintrobe, 1961), using standards obtained from the Laboratory for Disease Control in Ottawa. The blood was taken from the fish by severing the caudal peduncle and extracting it with heparinized capillary tubes. The blood was transferred into a small test tube, from which a measured amount was pipetted. The pipetted blood was transferred into tubes containing Drabkin solution; ten minutes were allowed for color development before the colorimetric readings were made.

The values that were obtained by this method were found to be slightly lower than those obtained by determinations based on total iron content. Therefore a correction

factor of $1.02 \times \text{Cyanmethemoglobin values} + 0.25$ was applied to all the hemoglobin values that were obtained (Larsen & Snieszko, 1961).

Measurements of Dry Weights and Fat Content:

The group of fifteen fish that were taken from each tank to be used for dry weight measurements, were immediately sacrificed with a blow on the head. These fish were blotted, weighed and placed into a drying oven at 65°C , for 7 days, after which the dry weight was determined. The dry samples were then stored in sealed boxes, with a dessicant, for later use in fat content analysis. The fat content was determined by ether extraction with a Labconco Goldfish Fat Extractor (Model 35003), using about 3.0 grams of dry material which had been subjected to a four hour reflux distillation.

Arsenic Residue Analysis:

The fish tissue used for residue determination was the same as that used for the fat extractions. The tissue sample consisted of oven dried fish that had been homogenized with a blender, therefore, containing muscle, bone, skin and scales, and other soft tissue.

An accurately weighed out 2.0 g. sample was placed into a porcelain crucible and ashed in a muffle furnace at

500°C for 16 hours. Upon cooling to room temperature the ashes were stored in dry, clean test tubes; then 5.0 ml. of fuming nitric acid was added to each of the test tubes. Two hours later 20.0 ml. of double distilled water was added. At this point, after agitation by inversion, most of the ashes had been dissolved by the nitric acid and water. These samples were then left overnight to settle out any undissolved materials before any determinations were made. This method of preparing the samples is similar to that proposed by Anderson (1972) and was modified by Dick (pers. comm., 1974).

For the arsenic determinations, a 20.0 μ l aliquot was injected into the graphite furnace of an atomic absorption spectrometer (Perkin-Elmer, Model 503). This flameless technique has been found to be the most sensitive method when using an atomic absorption spectrometer. Standards were prepared by adding known quantities of arsenic to samples prepared with tissue known to be arsenic free.

When the standards were analyzed, a negative absorbance reading was obtained, even though the background corrector was functioning properly. Upon observing these readings some experiments were performed to determine the source of the interference. This was achieved by adding arsenic, in the form of arsenic trioxide, to several samples of water

to obtain a final concentration 1.0 mg/l arsenic in the water. To the samples various metals were added to try to determine which one present in the tissue samples was creating the negative absorbance readings. It was found that both potassium and phosphorus greatly depressed the absorbance values of the arsenic water standards when each was added to a sample. When both chemicals were added, negative absorbance readings were obtained for the water samples that contained 1.0 mg/l arsenic. When this was discovered, wavelengths other than the recommended 193 m μ were used to try to overcome the effects of potassium and phosphorus. However, the above was not possible and at that point the arsenic residue analysis was terminated.

The explanation for the high incidence of potassium and phosphorus is as follows. The tissue used in these attempted determinations came from whole fish and included a large percentage of bones which would contain relatively large amounts of K and P. Also a 2.0 g. sample of dry fish tissue corresponds to approximately 8.0 g. of wet tissue, therefore, the non-combustible material would be largely made up of metals with concentrations much greater than normally found in wet tissue. It must also be remembered, as explained in the introduction, that phosphate groups and phosphorus react chemically similar to arsenate groups and arsenic, therefore, it was virtually impossible

to eliminate the phosphorus from the samples without also removing the arsenic.

Due to uncontrollable circumstances wet tissue could not be used for these analyses. It is felt that if wet tissue had been available, the interference by phosphates would not have been serious enough to hamper the arsenic determination by atomic absorption spectroscopy, since a large percent of the phosphates came from bone and scales which would not have been assayed. It had been intended to examine levels of arsenic in the liver, gills, gut and muscle tissues where phosphate levels would not have been sufficient to create the interference that was observed for the bone bearing tissue samples.

RESULTS

During the two week acclimation period, prior to the addition of the toxicants, the fish readily adapted to the test tanks and to the experimental diet. While the actual food consumption was not measured, it was apparent that the degree of general activity of the fish changed when the flow of toxicant was started. The fish exposed to 0.02 mg/l cyanide fed on the food presented to them more voraciously than did the control fish. In contrast, the fish exposed to all levels of arsenic, were less active than the control fish, and on occasion, there was undigested food left on the bottom of their tanks. During the three experiments performed mortality was minimal (see Table 2), and none of the control fish died.

Effects on Growth

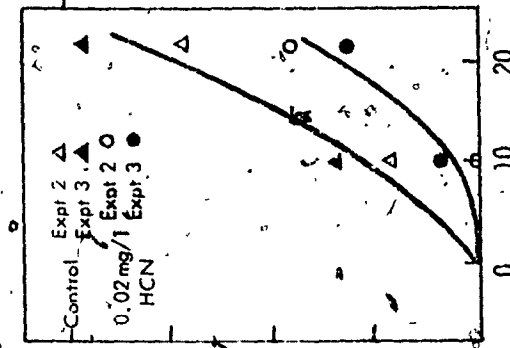
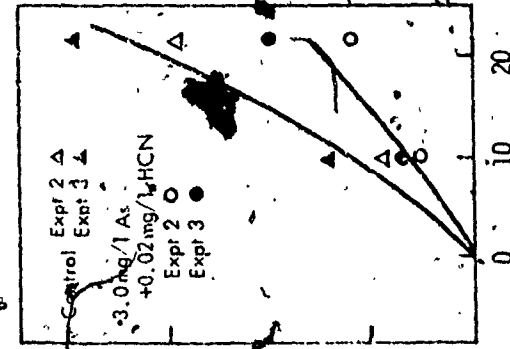
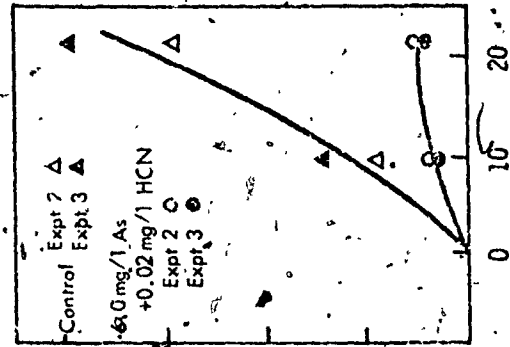
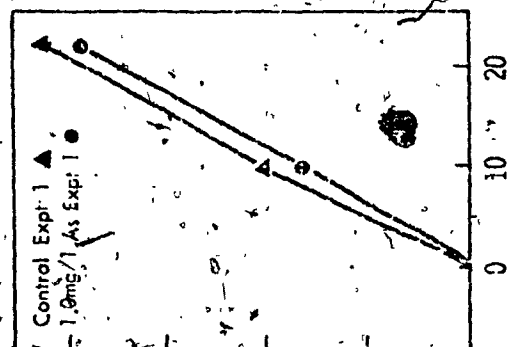
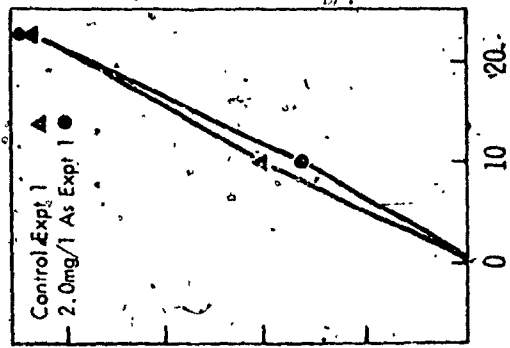
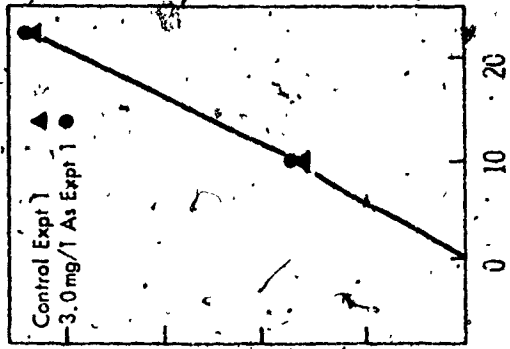
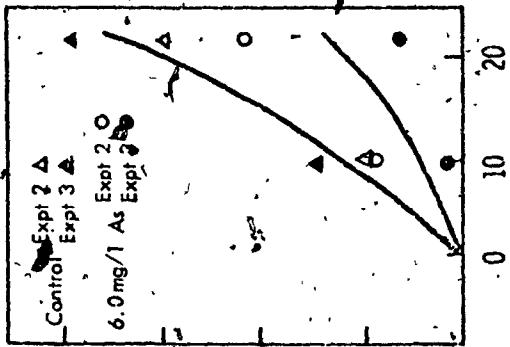
The growth was calculated from the sum of the individual weights taken at 0, 10 and 21 days and expressed as percent wet weight gain. These results are illustrated by eye fitted curves in Figure 5.

The results indicate that weight gains of 14 to 22 percent were attained in 21 days by the control fish. The control fish weight gains in experiment 2 were lower than those of the other experiments. However, the differences in the weight gains among the control groups, did not

Table 2. Mortality observed among yearling rainbow trout exposed to various concentrations of arsenic and/or cyanide for 21 days in a flow through system at 11°C.

| Expt. No. | Toxicant Concentration in mg/l | | No. of fish at beginning of expt. | Initial average weight of fish in g | No. of Mortalities |
|-----------|--------------------------------|------|-----------------------------------|-------------------------------------|--------------------|
| | As | HCN | | | |
| 1 | 0.0 | 0.0 | 43 | 20.66 | 0 |
| | 1.0 | 0.0 | 43 | 19.59 | 1 |
| | 2.0 | 0.0 | 44 | 19.90 | 0 |
| | 3.0 | 0.0 | 45 | 20.63 | 0 |
| 2 | 0.0 | 0.0 | 31 | 23.96 | 0 |
| | 3.0 | 0.02 | 31 | 27.85 | 0 |
| | 6.0 | 0.02 | 31 | 27.34 | 2 |
| | 0.0 | 0.02 | 31 | 27.08 | 0 |
| | 6.0 | 0.0 | 31 | 28.93 | 0 |
| 3 | 0.0 | 0.0 | 46 | 23.07 | 0 |
| | 3.0 | 0.02 | 44 | 24.09 | 1 |
| | 6.0 | 0.02 | 45 | 25.05 | 2 |
| | 0.0 | 0.02 | 44 | 24.67 | 1 |
| | 6.0 | 0.0 | 43 | 24.58 | 5 |

Figure 5. Relationship between the percentage wet weight gain of yearling rainbow trout and the time they were exposed to either arsenic, cyanide, or arsenic and cyanide during 21-day growth periods in a flow through system at 11°C.



WET WEIGHT GAIN (PERCENT)

TIME IN DAYS

Table 3. Comparison, using a Student "t" test, of percentage wet weight gains of the control fish versus the growth rates of the experimental fish over the 10 and 21-day exposure periods.

| Expt. No. | Toxicant Concentration in mg/l | | Percentage Weight Change | | "t" Values for Experimental Fish vs. Control | "t" Values for HCN group vs. As + HCN group |
|-----------|--------------------------------|------|--------------------------|------|--|---|
| | As | HCN | 0-10 | 0-21 | | |
| 1 | 0.0 | 0.0 | 10.3 | 21.7 | - | - |
| | 1.0 | 0.0 | 8.2 | 19.7 | 1.74 | 1.61 |
| | 2.0 | 0.0 | 8.1 | 22.8 | 0.59 | 3.52** |
| | 3.0 | 0.0 | 10.3 | 21.7 | 0.067 | |
| 2 | 0.0 | 0.0 | 4.5 | 14.7 | - | - |
| | 3.0 | 0.02 | 2.7 | 5.8 | 3.99** | 1.61 |
| | 6.0 | 0.02 | 1.2 | 2.3 | 6.32*** | 3.52** |
| | 0.0 | 0.02 | 0.0 | 9.3 | 2.66** | |
| 3 | 6.0 | 0.0 | 4.3 | 10.5 | 2.00* | |
| | 0.0 | 0.0 | 6.9 | 19.5 | - | - |
| | 3.0 | 0.02 | 3.5 | 10.0 | 3.99*** | 2.78** |
| | 6.0 | 0.02 | 1.4 | 2.3 | 7.30*** | 3.16** |
| | 0.0 | 0.02 | 2.0 | 6.3 | 5.36*** | |
| | 6.0 | 0.0 | 0.6 | 3.2 | 3.26** | |

* significant P < 0.05
 ** very significant P < 0.01
 *** very highly significant P < 0.001

appear to have any significance when the weight gains of poisoned fish were examined.

Arsenic, at concentrations of 1.0, 2.0 and 3.0 mg/l as As, produced no apparent effects on the growth of rainbow trout; their percentage weight gains being approximately the same as the control group's gain at the end of both the 10 day and the 21-day periods. However, the wet weight gains of the groups of fish exposed to 6.0 mg/l arsenic were noticeably less than those of the control groups; especially at the end of the 21-day growth period (see Figure 5), when the growth had been reduced by a factor of 55 percent.

Cyanide, at 0.02 mg/l, was seen to reduce the wet weight gains of rainbow trout by approximately 65 percent after 21 days of exposure. From the eye fitted curves in Figure 5 it can be seen that the reduction in weight gain occurred predominantly in the first 10-day period. It was also noted that the growth rate during the second growth period exceeded the rate recorded for the control fish. Essentially this indicates that the cyanide had its greatest effect on the fish during the first 10 days of exposure. It then appears that the fish recover and that growth is stimulated to an above normal degree. However, even though the rate of gain was greater for the poisoned fish than that of the controls in the second growth period it was observed that the overall gain was significantly lower than that

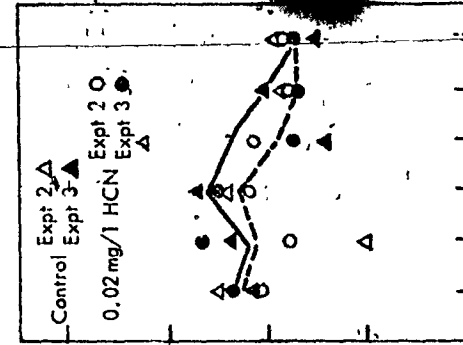
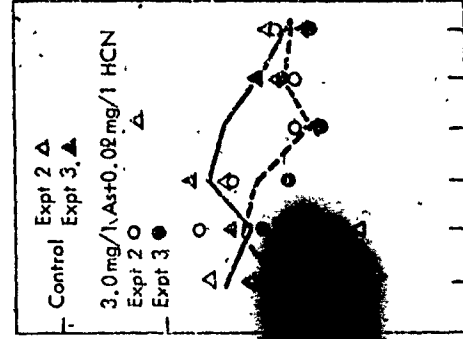
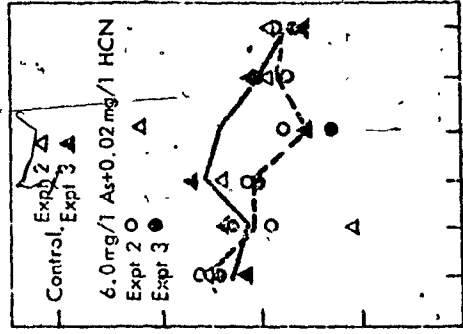
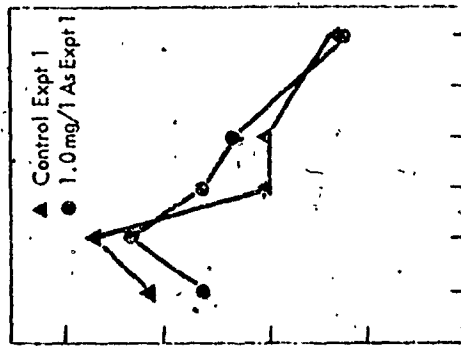
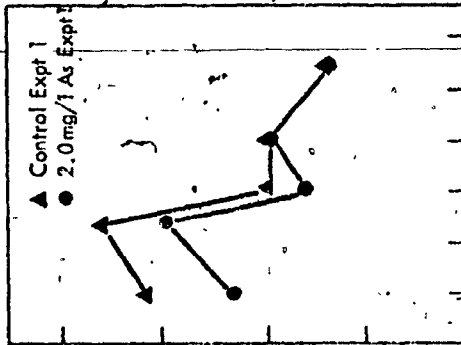
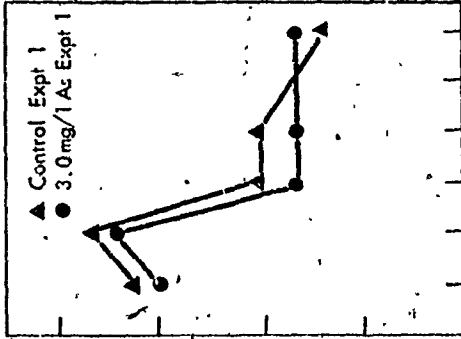
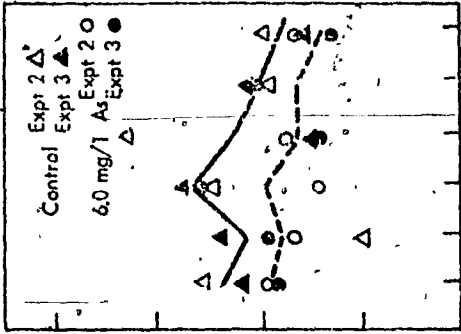
recorded for the control fish (see Table 3).

Upon examining the wet weight gains of the fish exposed to 3.0 mg/l arsenic plus 0.02 mg/l cyanide, three comparisons were made. This combination of toxicants produced a 55 percent reduction in growth after 21 days as compared to the control. This effect was similar however, to that observed for the fish exposed to only 0.02 mg/l cyanide. Also having observed that 3.0 mg/l arsenic did not affect the growth of rainbow trout during the 21-day growth period, it appears that the observed effect at 3.0 mg/l arsenic plus 0.02 mg/l cyanide, would be due to cyanide alone.

An 85 percent reduction in wet weight gain, after 21 days, was observed in the groups of fish exposed to 6.0 mg/l arsenic plus 0.02 mg/l cyanide. This reduction was greater than that elicited by exposure to either 0.02 mg/l cyanide or 6.0 mg/l arsenic. This suggests that, the two toxicants combined at the above concentrations, have a greater effect on wet weight gain of rainbow trout than when acting individually.

The results of the growth experiments were subjected to a statistical analysis presented in Table 3. Comparisons were carried out to determine whether differences between the control weight gains and the gains of the poisoned fish were significant. Also a comparison between the fish that had been exposed to only 0.02 mg/l cyanide

Figure 6. Relationship between mean oxygen consumption of yearling rainbow trout, and elapsed time after exposure to arsenic cyanide, or arsenic and cyanide for 21 days in a flow-through system at 11°C.



OXYGEN CONSUMED (mg/g/hr)

0 24 48 72 96 120 144 0 24 48 72 96 120 144 0 24 48 72 96 120 144 0 24 48 72 96 120 144

ELAPSED TIME IN HOURS

and those poisoned with both arsenic and cyanide were made. When the comparisons were made for the two individual experiments, as seen in Table 3, three out of the four analyses were significantly different. However, when the growth results for experiments 2 and 3 were pooled, only the group poisoned with 6.0 mg/l arsenic plus 0.02 mg/l cyanide proved to be different from the fish exposed to 0.02 mg/l cyanide alone.

Effects on Respiration

The metabolic rates of the control and poisoned fish, measured after the growth experiments, are shown in Figure 6, which illustrates the mean daily oxygen consumption for each group of five fish tested.

The respiration of the control fish varied considerably for the three experiments. In experiment 1, the control fish initially increased their respiration rates and then showed a marked drop after the second day. For experiments 2 and 3, the data of the control fish were pooled and the eye fitted curves for these groups of fish also display an initial increase in oxygen consumption but the later decrease was not as great as that observed for the control group of experiment 1. There was however, considerable variation in the respiration rates of the control fish in the two experiments (see Figure 6).

The respiration of the fish previously exposed to 1.0, 2.0 and 3.0 mg/l arsenic for 21 days, closely followed that of the control fish (see Figure 6), with rates initially increasing and then decreasing to the same level as the control fish. The fish that had been exposed to 6.0 mg/l arsenic had lower respiration rates than the control fish (see Figure 6). This group of fish did not display an initial increase in oxygen consumption, but rather unlike the control fish, kept their respiration rates at a relatively constant level for the first three days and then slightly decreased their oxygen consumption for the last three days.

Following the 21-day exposure to 0.02 mg/l cyanide, the poisoned groups of fish had respiration rates that closely followed those of the control groups of fish (see Figure 6). However, they did not initially increase their oxygen consumption to the same degree as did the control fish.

The group of fish exposed to 3.0 mg/l arsenic plus 0.02 mg/l cyanide displayed a slight initial increase in oxygen consumption for the first 48 hours of the experiment. Following the initial increase a gradual decrease in oxygen uptake occurred. The fish that had been previously exposed to 6.0 mg/l arsenic plus 0.02 mg/l cyanide displayed a decrease in oxygen consumption until a basal level was reached after 5 days. The respiration rates of the above

Table 4. Observed mean swimming times of rainbow trout tested at a water velocity of 1.54 ft/sec (47.0 cm/sec) and at 11°C after previous exposure to various concentrations of arsenic and/or cyanide for 21 days.

| Expt. No. | Toxicant Concentrations | | Average Wet Weight in g. | Average Length in cm. | Mean Swimming Time in min. | Confidence Interval for Mean in min. |
|-----------|-------------------------|------|--------------------------|-----------------------|----------------------------|--------------------------------------|
| | As in mg/l | HCN | | | | |
| 1 | 0.0 | 0.0 | 23.23 | 13.93 | 6.60 | +2.93 |
| | 1.0 | 0.0 | 23.97 | 13.80 | 10.40 | +5.97 |
| | 2.0 | 0.0 | 25.17 | 13.92 | 5.82 | +4.04 |
| | 3.0 | 0.0 | 24.62 | 13.88 | 5.37 | +2.53 |
| 2 | 0.0 | 0.0 | 27.54 | 14.80 | 19.74 | +16.40 |
| | 3.0 | 0.02 | 28.92 | 14.94 | 5.55 | +3.84 |
| | 6.0 | 0.02 | 26.90 | 15.10 | 6.87 | +5.97 |
| | 0.0 | 0.02 | 28.53 | 15.12 | 4.97 | +7.90 |
| | 6.0 | 0.0 | 32.83 | 15.14 | 23.37 | +12.18 |
| 3 | 0.0 | 0.0 | 27.36 | 14.46 | 23.32 | +11.97 |
| | 3.0 | 0.02 | 27.09 | 14.36 | 10.17 | +4.31 |
| | 6.0 | 0.02 | 25.76 | 14.82 | 9.20 | +2.36 |
| | 0.0 | 0.02 | 27.52 | 14.16 | 5.77 | +2.79 |
| | 6.0 | 0.0 | 26.36 | 14.50 | 20.11 | +16.85 |

1/ Fish in this study were tested on two occasions separated by an interval of three days due to a breakdown of equipment.

Table 5. Analysis of variance to compare the swimming stamina of rainbow trout exposed to arsenic and/or cyanide for a period of 21 days.

| Source | Sum of Squares | Degrees of Freedom | Mean Square | Significance |
|---------------------|----------------|--------------------|-------------|-----------------------|
| <u>Expt. 1</u> | | | | |
| Between Treatments | 100.30 | 3 | 33.43 | F_{20}^3 2.191 n.s. |
| Within Treatments | 305.08 | 20 | 15.25 | |
| Total | 405.38 | 23 | | |
| <u>Expt. 2</u> | | | | |
| Between Treatments | 1525.10 | 4 | 381.27 | F_{20}^4 3.82* |
| Within Treatments | 2093.67 | 20 | 99.69 | .025 > P > .01 |
| Total | 3618.78 | 24 | | |
| <u>Expt. 3</u> | | | | |
| Between Treatments | 1145.99 | 4 | 286.50 | F_{20}^4 4.79** |
| Within Treatments | 1197.07 | 20 | 59.85 | .01 > P > .005 |
| Total | 2343.06 | 24 | | |
| * significant | | P < 0.05 | | |
| ** very significant | | P < 0.01 | | |

group of fish were substantially lower than those of the control fish; however, they were higher than the respiration rates observed for the fish exposed to only 6.0 mg/l arsenic.

The groups of fish exposed to a combination of the two toxicants for 21 days, had lower respiration rates than the control fish. However, the differences were not as great as those elicited by the exposure to 6.0 mg/l arsenic, and were greater than those observed for fish that had been poisoned with 0.02 mg/l cyanide.

Comparisons were attempted, but due to the large variances encountered for the respiration means, none of the observed differences were proven to be significantly different. However, upon reviewing the data presented in Figure 6, it may be postulated that exposure of rainbow trout to 6.0 mg/l arsenic had the greatest effect on the respiration rates of the individuals tested.

Effects on Swimming

The results of the swimming stamina tests are shown in Table 4, which presents the mean swimming time endured by rainbow trout tested at a water velocity of 1.54 ft./sec (47.0 cm/sec). Since all the fish tested were of the same length and weight, there was no need to correct for size.

There was a difference in the mean swimming times endured by the control groups of fish. The control fish of experiment 1 had a mean swimming time of only 6.6 minutes, while the control groups of experiments 2 and 3 had mean times of 19.7 and 23.3 minutes respectively. This can most likely be attributed to different stocks of fish.

The results indicate that exposure to arsenic concentrations of 1.0, 2.0, 3.0 and 6.0 mg/l appeared to have had no significant effect on the swimming stamina of rainbow trout. These observations in experiments 1, 2 and 3 were further confirmed through an analysis of variance presented in Table 5.

Cyanide, at 0.02 mg/l HCN, reduced the swimming ability of rainbow trout by about 75 percent as compared to the control fish and this difference was found to be statistically significant when the results were analyzed (see Table 5).

The fish exposed to 3.0 mg/l arsenic plus 0.02 mg/l cyanide and those fish subjected to 6.0 mg/l arsenic plus 0.02 mg/l cyanide had significantly lower mean swimming times than the control fish and the groups of fish exposed to 6.0 mg/l arsenic. However, these swimming times were not significantly different from those times obtained from fish that had been previously exposed to 0.02 mg/l cyanide. This suggests that the observed reduction in the mean

Table 6. Mean blood hemoglobin content of yearling rainbow trout which had been exposed to arsenic and or cyanide for a 21-day period.

| Expt. No. | Toxicant Concentration in mg/l | | No. of Fish Sampled | Mean Hemoglobin Values in g/100 ml Blood (Variance). | | Control vs. Experimental Means "t" test (Sig) |
|-----------|--------------------------------|------|---------------------|--|--------------------------------------|---|
| | As | HCN | | Mean Hemoglobin | Values in g/100 ml Blood (Variance). | |
| 1 | 0.0 | 0.0 | 5 | 10.63 (2.91) | | |
| | 1.0 | 0.0 | 5 | 8.02 (1.05) | 2.93* | |
| | 2.0 | 0.0 | 5 | 7.64 (2.24) | 2.93* | |
| | 3.0 | 0.0 | 4 | 8.36 (0.64) | 2.64* | |
| 2 | 0.0 | 0.0 | 4 | 10.66 (0.66) | | |
| | 3.0 | 0.02 | 4 | 8.84 (1.63) | 2.44* | |
| | 6.0 | 0.02 | 4 | 8.86 (1.47) | 2.47* | |
| | 0.0 | 0.02 | 4 | 10.24 (0.39) | 0.82 ns | |
| 3 | 0.0 | 0.0 | 4 | 10.26 (0.78) | 0.71 ns | |
| | 0.0 | 0.0 | 4 | 9.75 (0.99) | | |
| | 3.0 | 0.02 | 5 | 7.83 (0.54) | 3.22* | |
| | 6.0 | 0.02 | 5 | 8.16 (2.41) | 1.86 ns | |
| | 0.0 | 0.02 | 4 | 8.07 (1.2) | 2.37 ns | |
| | 6.0 | 0.0 | 5 | 9.47 (0.71) | 0.45 ns | |

* significant P < 0.05

swimming time was predominantly due to the cyanide alone.

All the results were statistically tested with an analysis of variance to detect differences in treatment responses as seen in Table 5. Then using the data obtained from the ANOVA, least significant differences were determined to make further comparisons possible. Also, as seen in Table 4, confidence intervals were established for the mean times obtained.

Effects on Blood Hemoglobin Levels

The hemoglobin of the control fish, in all three experiments, were all approximately the same, even though 25-gram fish were used in experiment 1 and 35 gram fish were used in experiments 2 and 3. As seen in Table 6, the mean hemoglobin levels for the control fish were all approximately 10 g/100 ml of blood.

Hemoglobin levels of the fish exposed to 1.0, 2.0 and 3.0 mg/l arsenic were reduced by approximately 20 percent. This observed reduction was a significant decrease when the results were statistically tested with a "t" test as seen in Table 6. The fish subjected to 6.0 mg/l arsenic for 21 days, in both experiments, displayed no reduction in their blood hemoglobin levels.

The fish exposed to 0.02 mg/l cyanide, during the growth period, had a 10 percent reduction in hemoglobin levels. However, as seen in Table 6, this reduction did

Table 7. Comparison, using a "t" test, of the effect of a 21-day exposure to arsenic and/or cyanide on the mean percentage water content and the mean fat content of yearling rainbow trout.

| Expt. NO. | As mg/l | HCN | NO. of Fish Sampled | Mean Percentage Water Content | "t" test, Control vs. Experimental | No. of Fish Sampled | Mean Percentage Fat Content | "t" test, Control vs. Experimental |
|-----------|---------|------|---------------------|-------------------------------|------------------------------------|---------------------|-----------------------------|------------------------------------|
| 1 | 0.0 | 0.0 | 15 | 75.49 | - | 12 | 4.10 | - |
| | 1.0 | 0.0 | 15 | 75.58 | 0.24 | 9 1/ | 3.76 | 1.08 |
| | 2.0 | 0.0 | 15 | 75.92 | 1.30 | 12 | 3.78 | 1.64 |
| | 3.0 | 0.0 | 15 | 76.34 | 2.94** | 12 | 3.64 | 2.15* |
| 2 | 0.0 | 0.0 | 15 | 76.70 | - | 12 | 3.54 | - |
| | 3.0 | 0.02 | 15 | 79.72 | 3.25** | 12 | 1.68 | 4.05** |
| | 6.0 | 0.02 | 15 | 78.80 | 3.58** | 12 | 2.31 | 2.47* |
| | 0.0 | 0.02 | 15 | 78.35 | 1.48 | 12 | 3.04 | 1.15 |
| 3 | 6.0 | 0.0 | 15 | 77.85 | 0.94 | 12 | 2.90 | 1.74 |
| | 0.0 | 0.0 | 15 | 76.92 | - | 12 | 3.66 | - |
| | 3.0 | 0.02 | 15 | 76.95 | 0.05 | 12 | 3.06 | 2.74 |
| | 6.0 | 0.02 | 15 | 79.69 | 4.43*** | 12 | 3.21 | 1.66 |
| 4 | 0.0 | 0.02 | 15 | 77.99 | 2.06** | 12 | 3.36 | 0.82 |
| | 6.0 | 0.0 | 15 | 77.39 | 0.78 | 12 | 3.17 | 2.04 |

* Significant P < 0.05
 ** Highly significant P < 0.01
 *** Very highly significant P < 0.001
 1/ Numbers on weighing boats smudged beyond recognition, therefore identification of fish was made impossible.

not prove to be significantly different from the values obtained for the control fish.

The fish subjected to 3.0 mg/l arsenic plus 0.02 mg/l cyanide and 6.0 mg/l arsenic plus 0.02 mg/l cyanide all had their hemoglobin levels reduced by approximately 20 percent. These reduced levels were significantly lower than the controls with the exception of the level for the group of fish exposed to 6.0 mg/l arsenic plus 0.02 mg/l cyanide in experiment 3. Eventhough the observed reduction was 20 percent, the variance for the mean was too large to prove a significant reduction.

Effects on Percentage Water and Fat Content

The percentage water content, obtained from the dry weights, were recorded after seven days of drying of the fish at 70°C. Means were then established for each group of fish and presented in Table 7 as a mean percentage water content. The mean percentage fat content was obtained as outlined in the methods and is presented in Table 7.

It was observed that in each of the three experiments, the control groups of fish had the lowest percentage water content. These groups of fish, as seen in Table 7, also had the highest mean percentage fat contents.

The fish exposed to 1.0, 2.0 and 6.0 mg/l arsenic for 21 days, did not display any significant variations

from the control values for the percentage water content. It was also observed that the above groups of fish did not have significantly different percentage fat contents when compared to their respective controls. However, the fish that had been subjected to 3.0 mg/l arsenic, had a significant increase in the percentage water content and a significant decrease in the percentage fat content (see Table 7).

The results for the fish exposed to 0.02 mg/l cyanide, as seen in Table 7, indicate that they had a higher water content than did the control fish. In both instances they had at least 1.0 percent more water than the control groups, but due to a higher variance in experiment 2, this difference was not significant. However, in both experiments 2 and 3, the fat contents of the fish exposed to 0.02 mg/l cyanide, did not vary significantly from the control values.

The fish subjected to a combination of the two toxicants, all displayed significant increases in percentage water content with the exception of the group exposed to 6.0 mg/l arsenic plus 0.02 mg/l cyanide in experiment 3. The increases consisted of a 1 to 2 percent higher percentage water composition. When the mean percentage fat contents were examined, as seen in Table 7, all the groups

showed a significant reduction in fat content, but, there was also one exception; this being the group of fish that had been poisoned with 3.0 mg/l arsenic plus 0.02 mg/l cyanide.

DISCUSSION

Physiological Implications

In this study of the effects of arsenic and/or cyanide on the growth, swimming stamina and respiration of rainbow trout, it was found that arsenic trioxide affected the fish only at the highest concentration tested, 6.0 mg/l as As and then it only had an effect on the rate of growth. However, arsenic used in combination with cyanide caused a greater reduction in growth than did either of these toxicants used alone. Exposure to 0.02 mg/l of cyanide markedly reduced growth and the swimming stamina which was not affected by arsenic.

The question of whether arsenic and cyanide chemically interact in the water had to be investigated before the results of this study could be properly interpreted. Broderius (pers. comm., 1974, see Appendix II for details), states that arsenic and cyanide, in the presence of each other in water, do not form any chemical complexes. However, arsenic trioxide will form arsenate and arsenite ions when dissolved in water; this author then points out that the arsenate ion, a strong oxidizing agent, may oxidize HCN to cyanogen (C_2N_2) and cyanate (CNO^-), which are less toxic than HCN. This oxidation of cyanide to cyanogen and cyanate, could explain why the pyridine-pyralazone method used to

monitor levels of HCN present in the tanks, consistently produced slightly lower values than expected, since this method was not designed to measure cyanogen and cyanate concentrations in the water. Ford-Smith (1964) also studied the chemistry of cyanide complexes and found no complexation occurring between cyanide and arsenic. We therefore assumed that in the current study of the effects of arsenic and cyanide, on rainbow trout, that no chemical complexation occurred between the two chemicals.

In the present study, the growth, swimming ability and respiration of rainbow trout exposed to sublethal concentrations of arsenic and/or cyanide, were used as criteria to measure the effects of chronic exposure to the toxicants.

As seen in Figure 5, arsenic at the concentration of 1.0, 2.0 and 3.0 mg/l did not seem to impair the growth of rainbow trout, while 6.0 mg/l of arsenic was seen to reduce the growth by 65 percent. Gilderhus (1966) reports that the 96 hour LC_{50} for rainbow trout is 25.6 mg/l of arsenic; 6.0 mg/l arsenic would therefore be approximately 0.23 of the LC_{50} .

There is only little information of the chronic effects of arsenic on aquatic fauna. Gilderhus (1966), using bluegills (Lepomis macrochirus), studied the effects of sodium arsenite on various physiological and histopathological processes. He exposed his test fish to the

herbicide by slug doses applied in concrete test pools. He found that over a 16-week period there was a reduction in the growth of the fish. Lawrence (1958), using a similar experimental design, poisoned his ponds with 4.0 and 8.0 mg/l slug doses applied once a month over a two month period, also obtained reductions in the growth of the arsenic poisoned fish. Lunde (1972), using rainbow trout fed on food rations supplemented with both organic and inorganic arsenicals, found that the growth rate of his test fish was also reduced even though this was not the aim of his experiment. In the present study a reduction in the growth of the test fish was also observed, but only at the highest concentration tested. Even though arsenic uncouples oxidative phosphorylation (Lehninger, 1972, p. 324), it may have not had enough of an effect on the growth rate to reduce it when the fish were exposed to it at 1.0, 2.0 and 3.0 mg/l; however, at 6.0 mg/l, the arsenic may have sufficiently inhibited the metabolic pathways to reduce the growth of the test fish.

The cyanide concentration tested in this study (0.02 mg/l HCN) was also approximately 0.25 of the LC_{50} as established for rainbow trout by Brown (1968). Cyanide at the above concentration, as seen in Figure 5, reduced the growth by approximately 55 percent. It was also observed that the HCN had its greatest effect during the first 10 days of the experiment and that after this period, some

adaptation to cyanide seemed to occur as the growth rate was seen to be greater than that of the controls. This phenomenon was also observed by Leduc (1966) working with coho salmon (Oncorhynchus kisutch) and cichlid fish (Cichlasoma bimaculatum). (Using the following concentrations of cyanide; 0.01, 0.02, 0.04 and 0.08 mg/l HCN, Leduc (1966) found that after 24 days the growth of juvenile coho salmon, kept in flowing water at 16°C, was reduced as compared to the controls. He found that during the second half of the 24-day experiment, the salmon exposed to cyanide concentrations of 0.02 to 0.08 mg/l HCN, grew faster than the controls. When cichlids were tested, a similar reaction was observed. Dixon (pers. comm., 1974), working with rainbow trout at cyanide concentrations of 0.01, 0.02, and 0.03 mg/l at 12°C, also noted this reaction of the fish to the exposure to HCN during 18-day growth experiments.

The combination of arsenic and cyanide, 3.0 mg/l As plus 0.02 mg/l HCN, produced no different effect on growth than did 0.02 mg/l HCN alone. Assuming that arsenic and cyanide work at different sites in the metabolic pathways, then this would have been expected since no reduction was observed for the fish exposed to 3.0 mg/l of arsenic. The fish exposed to 6.0 mg/l As plus 0.02 mg/l HCN produced the greatest reduction in growth, 85 percent less wet weight gain as compared to the control fish.

The fat and water contents of the fish were also used as criteria to interpret the nutritional state of the fish. Arsenic, at 1.0, 2.0 and 3.0 mg/l, did not appear to alter the fat and water contents of the rainbow trout as compared to the values obtained from the control fish. However, exposure to 6.0 mg/l of arsenic did increase the water content and decreased the fat content; both of these differences, as seen in Table 7, were not proven to be statistically significant. Similarly, exposure of test fish to 0.02 mg/l HCN increased the water content by approximately 1.0 percent and reduced the fat content by less than 1.0 percent. The combination of the toxicants produced significant differences in both the percentage water and the percentage fat contents. This is indicative of an addition of the effects of the toxicants since the single actions of the poisons were not great enough to produce these types of responses.

An additive effect was only noted in the growth of the fish exposed to 6.0 mg/l As plus 0.02 mg/l HCN where the percent wet weight gain was reduced by 85 percent as compared to the control weight gains. The group exposed to 3.0 mg/l As plus 0.02 mg/l HCN also had a reduced wet weight gain, however, it was not less than that of the group exposed to 0.02 mg/l HCN. This group did not display the same type of initial growth depression as did the group of fish that were exposed to 0.02 mg/l of cyanide,

nor did it show a greater rate of growth after the first 10 days of exposure to the toxicants. This would indicate that the arsenic interfered with the action of the cyanide on the fish and that possibly over a longer period of time this group would have shown less growth than the fish poisoned with 0.02 mg/l HCN and therefore an additive effect of the toxicants would have been observed.

The results exemplify the fact that arsenic has a different mode of action on the fish than does cyanide. Whereas the fish can overcome cyanide poisoning as far as wet weight gain is concerned, they cannot do the same for arsenic. The results suggest that a threshold must be reached before arsenic has an effect on the growth of fish. This threshold seems to be both time related and concentration related. This assumption was made on the data obtained in this study and that presented by Lawrence (1958) and Gilderhus (1966). It was seen that in this study, exposure to 3.0 mg/l of arsenic for 21 days did not affect the growth of rainbow trout and those exposed to 6.0 mg/l suffered a reduction in growth; this indicates a concentration threshold. However, Lawrence (1958) found that bluegills poisoned with 4.0 mg/l of arsenic for two months, showed a considerable decrease in growth. Gilderhus (1966), working with both higher and lower concentrations of arsenic than Lawrence, over a 16-week period, also noted

reductions in the growth of his test fish. Since both of these studies were longer in duration than the present one, it would be valid to suggest that there is a time factor involved with the toxicity of arsenic. This time factor would be related to the concentration and the amount of the arsenic accumulated by the fish. However, it must also be noted that in both of the above experiments, the fish were also exposed to arsenic in their food and from the bottom sediments. In our study the fish were only exposed to arsenic dissolved in the water. Another explanation for why the fish in Lawrence's and Gilderhus' studies showed a decreased growth rate could be the accumulation of both inorganic and organic compounds of arsenic. Lunde (1972), showed that organic arsenicals accumulated to a much greater degree than the inorganic forms. Since the blue-gills obtained their nutrition from invertebrates living in the pools, they could have accumulated organic arsenicals from their food supply.

Warren (1971, p. 153) suggests that when metabolic pathways are partially inhibited by toxic substances, growth will be reduced. A certain amount of energy is always required for standard metabolism, anything in excess will be used for activity and growth. Therefore, metabolic inhibitors such as arsenic and cyanide, retard metabolic processes and reduce the available energy normally used for

activity and growth. As noted in the results, the fish poisoned with arsenic or, arsenic and cyanide, at all concentrations of arsenic, appeared to be more sluggish and fed less than the other groups. Therefore, since there was a decrease in food consumption, it would be expected that these fish would undergo less growth and reduced activity. This was the case for the fish exposed to 6.0 mg/l As, 3.0 mg/l As plus 0.02 mg/l HCN and 6.0 mg/l As plus 0.02 mg/l HCN, while there were no measurable effects on the fish exposed to 1.0, 2.0 and 3.0 mg/l of arsenic.

Swimming stamina tests were performed to evaluate the effects of arsenic and/or cyanide on the physical abilities of rainbow trout. The results indicate that previous exposure to cyanide, either alone or in combination with arsenic can seriously reduce the swimming ability of trout tested at a water velocity of 1.54 ft/sec. (47.0 cm/sec). This decrease was expected since various other researchers (Neil, 1957; Leduc, 1966; Bröderius, 1970) had also reported decreases in swimming stamina due to previous exposure to sublethal levels of cyanide. Neil (1957) tested brook trout (Salvelinus fontinalis) at 1.86 ft/sec. that had been previously exposed to 0.01, 0.03 and 0.05 mg/l CN⁻ for 15 days and observed reductions in swimming ability from 75 to 98 percent. Leduc (1966), testing

the maximum sustained swimming speed of cichlids observed a reduction in the groups of fish previously exposed to 0.04 mg/l HCN. Broderius (1970) tested the swimming to exhaustion of coho salmon that had been previously exposed to 0.01, 0.03 and 0.05 mg/l HCN for 8 days before the swimming tests. He also performed swimming experiments exposing the salmon to the above concentrations during the tests. He observed reductions in swimming times that were inversely proportional to the cyanide concentrations; that is the higher the cyanide levels, the less time the fish were able to swim and the more time necessary to recover to the control levels after termination of exposure to cyanide.

It must be noted however, that in this study the fish were only subjected to relatively short periods of swimming at a water velocity of 1.54 ft/sec. until fatigued. The short test period might explain why no noticeable effects on swimming ability were noted for the rainbow trout that had been exposed to arsenic alone. If the test had been longer in duration, then perhaps exposure to arsenic may have shown to be deleterious to the swimming ability of rainbow trout.

The swimming ability of fish may be reduced for a variety of reasons. If the metabolic pathways are inhibited by toxicants then the available energy would be reduced and

a decreased swimming stamina would be the end result. Such was the case for fish poisoned with cyanide, both in this study and those of Neil, Leduc and Broderius. A second reason for a reduction in swimming stamina would be an interference of the $O_2 - CO_2$ exchange at the gills. This could be brought about in the following ways; physical agents such as pulp fiber have been reported to obstruct normal gill ventilation of fish by MacLeod and Smith (1966) and therefore reduced the swimming performance of fish. Another manner by which the gas exchange at the gills may be interfered with is a histopathological condition produced by a toxicant. Gilderhus (1966), discovered a higher incidence of hemorrhagic globes on the gills of bluegills exposed to sodium arsenite than those of his control fish. Since no histological work was performed in this study, it cannot be said that this was also the case for the rainbow trout exposed to arsenic, however, the possibility does exist. It is apparent from the results that even if this histopathological condition did exist in the test fish, it did not disrupt the gas exchange sufficiently to reduce the swimming ability of the trout tested in this study.

The swimming tests were designed to measure the effects of the toxicants on the active metabolic rate of the fish. Respiration studies were performed to examine

the consequences of arsenic and/or cyanide poisoning on the standard or basal metabolism of rainbow trout. At 6.0 mg/l of arsenic (Figure 6), the greatest differences between the control and poisoned fish were observed, however, these differences were not statistically significant. Similarly all the other differences were also not significantly different.

After previous exposure to the toxicants, the fish in the respirometry experiments in most instances, displayed an initial increase in oxygen consumption with a drop in respiration occurring after the second or third day. This suggests an initial increase in respiration due to the different surroundings and an eventual acclimation after a period of time. This was more pronounced in the groups of fish tested during the first experiment; possibly because of their smaller size. The rainbow trout toxified with 0.02 mg/l of cyanide had oxygen consumption rates that closely followed those of the control fish throughout the respiration studies. Dixon (pers. comm., 1974), found in his respiration studies that previous exposure to cyanide, also at 0.02 mg/l, just significantly affected the oxygen consumption of his 12-gram rainbow trout. In the present study, rainbow trout with an average weight of 28 grams were used and this is most likely why no similar results were obtained. The toxicity of cyanide seems to be size

related since 5 gram fish used in another experiment by Dixon had a greater difference in their respiration rates from the control fish oxygen consumption, than did the 12 gram trout. It must be noted at this point that the swimming times of the cyanide treated fish were greatly reduced as compared to the control fish. These results would lead to the assumption that the cyanide did not depress the basal metabolic rate, but it did however, inhibit the active metabolism of the fish, even though they seemed to acclimatize to the toxicant when their wet weight gains were examined.

The hemoglobin levels obtained may not be conclusive enough to make any valid statements or comparisons. The number of fish sampled was actually too small, since the differences within one group tended to influence the mean value with too much weight. This is emphasized by the fact that the groups of fish poisoned with 1.0, 2.0 and 3.0 mg/l of arsenic displayed reduced hemoglobin levels while those exposed to 6.0 mg/l showed no reduction in the hemoglobin levels of their blood. This seems to be a highly unlikely occurrence, since arsenic tends to promote hemolysis of the blood and will also reduce the hemoglobin content (Fowler and Weissberg, 1974). However, while cyanide alone had no effect on hemoglobin levels, the combination of arsenic and cyanide did. Also there does not seem to be any correlation between the reduced hemoglobin levels and

and the swimming stamina of the test trout, since only the fish that had been treated with cyanide, either alone or with arsenic, displayed reductions in their swimming ability.

The fish exposed to 6.0 mg/l showed a decreased growth rate, but their respiration and their swimming ability as previously mentioned, did not differ significantly from the control fish. This suggests that their metabolic pathways had not been sufficiently inhibited to fully restrict either the basal or the active metabolism.

Bilinski (1969) suggests that for prolonged swimming, such as undertaken by migrating salmon, fish rely on the aerobic metabolism of fat in the red muscle as a source of energy. Therefore if fish exposed to 6.0 mg/l of arsenic were able to maintain a certain degree of active metabolism they would be able to endure a maximum sustained swimming speed for longer periods of time than those exposed to cyanide, or cyanide and arsenic, since cyanide greatly inhibits aerobic respiration. This was evident from the results of the growth and swimming experiments. Kruzynski (1972) observed similar results when fish exposed to the higher concentrations of dietary methoxychlor, showed longer maximum sustained swimming times than the controls. He suggested that an increase in anaerobic metabolism was responsible for the better performance of these fish. Studies by Black

et al., (1962), on rainbow trout, and by Beamish (1968) on cod, in both cases showed that these fish utilized more than 80 percent of their muscle glycogen during fifteen minutes of continuous strenuous activity. It is also recognized that at cruising speeds aerobic active metabolism is the main energy source. Smit et al., (1971), from their experiments on goldfish (Carassius auratus), suggested that when a fish exceeds a swimming speed of 3.4 body lengths per second, which is greater than maximum cruising speed, it maintains its sustained swimming speed through both aerobic and anaerobic metabolism in the red and white muscle fibers. However, the water velocity used in this study was higher than the cruising speed and lower than burst speed, since the fish swam at approximately 3.5 body lengths per second (Brett, 1964). Therefore, it is possible that a phenomenon similar to what Smit et al., observed occurred in the group of fish exposed to 6.0 mg/l of arsenic, and this would explain why the poisoned fish displayed the same swimming abilities as the control fish, especially if there was increased anaerobic metabolism occurring due to the exposure to the toxicant.

Ecological Implications

The release of arsenic from mine sites into the surface waters like in the Yellowknife area of the Northwest Territories, has reached dangerous levels. Kam Lake, which

measures approximately 2.5 miles in length, has been thoroughly contaminated with the arsenic discarded along with other mine tailing effluents. Levels as high as 4.0 mg/l of arsenic in the water, 3.3 ppm in the muscle of Northern pike (Esox lucius), and up to 16,700 ppm in bottom sediments have been reported by Falk et al., (1973). Long after the source of arsenic will have been arrested, the bottom sediments will continue to leach out arsenic and pollute this aquatic environment. With these high levels of arsenic present; bioaccumulation of the element by phytoplankton and zooplankton, will promote higher uptake of arsenic by the fish; eventually reaching man and being a potential health hazard.

Since arsenic strongly accumulates in the bottom sediments (Lis and Hopke, 1973; Falk et al., 1973), there seems to be a direct effect on the benthic invertebrates. Gilderhus (1966), found that there was a decrease in both the diversity and the number of representatives of each species of invertebrates in his test pools and that this was proportional to the arsenic concentrations in the sediments.

In the present study, fish were only exposed to arsenic dissolved in water. It had been intended to compare the level of arsenic accumulation in the test fish to the levels recorded in other studies, but as explained in the method section, the arsenic residue analysis was not

possible to perform. However, it may be valid to assume that any of the results obtained in this study would be magnified where fish are exposed to arsenic both in the water and in their diet. The growth rate would possibly be retarded in a greater manner than was observed in this study, the respiration rate could be reduced to a greater extent and possibly the swimming stamina would also be affected in a deleterious manner.

It must be noted at this time that the question of the action of cyanide has not yet been discussed. The mining operations in the Yellowknife area use large quantities of cyanide to leach out the gold from the ore. Cyanide is extremely toxic and when levels as high as 10 mg/l and higher occur, one does not expect to find any aquatic fauna that are still alive (Bérubé. 1971). However, further downstream from the mines, the cyanide concentrations drop and the question of multiple toxicity at sublethal levels arises.

When the fish are subjected to cyanide as well as to arsenic, as was seen in this study, the growth of the fish was reduced to a greater degree than that was observed for each of the toxicants acting singly. Also, since the fish in the natural conditions are exposed to arsenic in both the water and in their diet, it might be suspected that the action of arsenic and cyanide on these fish would be greater than that observed in this study.

Upon exposure to arsenic and cyanide, histopathological conditions have been shown to occur in fish. Both of these toxicants have been known to create histopathological disorders in the reproductive systems of fish (Gilderhus, 1966; Ruby and Dixon, 1974). Gilderhus found that in the pools treated with the higher concentrations of arsenic, the fish histologically examined had ovaries that displayed 50 percent of the oogonia, primary oocytes and secondary oocytes as encapsulated cells with a rim of cytoplasm of varying density around the inner surface of the vitelline membrane. Ruby and Dixon observed a decrease in spermatogenesis in immature rainbow trout that was proportional to the concentration of cyanide that they had been exposed to. If the reproduction of the fish is retarded then eventually the fish population of a lake would disappear because of the lack of new recruits. Also with a reduction in the swimming ability these fish would be better prey for a predator species and would also have their scope of activity reduced.

This study was performed under very artificial conditions, but it has shown some effects of arsenic and cyanide that may have important ecological implications. It would be more ecologically significant if in future work the trout were exposed to arsenic in both their water and in their diet, since under natural conditions this is what

occurs. It would also be important to measure the accumulation of arsenic by both phytoplankton and zooplankton.

SUMMARY

Three experiments were carried out to determine the effects of chronic arsenic and/or cyanide poisoning on the growth and swimming stamina of rainbow trout (Salmo gairdineri).

The experiments were performed in white, translucent polyethylene flow-through tanks designed to hold 120 l each at 11.0°C. Each experiment had a duration of 21 days during which the fish were exposed to 1.0, 2.0, 3.0 and 6.0 mg/l arsenic, 0.02 mg/l cyanide, 3.0 mg/l As plus 0.02 mg/l HCN and 6.0 mg/ As plus 0.02 mg/l HCN in their water supply.

The results suggest that arsenic at 6.0 mg/l had deleterious effects on the growth of the test trout. It was also seen that cyanide seriously reduced the growth and that fish exposed to 6.0 mg/l As plus 0.02 mg/l HCN, had a greater reduction in growth than either of the above groups. Swimming stamina was not affected by arsenic alone but was by exposure to cyanide and a combination of arsenic and cyanide. The respiration results are inconclusive and to derive any conclusions is difficult. Significant differences in the hemoglobin levels and in the fat and water contents were obtained and some of these differences support the results obtained for the growth and swimming stamina experiments.

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APPENDIX I

Breakdown of the components of the Purina Trout Chow.

| | | |
|----------|---|-------|
| Protein | - | 40.0% |
| Fat | - | 2.5% |
| Fiber | - | 5.5% |
| Ash | - | 13.0% |
| Minerals | - | 3.0% |
| Water | - | 36.0% |

APPENDIX II

Personal communications from Dr. Steven Broderius,
Department of Entomology, Fisheries and Wildlife, University
of Minnesota. (see following pages).



UNIVERSITY OF MINNESOTA
TWIN CITIES

Department of Entomology, Fisheries, and Wildlife
St Paul, Minnesota 55101

April 22, 1974

Mr. Menno R. Speyer
Biology Department
Sir George Williams University
1455 de Maisonneuve Blvd., West
Montreal 107, Quebec

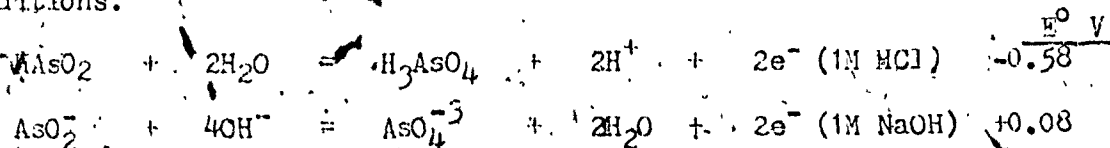
Dear Mr. Speyer:

In response to your letter of April 3rd, I have outlined a possible explanation for your observed results and some suggestions to confirm the effect which arsenic compounds may have on cyanide solutions.

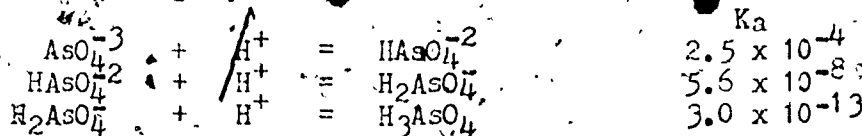
Upon review of some publications which discuss those metals which complex with cyanide, it can be concluded that arsenic does not form a cyanide complex. However, an explanation of your observed phenomenon that cyanide toxicity is reduced in the presence of arsenic may be related to various possible chemical reactions occurring between arsenic species and free cyanide.

As you are probably aware, the principal oxidation states of arsenic are +3 and +5. In the +3 state arsenic forms arsenious oxide (As_2O_3 or As_4O_6). When treated with water, As_2O_3 gives a slightly acid solution which is thought to contain the hydroxide $As(OH)_3$ or H_3AsO_3 (also written $HAsO_2$). This hydroxide is amphoteric; it can neutralize acids to give solutions containing $As(OH)_2^+$, and it can neutralize bases to give solutions containing arsenite ions (variously written as $H_2AsO_3^-$, AsO_2^- , or $As(OH)_2^-$).

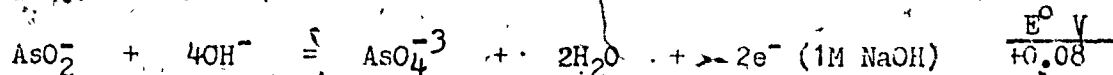
The arsenic trioxide (As_2O_3) dissolves very slowly in water or acid but readily in sodium hydroxide. Your arsenic stock solutions should be prepared by dissolving a known amount of the pure substance dried at 110°C in an excess of sodium hydroxide and adding hydrochloric or sulfuric acid until the reaction is neutral or slightly acid. Neutral or weakly acid solutions are quite stable and can be kept unchanged for long periods of time. However, an alkaline solution of the arsenic trioxide should not be kept, because arsenite is slowly oxidized to arsenate by air under these conditions.



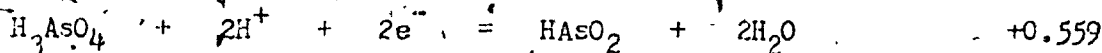
In the +5 state the principal compounds of arsenic are arsenic acid and its derivatives, the arsenates. Arsenic acid is primarily orthoarsenic acid, H_3AsO_4 , a triprotic acid. The hydrogen ion concentration plays an important role in determining the state of equilibrium. With a change in pH the following equilibria must be considered as well:



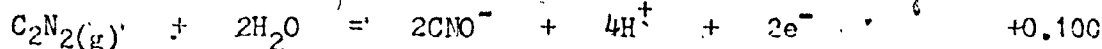
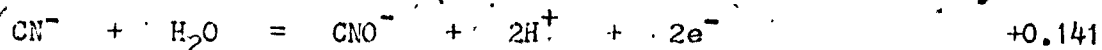
Arsenate ion is considered to be a strong oxidizing agent and in the presence of cyanide it is conceivable that it could oxidize cyanide to cyanogen (C_2N_2) and cyanate (CNO^-), which are much less toxic than HCN. Therefore, if your arsenic trioxide stock solutions are alkaline a mechanism for the conversion of cyanide in your test aquaria to an essentially non toxic cyanogen compound is most probable. The mechanism equations involved are as follows:



Upon dilution of the stock arsenic solution by the test water containing cyanide:



This reaction can then be coupled with a number of possible reactions involving the oxidation of cyanide:



My research with various cyanide species over the last seven years has convinced me that a method for determining the cyanide concentration in test solutions is essential in order to do meaningful research with this toxicant. In your instance direct use of the pyridino-pyrazolone method for cyanide, or some other appropriate method, would be applicable for determining free cyanide (i.e., CN^- and HCN) in test solutions where arsenic is present since an arsenic-cyanide complex is not formed. Therefore, I suggest that your future research plans incorporate analytical determination of not only cyanide but also arsenic in the test solutions to see if the above explanation for reduced cyanide affect in the presence of arsenic is appropriate.