NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

Canada

Investigations on the Toxicokinetics of Cyanide in Juvenile Rainbow Trout

Yves Bois

A Thesis

in

The Department

of

Biological Sciences

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
Concordia University
Montréal, Québec, Canada

January 1988

ISBN 0-315-41631-9

ABSTRACT

Investigations on the toxicokinetics of cyanide in juvenile rainbow trout (Salmo gairdneri).

Yves Bois

The object of this research project was to examine the toxicokinetics of cyanide and thiocyanate in relation to their possible effects on the thyroid gland of rainbow trout. Juvenile trout were exposed to sublethal concentrations of cyanide 0,01 mg/L HCN (0,34 uM HCN) and 0,02 mg/L HCN (0,74 uM HCN), at different times of the year under laboratory conditions for periods of up to 30 days.

We measured the bioaccumulation of cyanide (HCN) and thiocyanate (SCN) in the blood plasma of trout. The concentrations of plasma cyanide reached their maxima within the first 48 hours of exposure and remained constant until the end of the experiment. Plasma concentrations in the summer were about 2 times higher then in the winter. Intriguingly, fish showed an apparent ability to bioconcentrate cyanide from the water which was also affected by season. No dose-response relationships could be demonstrated between the plasma concentrations and the cyanide levels in the water.

Higher concentrations of thiocyanate were observed during the summer then in the winter. During winter, maximum concentrations were established by day 15 and remained

constant until the end of the experiment. No dose-response relationships were established between the plasma SCN concentrations and the cyanide levels in the water.

The clearance of thiocyanate from plasma was also monitored for 15 days following a 30-day exposure period to cyanide. Seven to 15 days were required for the complete elimination of thiocyanate.

The height of the epithelial cells of the trout thyroid follicles were also measured. After 15 days of cyanide exposure during the summer, the highest exposure concentration (0,02 mg/L HCN) produced a 13 % increase in their height.

ACKNOWLEDGEMENTS

I wish to express my thank to Dr. G. Leduc for his constructive criticism in the preparation of this thesis. I am also grateful to the other committee members, Dr. S.M. Ruby and Dr. B. Hill. The contribution of Dr. P.D. Anderson and Dr. D. Fairbairn was also much appreciated.

The daily support of my friends from the fish lab greatly facilitated the achievement of this thesis. Thanks for those never ending suppers.

I also want to thank Alyne Groleau for her patience and her comprehension during the completion of this degree.

Finally, I would like to express my appreciation to my parents as well as to my family for their constant support and encouragement throughout all my studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (Cont'd)

LIST OF FIGURES

INTRODUCTION

This study was undertaken in an attempt to enlarge our understanding of the sublethal toxicokinetics of cyanide in rainbow trout (Salmo gairdneri) and of its possible effects on thyroid function. We also investigated the effect of season on the bioaccumulation of cyanide and thiocyanate.

Cyanide toxicity has received much interest in fish toxicology research. Cyanide sublethal effects have been described under various exposure conditions and suggest an effect on the intermediary metabolism. The effect of cyanide exposure on growth rate (Dixon and Leduc, 1981, Leduc, 1966b, McCraken and Leduc, 1980, Kovacs and Leduc,1982b, McGeachy, 1984), oxygen consumption (Dixon and Leduc,1981), and, among other parameters, swimming ability (Kovacs and Leduc,1982b, McGeachy, 1984), have been traditionally attributed to the action of the cyanide ion on the respiratory chain.

It is only recently that Raymond et al (1986) directly tested this hypothesis by measuring liver cytochrome oxidase activity in trout exposed to various cyanide concentrations which have been shown to induce different physiological responses. Since maximum inhibition was achieved at the lowest exposure concentration, it suggests that cytochrome oxidase inhibition can hardly explain the variation in sublethal responses observed at the different exposure concentrations.

It has also been demonstrated that a portion of the plasma cyanide load is transformed into thiocyanate in the fish body (McGeachy, 1984, Raymond et al, 1986). Consequently, the cyanide exposure concentration can be suspected of not reflecting the effective concentration to which the fish are exposed. No report of the plasma cyanide concentrations established under cyanide exposure was found in the literature.

Thiocyanate accumulates in plasma presumably because of its reabsorption at the kidney level. However, thiocyanate has been shown to be excreted once it reached a sufficient plasma concentration (Hemming et al,1985). This leakage may occur in fish exposed to low cyanide concentrations due to kidney tissue necrosis (Dixon and Sprague,1981). Sudden drops in plasma thiocyanate reported in earlier works (McGeachy, 1984, Raymond et al, 1986) could possibly be explained, as these authors suggested; by the kidney condition. If this is true, thiocyanate would be excreted rapidly from the plasma and significant concentration of plasma thiocyanate would not be maintained for very long after cyanide exposure is arrested.

Although thiocyanate is less toxic then cyanide, it has been shown to be a powerful hypothyroidal inducing agent in mammals (Kreutler et al, 1977, Bourdoux et al, 1978). Thiocyanate was shown to compete with iodide for its incorporation at the thyroid follicle level in rainbow trout

(Eales and Shostak, 1983). Cyanide, through its possible effect on thyroid peroxidase can also depress thyroid function (Ohtaki et al, 1985, Turner and Bagnara, 1976). Administration of thyroid hormones were shown to facilitate salt water adaptation of juvenile rainbow trout (Leatherland, 1985) and to modify growth rate (Higgs et al, 1982). These physiological processes were shown to be affected under cyanide exposure (Leduc, 1984). Consequently the possible interaction between thyroid function and thiocyanate and/or cyanide might help to understand some of cyanide's sublethal effects.

Season of experimentation was observed to modify the lethal toxicity of cyanide with a maximum sensitivity occurring during winter (McGeachy and Leduc, 1988). Since some of the factors controlling the effective dose of a toxicant (uptake, biotransformation, clearance) are affected by the organism metabolic rate which in turn is modified by season (Beamish, 1964), season might affect cyanide and thiocyanate bioaccumulation.

We tried to answer the following questions:

Could blood plasma cyanide concentrations explain the cytochrome oxidase inhibition in fish exposed to sublethal concentrations of cyanide? How are these levels affected by season of experimentation?

What is the pattern of plasma thiocyanate accumulation and how is it affected by season of experimentation?

Does cyanide exposure affect trout thyroid function?

In order to answer these questions, groups of fish were examined at different time intervals over 30 days of exposure to 2 concentrations of cyanide as well as at different times of the year. Groups of control fish were also maintained. Plasma cyanide, thiocyanate and hematocrit were measured as well as the histological appearance of the thyroid. One experiment was followed by a clearance period of 15 days during which plasma thiocyanate, cyanide and hematocrit were measured. We also monitored changes in total plasma iodide concentration and in the height of the thyroid follicles epithelial cells in a final 30-day experiment.

MATERIALS AND METHODS

FISH

Parr stage rainbow trout (Salmo gairdneri) used in the first experiment were obtained from "La pisciculture Mt-Sutton", Sutton, Québec. Trout for the subsequent experiments were acquired from "La pisciculture des Landes", St-Felix de Kingsey, Québec, in order to obtain fish of similar size (30 g). In both instances, fish were transported in plastic bags pressurized with oxygen. Upon arrival at the laboratory, the fish were given a 10-day adaptation period to laboratory conditions in 200-L oval shaped fiberglass tanks at an approximate stocking density of 150 fish per tank. A continuous flow of dechlorinated city of Montréal water (2L/min), maintained at a constant temperature of 12 +/- 1°C, was delivered to the tanks by plastic PVC piping.

The photoperiod established during the holding period (12 h dark:12 h light) was identical to the one maintained during experiments. All tanks were illuminated with rows of fluorescent lights. No mortality occurred during the holding period.

At the end of the holding period, the fish to be tested were randomly selected and distributed to the bioassay tanks for an additional two weeks of adaptation to the test tanks and water.

EXPERIMENTAL CONDITIONS

Tanks

All experiments were conducted in 90-L rectangular white polyethylene tanks. Each tank was covered with a black plastic cover in order to minimize disturbance of the fish.

Water

Each tank was supplied with a continuous flow of water (1,7 L/min) controlled by predictable flowmeters. This flow of dilution water gave a 99% replacement in 4 h as calculated from Sprague (1973).

The water temperature, dissolved oxygen concentration and pH were measured every other day in the tanks. Oxygen was measured with a YSI model 54 oxygen meter and the pH with a combined electrode pH meter (Radiometer, #29). Water hardness and alkalinity data were obtained from the city of Montréal. The iodide concentration was also considered since it was shown to affect the plasma iodide concentration (Gregory and Eales,1974). This parameter was also obtained through the city of Montréal laboratories. The mean values for these water quality parameters are reported in Table 1.

Toxicant

Stock solutions of cyanide were prepared from technical grade KCN (Anachemia Chemicals). The cyanide stock solution concentration was assayed weekly by titration with standard

0,1 N silver nitrate solution (Vogel, 1955,p263). Mariotte bottles, previously acid washed, were utilized to meter the toxicant into the tanks (Leduc, 1966a). On day 0, the desired cyanide concentrations were established by the addition of a calculated volume of the stock solution and later maintained with the Mariotte bottles. The test concentrations were verified 5 time a week by colorimetric determination (Lambert et al,1975). The mean observed concentrations of cyanide in the different tanks as well as their standard deviation are reported in Table 2.

Food

The iodide content of some commercially available fish food is unrealistically high as compared to natural food (Gregory and Eales, 1974). Because dietary iodide has a major impact on plasma iodide levels (Gregory and Eales,1974) a low iodide experimental diet was prepared as per Kruzynski (1972). The diet consisted of a mixture of 10 parts of distilled water, 4 parts of a mixture of beef heart, beef liver and fish food (3:3:1 wet weight basis) and 1 part of gelatin (dry weight basis). This diet was prepared for each experiment and kept frozen at -20 °C. The fish food utilized in the mixture was the Purina brand pelleted fish food; size 3 pellets.

During the experiments the fish were fed daily, in late morning, with a maintenance ration of 1,4% of the body weight except for the day prior to sampling when no food was given.

Table 1. Physico-chemical parameters of the dilution water during the different experiments.

PARAMETER	EXPERIMENT AND DATE		
	#1 August 1986	#2 Nov-Dec. 1986	#3 Nov-Dec 1986
pH	7,11	7,64	7,64
TOTAL HARDNESS (mg/L as $CaCO_3$) 1/	127	133	133
ALKALINITY (mg/L as $CaCO_3$) 1/	85	90	90
TEMPERATURE (^{o}C)	13,1	12,4	12,4
DISSOLVED OXYGEN (% SATURATION IN TANK)	93	89	87
TOTAL IODIDE (ng/ml) 1/	6,2	6,1	6,1

1/: Obtained through the city of Montréal.

Table 2. Means and standard deviations of the observed cyanide concentrations in the various replicates of the different experiments. Fish mortality during the different exposure periods is also given 1/.

EXPERIMENT AND DATE	NOMINAL CYANIDE CONCENTRATION (uM HCN)	OBSERVED CYANIDE CONCENTRATION (uM HCN) MEAN	(S.D.)	N	FISH MORTALITY N 1/
# 1 August 1986	0,37	0,36	0,02	(11)	0
		0,36	0,03	(13)	0
		0,34	0,04	(14)	0
	0,74	0,75	0,04	(11)	0
		0,69	0,11	(12)	2
		0,72	0,07	(13)	0
# 2 November-December 1986	0,37	0,41	0,08	(11)	0
		0,35	0,09	(12)	1
		0,35	0,07	(13)	0
	0,74	0,72	0,06	(15)	0
		0,72	0,04	(16)	0
		0,73	0,05	(15)	0
#3 November-December 1986	0,37	0,38	0,06	(17)	0
		0,38	0,07	(18)	0
	0,74	0,74	0,06	(18)	0
		0,71	0,10	(17)	1

1/: Deaths from the accidental exposure to lethal concentrations of cyanide on day 20 of experiment #1 are not reported.

EXPERIMENTAL DESIGN

Three experiments were conducted , one in August 1986 (#1) and the two others (#2 and #3), simultaneously, in late November and December 1986. The concentrations of cyanide tested were 0,37 uM HCN (0,01 mg/L HCN) and 0,74 uM HCN (0,02 mg/L HCN). These are sublethal levels and known to induce different physiological responses (Leduc, 1984). In each experiment, there was a control group submitted to the same conditions except for cyanide.

Experiments #1 and #2 were designed to investigate the kinetics of cyanide and thiocyanate bioaccumulation during a period of 30 days in summer and winter. In both instances, blood cyanide, thiocyanate and hematocrit were measured. In addition, the thyroid tissues were taken for histological preparation. In the first experiment, the gonads were also removed to establish the sex of the fish and to determine if there were any sex related responses.

The protocol involved exposure of three groups of 28 fish to each concentration of cyanide . On each sampling day, 3 fish were randomly removed from each tank providing a sample size of 9 fish per treatment level . This design allowed for comparison among tanks and alleviated the potential bias that could have been introduced by the spatial distribution of the tanks. It also allowed for larger sample sizes without undue crowding of the fish.

For the first experiment (summer), sampling was originally scheduled on days 0, 2, 7, 15, 20 and 30. Unfortunately, all cyanide exposed fish died on day 20 because of an accidental exposure to lethal concentrations of cyanide. Since the patterns of plasma thiocyanate accumulation for the first two weeks of cyanide exposure were already well documented (McGeachy, 1984, Raymond et al, 1986), the samplings for the second experiment (winter) were performed on days 0, 15, 20, and 30 after which the metering of cyanide was arrested. The depuration pattern of thiocyanate and cyanide were subsequently monitored on days 32, 37, 40 and 45. Due to technical difficulties in analytical equipment no value of plasma cyanide could be determined on day 0, 30, 32, 37, 40 and 45.

The third experiment (winter) was designed to test the possibility that cyanide exposure could affect the thyroid function. For that purpose we measured the plasma total iodide levels as well as the height of the epithelial cells of follicles taken from the subpharyngeal region of the fish. This portion of the study involved duplicate exposure of groups of 30 fish to the same concentrations of cyanide as in the first two experiments. On days 0, 2, 7, 15 and 30, 5 fish from each tank were sampled giving a total sample size of 10 fish per treatment level per sampling day. Blood samples were also collected for hematocrit measurements.

SAMPLING PROTOCOL

On the prescribed day the fish were sampled between 10h00 and 13h00. Each fish was individually anesthetized in a 0,012 % solution of 3-aminobenzoic acid ethyl esther methanosulfate (MS-222; Sandoz).

Each fish was blotted dry, the fork length measured at +/- 0,01 cm and weighed to the nearest 0,01g. Blood samples were then collected by severing the caudal peduncule at a point approximatively 1 cm posterior to the anal opening. About 0,1 ml of blood was first collected with a disposable heparin-coated microhematocrit tube which was then closed at one end with critoseal. The capillary tubes were centrifuged at 6000 rpm for ten minutes and the percent of packed cell volume measured. Blood samples for cyanide, thiocyanate and iodide analysis were collected with a 5-ml disposable syringe (Becton-Dickinson) previously rinsed with a 0,07% solution of sodium heparin. These blood samples were immediately transferred to a 1,5-ml micro Eppendorf tube which was kept on ice.

The fish was then killed by sectioning the spinal cord with a scalpel. The subpharyngeal region was subsequently removed and was rapidly transfered into 20 ml of freshly prepared Bouin's fixative. These samples were used for measurement of the epithelial cells of the thyroid follicles.

The blood samples were later centrifuged in a cold room (4 ^{o}C) for 4 min, the plasma transferred into clean microcentrifuge tubes and immediatly frozen in liquid nitrogen. These samples were kept frozen at -20 ^{o}C until analysis.

PLASMA CYANIDE MEASUREMENTS

Since numerous studies on mammalian blood have demonstrated that 99% of the blood cyanide content is bound to the erythrocytes (Vesey and Wilson, 1978), any plasma sample showing visible signs of hemolysis was discarded to prevent any contamination.

The free cyanide was first isolated from the plasma sample by a diffusion procedure conducted in polyethylene Conway micro diffusion cells (Ganjeloo et al, 1980). A 0,1 or 0,2-ml aliquot of plasma was added to one end of the outer chamber of the diffusion cell and 1 ml of H_2SO_4 was tranferred to the other end. A 0,4-ml aliquot of 0,1N NaOH was added to the inner chamber. The cell was then covered with a lid coated with Lubriseal and the liquid contained in the outer chamber were finally mixed by gentle rotation. Upon acidification, cyanide is driven out of the plasma and is absorbed by the NaOH solution. Diffusion time was set at 4 h at room temperature (20 ^{o}C) and each cell was mixed at 30-min intervals by a gentle tilt. The efficiency of this method was first ascertained by a series of recovery tests in

which different quantities of cyanide and/or thiocyanate were added to plasma samples. Mean recovery was 97%.

The NaOH solution was then assayed by the modified method of Toida et al (1981). An aliquot of 0,8 ml of acetate buffer (pH 5, 0,3M) was slowly added to a 0,2ml aliquot of the NaOH solution which had been previously transferred into a test tube. The two solutions were gently shaken and 0,2 ml of a 0,1% chloramine T solution added to the test tube. Chlorination time was set at 30 sec. Finally, 0,2 ml of the barbituric acid, hydrochloric acid and pyridine reagent was pipetted in a timed sequence. The tubes, covered with parafilm, were shaken once more before being incubated at room temperature for 15 min. The percent relative fluorescence was measured in an Amico-Bowman spectrofluorometer (excitation:emission; 583-607 nm). The relative fluorescence values were compared to a standard curve established daily from cyanide solutions mixed in 0,1 N NaOH. The detection limit of this technique was 0,005 uM HCN.

PLASMA THIOCYANATE MEASUREMENTS

The plasma samples were first deproteinized as per Raymond et al (1986). Two analytical techniques had to be used due to difficulties encountered with the spectrofluorometer. In the first experiment, the samples were assayed for SCN following the method of Toida et al

(1981) while in the second, they were analysed as per Raymond et al (1986). The two methods agreed on the measured values.

TOTAL PLASMA IODIDE

All glassware utilized for the measurement of iodide was soaked for a minimum of 12 h in a chromic acid bath. The plasma samples were first prepared by a modification of the acid digestion technique developed by Aysola et al (1987). A 0,1-ml aliquot of the plasma sample was added to a 60-ml Teflon PFA container (Savillex corp.). Next, a 1-ml volume of an acid mixture (H_2SO_4 : HNO_3 : $HClO_4$; 3:1:1) was pipetted into the vessel. The lid was then screwed tightly and the vessel transferred into a larger sealable plastic container. The container was placed in a commercial microwave oven alongside a beaker containing about 20 ml of distilled water and a few boiling chips. After being heated at maximum power setting for 2 min, the samples were cooled on ice for 5 min. Since a yellow colored-liquid, presumably nitrous oxide, is produced upon heating and could interfere with the colorimetric determination of iodide, the vessel and its contents were heated for an additional 105 sec as before with the exception that the lid was placed ajar on the inner vessel. The samples were finally diluted in 25-ml volumetric flasks with distilled water before being assayed. The efficiency of this digestion method was confirmed by a series of spiking tests. The mean recovery was 96 %.

The diluted samples were assayed by the technique of Faulkner (1961). The reaction relies on the reduction of ceric ammonium sulfate by arsenic in presence of iodide which acts as a catalyst. However, the brucine sulfate addition step was omitted since it did not give reproducible results. The absorbance was read on a spectrophotometer (Spectronic 20) at 410 nm after a 15-min reaction period and the iodide concentration derived from a standard curve established weekly.

HISTOLOGY

The tissues kept in Bouin's fluid were cut into 3-mm pieces, washed in 70 % ethyl alcohol, infiltrated with paraffin (Fisher, m.p.:56-57 ^{o}C) and embedded. Sections of 8 um sectioned from the immediate anterior portion of the bulbus arteriosus were prepared for each fish. Five slides were prepared for each fish by staining with Harris' modified hematoxylin and eosin Y and mounted in permount. For each fish, the heights of 5 different epithelial cells in 5 separate intact follicles were measured with an eyepiece micrometer under oil immersion (1000X).

STATISTICAL ANALYSIS

Statistical analysis of the data was carried out with the Statistical Package for the Social Sciences (SPSS) available on the Concordia University Cyber 1. Raw data were analysed

in the following way; homoscedasticity was first ascertained with the help of the Bartlett's tests (p=0,05) before being analysed with a two-way ANOVA with 2 nested levels of factors. Data were log transformed when the variance of the raw data were shown to be heteroscedastic. Blood plasma thiocyanate, cyanide and total iodide data required this transformation. When a statistically significant difference was observed, an LSD mod test was utilised a posteriori to determine which treatment mean was different from the other. The effect of season on plasma thiocyanate accumulation was evaluated by comparing the log transformed data (pooled by treatment and experiment) with a three-way ANOVA. To test for a cyanide effect on the depuration curve of thiocyanate, a two-way ANOVA was carried out on the pooled data without using the controls. Other tests were used to ascertain secondary hypothesis and are described in the Results section.

RESULTS

The effect of season on the patterns of blood plasma cyanide and thiocyanate accumulation was assessed during two experiments conducted at different times of the year. The August 1986 experiment used fish averaging 33,20 g while the November and December 1986 experiment was carried out with fish weighing 30,02 g (Table 3).

CYANIDE ACCUMULATION

Blood plasma concentrations of cyanide measured during this study are reported in Tables 4 and 5 for the summer and the winter experiments respectively and illustrated in Figures 1 and 2.

In both experiments the background levels of cyanide in control fish remained constant over time at about 0,25 uM HCN with no significant difference between seasons ($p > 0,05$). For the winter experiment (Figure 2), due to technical difficulties, no plasma concentrations of cyanide in control nor cyanide exposed fish were available for day 0. None of the replicates within each treatment concentration of each experiment were shown to differ significantly from the others at any given time ($p > 0,05$).

The plasma cyanide concentrations observed in both experiments were higher in the exposed fish then in controls ($p < 0,001$). During the summer (Figure 1), the plasma cyanide concentrations increased rapidly after the onset of exposure to reach an apparent plateau within the first 48 h

and from thereon, the means remained constant ($p > 0,05$). There was a similar trend observed in the winter experiment since here again there was no difference observed between day 7 and 15 ($p > 0,05$), suggesting the occurrence of a plateau.

No dose-response relationship could be established ($p > 0,05$). However, the results suggest higher cyanide plasma level at higher concentration of HCN in the water. During the summer plasma cyanide concentrations were, at 0,37 and 0,74 uM HCN on day 15, 698 and 973 % higher then in the controls. During winter the increase was only of 286% and 493%.

Fish showed an apparent ability to concentrate cyanide in the plasma from the water. During summer, the fish exposed to 0,37 uM HCN had a plasma concentration of cyanide about 6,3 times greater then the water, while at 0,74 uM HCN the plasma level was 4,1 higher. During the winter, the bioconcentration factor dropped to 1,8 and 1,7 times respectively for the lowest and the highest exposure concentrations.

Season had a significant effect on the bioaccumulation of cyanide ($p < 0,05$). On day 15, the summmer values are respectively 254% and 144% higher then there are in winter at the lowest and highest exposure concentrations respectively.

Table 3. Number, mean wet body weight and length of juvenile rainbow trout at the beginning of the three experiments.

EXPERIMENT AND DATE	NUMBER OF FISH	MEAN WET BODY WEIGHT (g) AND STANDARD DEVIATION	MEAN LENGTH (cm) AND STANDARD DEVIATION
#1 AUGUST 1986	188	33,20 5,85	14,5 Ø,9
#2 AND #3 NOVEMBER - DECEMBER 1986	369	30,02 5,60	13,6 Ø,9

Table 4: Mean blood plasma cyanide concentrations , standard deviations and their 95 % confidence limits in juvenile rainbow trout held at 12°C and exposed for 15 days to sublethal concentrations of cyanide during august 1986.

EXPOSURE CONCENTRATION (uM HCN)	DAYS OF EXPOSURE	SAMPLE SIZE N	MEAN PLASMA HCN CONCENTRATION (uM) (S.D.)		95% CONFIDENCE LIMITS	
					L1	L2
0,00	0	9	0,38	(0,14)	0,29	0,47
	2	8	0,38	(0,11)	0,30	0,45
	7	8	0,27	(0,31)	0,07	0,48
	15	9	0,30	(0,15)	0,21	0,39
0,37	0	9	0,37	(0,10)	0,31	0,43
	2	9	2,17	(1,02)a	1,54	2,80
	7	9	1,79	(0,99)a	1,17	2,40
	15	8	2,34	(0,81)a	1,80	2,88
0,74	0	8	0,36	(0,09)	0,31	0,43
	2	9	2,75	(1,83)a	1,62	3,89
	7	9	2,16	(0,69)a	1,73	2,58
	15	9	3,05	(0,72)a	2,61	3,49

a= Significantly different from all controls ($p<0,01$)

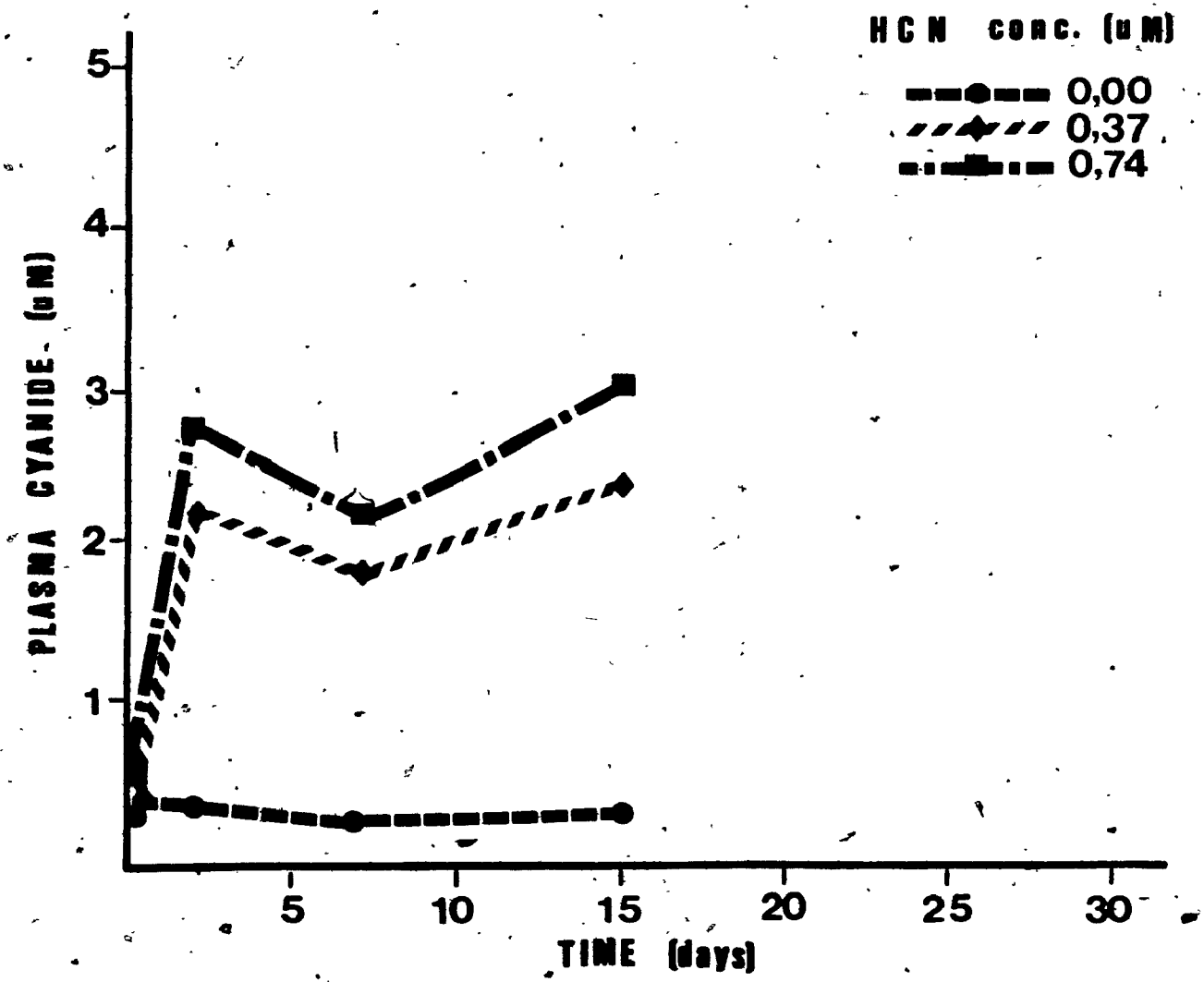

Figure 1: Mean blood plasma cyanide concentrations, in juvenile rainbow trout held at 12 ^{o}C and exposed for 15 days to sublethal concentrations of cyanide during August 1986

Table 5: Mean blood plasma cyanide concentrations, standard deviations and their 95 % confidence limits in juvenile rainbow trout held at 12°C and exposed for 15 days to sublethal concentrations of cyanide during November-December 1986.

EXPOSURE CONCENTRATION (uM HCN)	DAYS OF EXPOSURE 1/	SAMPLE SIZE N	MEAN PLASMA HCN CONCENTRATION (uM) (S.D.)		95% CONFIDENCE LIMITS	
					L1	L2
0,00	7	9	0,17	(0,09)	0,12	0,22
	15	9	0,19	(0,09)	0,13	0,25
0,37	7	7	0,97	(0,22)a	0,81	1,13
	15	9	0,66	(0,46)a	0,38	0,95
0,74	7	8	1,27	(0,46)a	0,96	1,58
	15	9	1,25	(0,99)a	0,64	1,86

1/ No measurements on day 0 and 2 due to a technical problem with the analytical apparatus.

a= Significantly different from all controls ($p<0,01$)

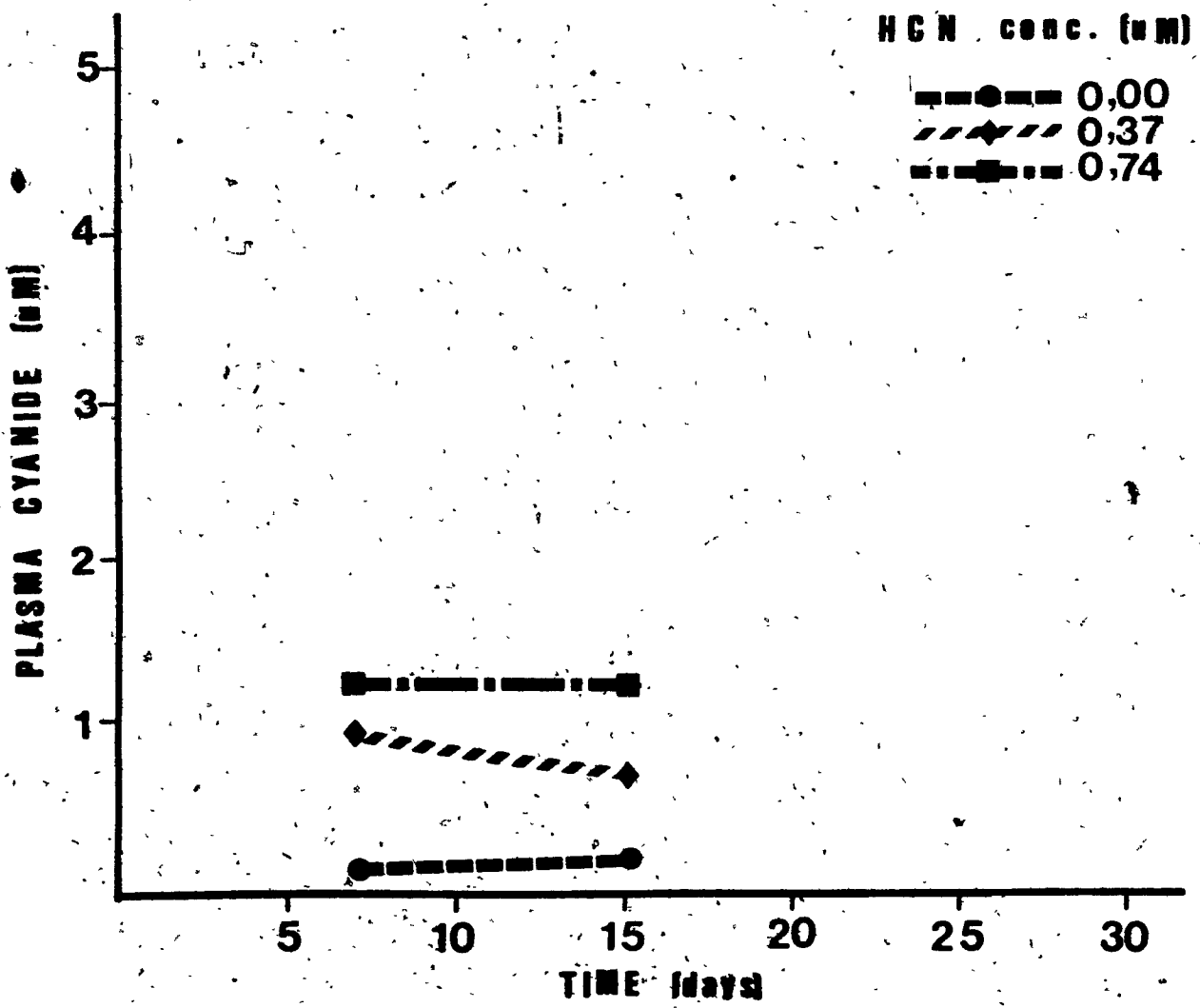

Figure 2: Mean blood plasma cyanide concentrations in juvenile rainbow trout held at 12 °C and exposed for 15 days to sublethal concentrations of cyanide during November-December 1986.

THIOCYANATE ACCUMULATION

The blood plasma thiocyanate concentrations measured during this study are reported in Tables 6 and 7 for the summer and the winter experiments respectively and illustrated in Figures 3 and 4.

In both experiments the background levels of thiocyanate remained constant over time, with an exceptional rise on day 2 of the summer experiment ($p < 0,01$). Also, none of the replicates within each treatment level in any experiment was shown to differ significantly from the others ($p > 0,05$). There was no seasonal effect on the plasma background concentrations ($p > 0,05$).

In the summer (Figure 3), cyanide induced a significant rise of SCN during the first two weeks ($p < 0,001$). On day 2, at 0,74 uM HCN, SCN concentrations were significantly higher than in the controls ($p < 0,01$) but at 0,37 uM HCN a significant increase did not occur until day 7. On day 15, thiocyanate levels in fish exposed to 0,34 and 0,74 uM HCN were respectively 1014 and 1797% higher than in the controls. During the summer, doubling the exposure concentrations from 0,37 to 0,74 uM HCN produced a 63% increased in thiocyanate on day 15. Although there is a tendency towards a dose-response relationship the differences, on day 15, are not significant ($0,10 > p > 0,05$).

In the winter (Figure 4), thiocyanate levels in the exposed fish increased significantly ($p < 0,001$) from day 0

to 15 and remained stable until day 30. No dose response relationship could be demonstrated despite the apparent difference in concentration. On day 30, the plasma thiocyanate levels at 0,37 and 0,74 uM HCN were respectively 483% and 894% higher then in the controls and doubling the exposure concentration during the winter months produced a difference of 58% in the plasma concentrations.

However season had a significant effect on the bioaccumulation of thiocyanate ($p < 0,05$). On day 15, the summmer values were respectively 83% and 110% higher then they were in winter at the lowest and highest exposure concentrations respectively.

INFLUENCE OF SEX

The 95 % confidence intervals reported in Tables 4, 5, 6, 7 and 8 suggest a large variability in the response of individual fish to cyanide exposure. In attempt to explain some of that variability the role of sex was tested but revealed no relationship between the plasma concentrations of cyanide or thiocyanate levels and the sex of the individual fish.

Table 6. Mean blood plasma thiocyanate concentrations, standard deviations and their 95 % confidence limits in juvenile rainbow trout held at 12°C and exposed for 15 days to various concentrations of cyanide in August 1986.

EXPOSURE CONCENTRATION (uM HCN)	DAYS OF EXPOSURE	SAMPLE SIZE N	MEAN PLASMA SCN CONCENTRATION (uM) (S.D.)		95% CONFIDENCE LIMITS	
					L1	L2
0,00	0	9	5,38	(1,92)	4,19	6,57
	2	8	9,34	(1,31)a	8,47	10,22
	7	9	3,69	(1,99)	2,46	4,92
	15	9	3,02	(1,02)	2,39	3,55
0,37	0	9	5,74	(1,85)	4,59	6,88
	2	8	12,73	(3,18)	10,61	14,86
	7	9	14,96	(9,16)b	9,28	20,63
	15	9	31,92	(9,95)b	25,27	38,57
0,74	0	8	5,64	(1,73)	4,48	6,80
	2	8	16,20	(3,50)b	13,86	18,54
	7	8	24,71	(5,99)b	20,71	28,71
	15	9	51,92	(15,41)b	42,37	61,48

a= Significantly different from all controls ($p<0,01$).
b= Significantly different from respective control ($p<0,01$).

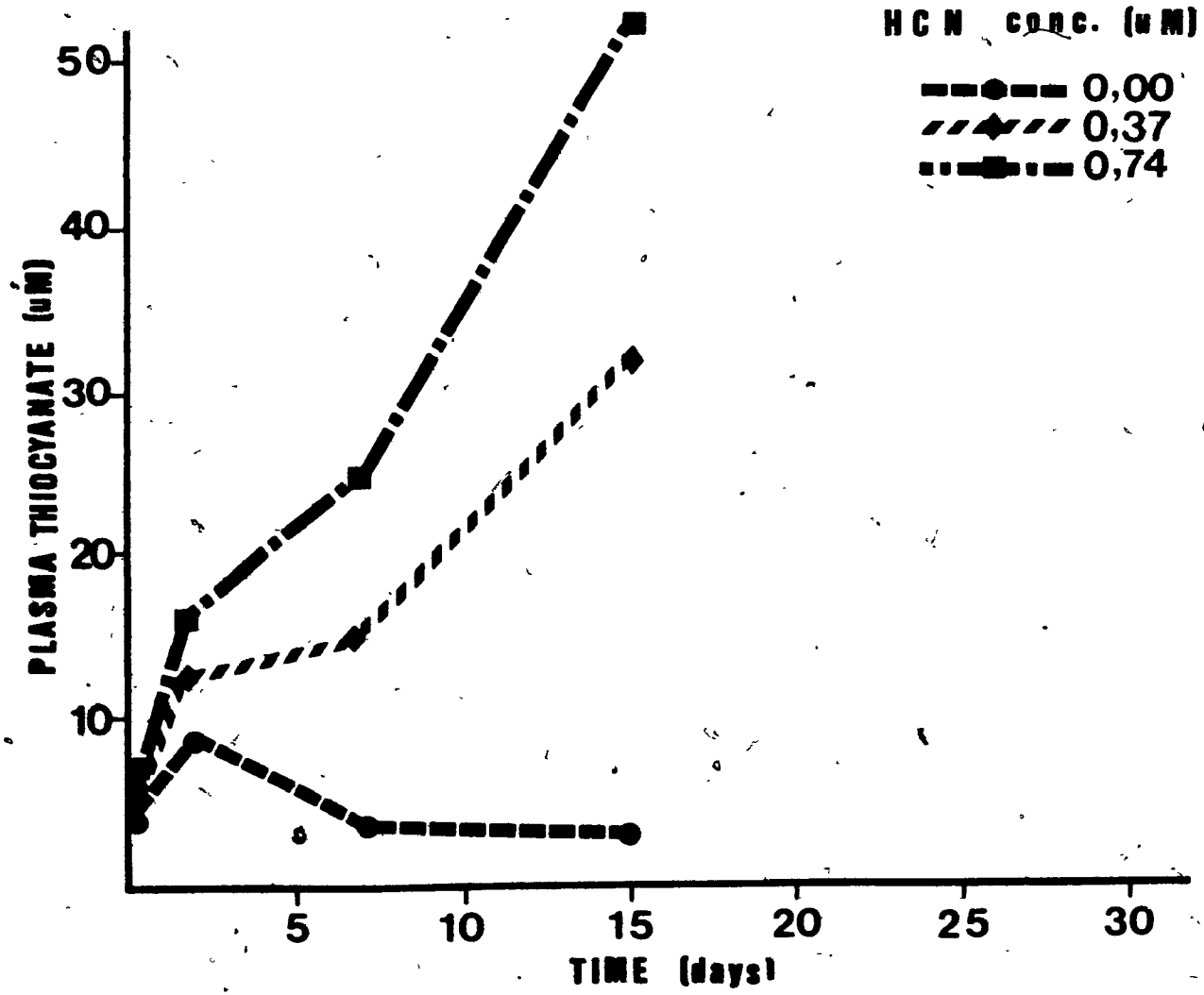

Figure 3: Mean plasma thiocyanate concentrations in juvenile rainbow trout held at 12 °C and exposed to various concentrations of cyanide in August 1986.

Table 7: Mean blood plasma thiocyanate concentrations, standard deviations and their 95 % confidence limits in juvenile rainbow trout held at 12°C and exposed for 30 days to various concentrations of cyanide in November-December 1986.

EXPOSURE CONCENTRATION (uM HCN)	DAYS OF EXPOSURE	SAMPLE SIZE N	MEAN PLASMA SCN CONCENTRATION (uM) (S.D.)		95% CONFIDENCE LIMITS	
					L1	L2
0,00	0	6	5,93	(1,49)	4,70	7,15
	15	7	4,33	(2,24)	2,68	5,98
	20	9	4,56	(1,90)	3,38	5,74
	30	7	2,30	(1,01)	1,55	3,04
0,37	0	9	3,98	(1,10)	3,30	4,67
	15	9	18,12	(7,38)a	13,54	22,70
	20	9	23,83	(9,73)a	17,80	29,86
	30	9	25,59	(11,03)a	18,75	32,43
0,74	0	9	4,25	(2,29)	2,83	5,67
	15	9	28,60	(8,12)a	23,57	33,63
	20	9	24,75	(10,55)a	18,21	31,29
	30	9	28,82	(12,18)a	21,27	36,38

a= Significantly different from all controls ($p<0,01$).

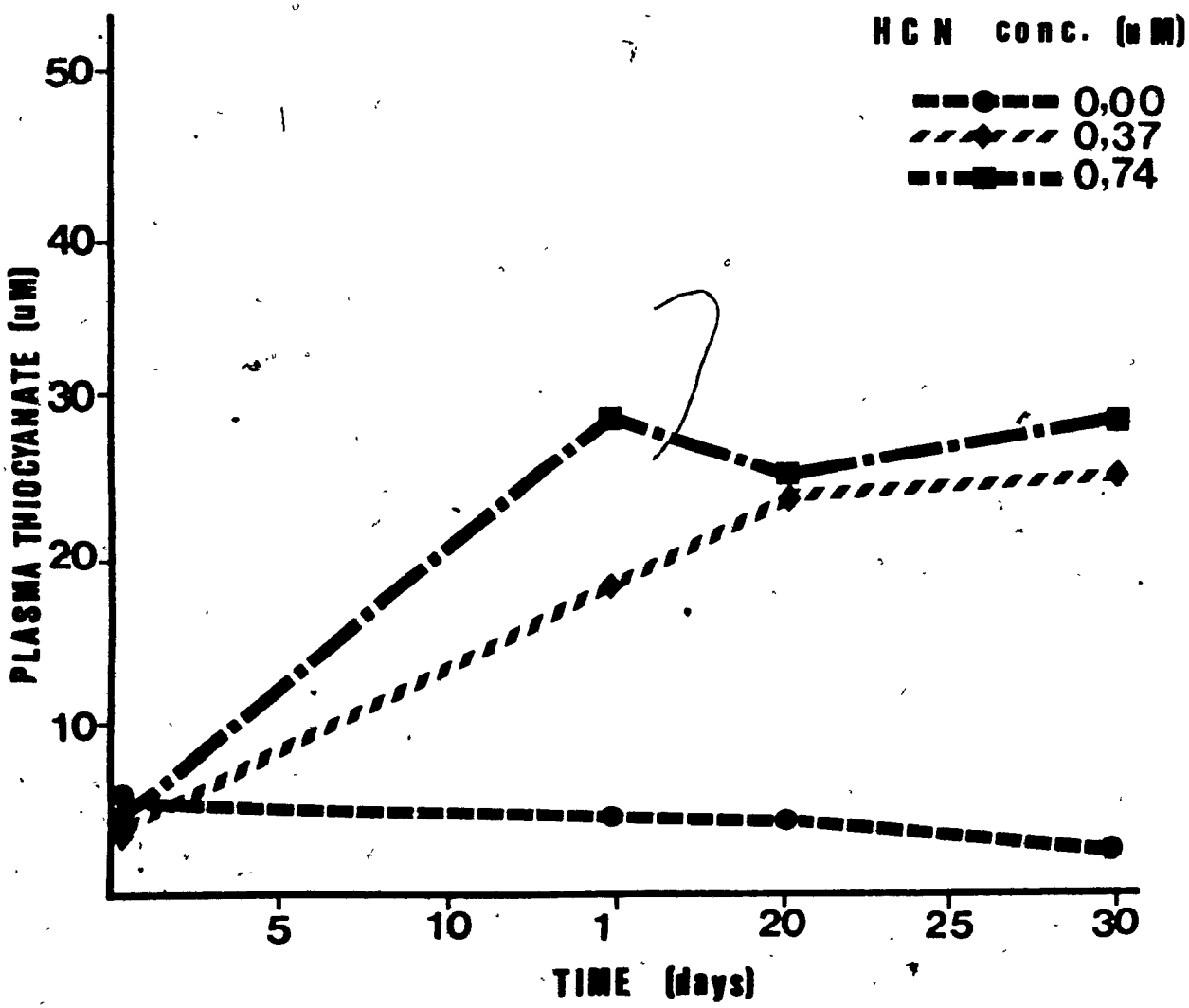

Figure 4: Mean blood plasma thiocyanate concentrations in juvenile rainbow trout held at 12°C and exposed to various concentrations of cyanide in November-December 1986.

CLEARANCE OF THIOCYANATE AND CYANIDE

At the end of the 30 days exposure to cyanide during winter, the fish were kept in cyanide-free running water for 15 days to monitor the disappearance of both cyanide and thiocyanate from the blood plasma. However, due to technicals problems the plasma cyanide concentrations could not be measured. The thiocyanate concentrations are reported in Table 9 and illustrated in Figure 5.

Throughout this portion of the experiment, the background levels of thiocyanate in control fish remained constant ($p > 0,05$) at about 3 uM SCN. None of the replicates within each treatment concentration were shown to differ from the others at any given time ($p > 0,05$).

Plasma thiocyanate concentrations in fish previously exposed to 0,37 uM HCN rapidly declined to reach control levels by day 7. A similar pattern occurred at 0,74 uM HCN although between 7 to 14 days were required for the same response. Since there were no significant differences in the plasma SCN at the two exposure levels on day 0 but there was on day 7 the slope of the curves suggest a faster elimination rate in fish previously exposed to 0,37 uM HCN. A two-way ANOVA was then conducted on the log transformed data (replicates were pooled) without considering the controls. Day 15 observations were not included since the 0,74 M HCN exposed fish might have reached control levels much earlier then by day 15. No difference in between the two exposure concentrations was detected on this set of data ($0,10>p>0,05$).

Table 8. Mean blood plasma thiocyanate concentrations, standard deviations and their 95 % confidence limits in juvenile rainbow trout held at 12 °C during a 15 -day clearance period following a 30-day exposure to various cyanide concentrations in November-December 1986.

EXPOSURE CONCENTRATION (uM HCN)	DAYS OF CLEARANCE	SAMPLE SIZE N	MEAN PLASMA SCN CONCENTRATION (uM) (S.D.)		95% CONFIDENCE LIMITS	
					L1	L2
0,00	0	7	2.30	(1,01)	1,55	3,04
	2	9	3,85	(1,13)	3,15	4,54
	7	8	3,69	(2,95)	1,72	5,66
	15	9	3,88	(2,00)	2,64	5,12
0,37	0	9	25,59	(11,03)a	18,75	32,43
	2	9	20,36	(9,66)a	14,37	26,35
	7	9	7,34	(6,04)	3,60	11,09
	15	9	6,27	(2,76)	4,55	7,98
0,74	0	9	28,82	(12,18)a	21,27	36,38
	2	9	25,85	(10,68)a	19,22	32,47
	7	9	16,54	(7,12)a	12,13	20,96
	15	8	6,44	(3,13)	4,35	8,52

a= Significantly different from all controls (p<0,01).

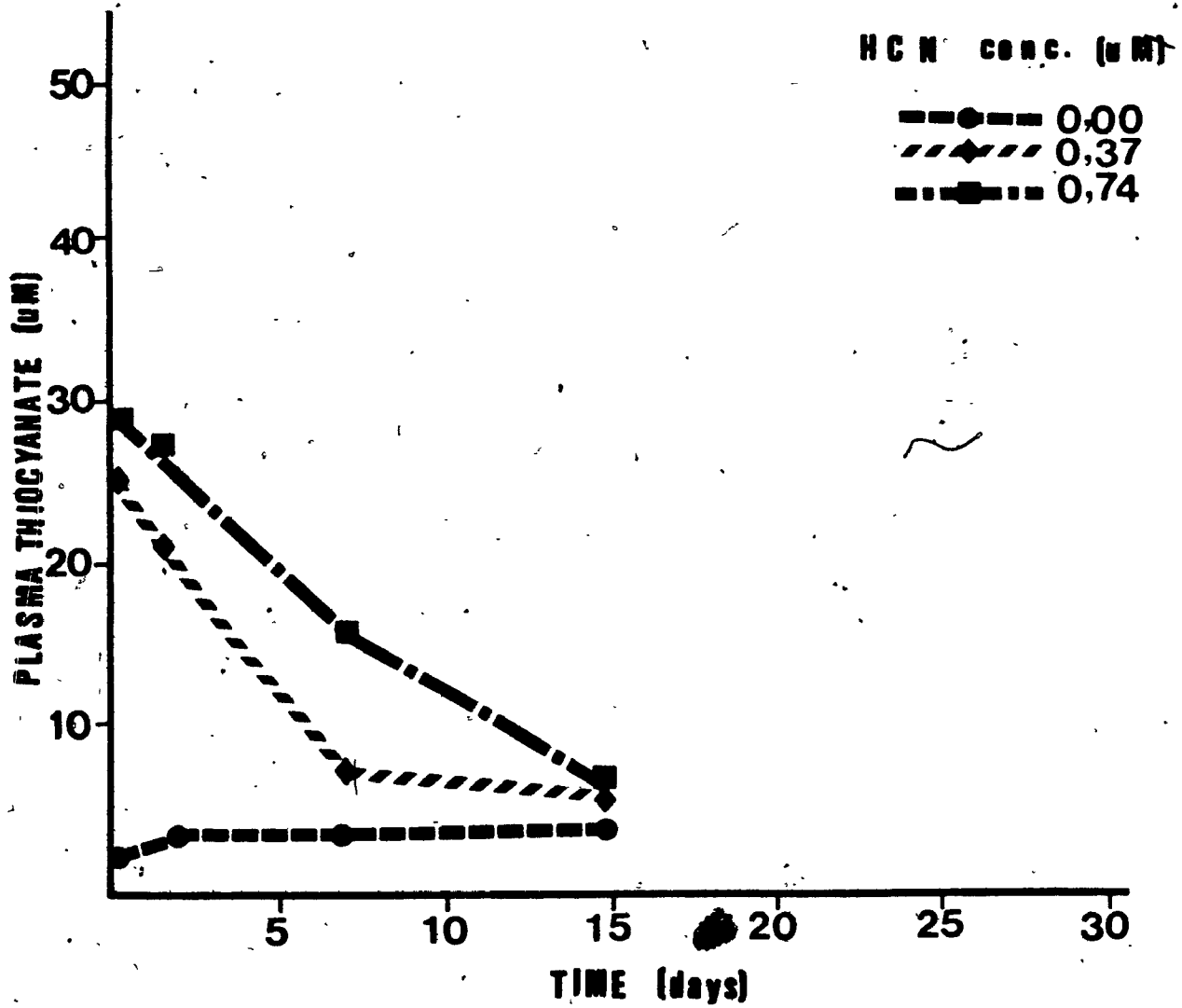

Figure 5: Mean blood plasma thiocyanate concentrations in juvenile rainbow trout held at 12°C during a 15 days clearance period following a 30-day exposure to various cyanide concentrations in November-December 1986.

EFFECTS OF CYANIDE ON TROUT THYROID FUNCTION.

The effect of cyanide exposure on rainbow trout thyroid function was assessed with a third experiment conducted in November-December 1986 with fish averaging 30,02 g. The mean wet body weight, length and the respective standard deviation of the rainbow trout used are reported in Table 3 whereas the iodide levels are in Table 9. The plasma concentrations of total iodide were not affected by cyanide exposure ($p>0,05$) under our experimental conditions. The statistical analysis of the data indicates that the variations observed in Table 9 mainly reflect random variation since no individual source of variation was shown to contribute significantly to the total error term.

The effect of cyanide exposure on the histological appearance of the thyroid follicles was evaluated during the summer experiment through the measurement of the height of the epithelial cells (Table 10). The fish exposed to 0,37 uM HCN were not affected. The highest treatment level (0,74 uM HCN) induced a 13 % increase in the height of the epithelial cells of the follicles ($p < 0,01$) aft[illegible] days of exposure.

HEMATOCRIT

Cyanide had no effect on the hematocrit value of the exposed fish. The mean packed cell volume was of 49%.

Table 9. Mean blood plasma total iodide concentrations, standard deviations and their 95 % confidence limits in juvenile rainbow trout held at 12 °C and exposed for 30 days to various cyanide concentrations in November-December 1986.

EXPOSURE CONCENTRATION (u M HCN)	DAYS OF EXPOSURE	SAMPLE SIZE N	MEAN PLASMA IODIDE CONCENTRATION (ug/ml)	(S.D.)	95% CONFIDENCE LIMITS L1	L2
0,00	0	6	0,28	(0,21)	0,12	0,44
	2	6	0,20	(0,08)	0,14	0,26
	7	9	0,20	(0,21)	0,08	0,33
	15	7	0,17	(0,07)	0,12	0,22
	20	8	0,14	(0,05)	0,11	0,17
	30	9	0,18	(0,06)	0,15	0,22
0,37	0	7	0,23	(0,21)	0,08	0,39
	2	6	0,20	(0,07)	0,14	0,26
	7	6	0,16	(0,06)	0,08	0,22
	15	9	0,27	(0,17)	0,17	0,37
	20	8	0,27	(0,36)	0,04	0,51
	30	9	0,21	(0,08)	0,16	0,26
0,74	0	5	0,22	(0,14)	0,09	0,35
	2	9	0,20	(0,19)	0,12	0,27
	7	8	0,20	(0,04)	0,17	0,22
	15	10	0,39	(0,28)	0,23	0,55
	20	8	0,18	(0,06)	0,13	0,23
	30	10	0,20	(0,09)	0,15	0,25

Table 10. Mean height, standard deviation and 95% confidence limit of the height of the thyroid epithelial cells of juvenile rainbow trout during exposure to various concentrations of cyanide for 15 days at 12 °C in August 1986.

EXPOSURE CONCENTRATION (uM)	DAYS OF EXPOSURE	NUMBER OF FISH N	THYROID CELL HEIGHT (uM) (S.D.)	95% CONFIDENCE INTERVALS L1	L2
0,00	0	6	2,73 (0,18)	2,58	2,88
	7	5	2,72 (0,14)	2,58	2,86
	15	6	2,72 (0,35)	2,44	3,00
0,37	0	6	2,73 (0,24)	2,53	2,93
	7	5	2,73 (0,39)	2,36	3,10
	15	6	2,74 (0,18)	2,60	2,88
0,74	0	6	2,73 (0,33)	2,46	3,00
	7	5	2,88 (0,15)	2,73	3,03
	15	6	3,09 (0,28)a	2,86	3,32

a= Significantly different from all controls ($p<0,01$).

DISCUSSION

CYANIDE ACCUMULATION

This study clearly showed that significant plasma concentrations of cyanide are established within the first 48 h of cyanide exposure at a time when a steady state is established.

No report of plasma cyanide concentrations in fish exposed to cyanide was found in the literature but the concentrations measured in our control fish are similar to those reported in control trout by Hemming et al (1985) in their study on thiocyanate toxicity. Since no cyanide was found in the dilution water these background levels may be attributed to cyanoglycosides contained in the food (Solomonson, 1981, p 11-28).

In both experiments, the plasma cyanide concentrations in exposed fish were, at steady state, higher then the cyanide levels in water. This was unexpected since hydrogen cyanide was believed to diffuse passively through the gills membrane to reach an equilibrium in the blood plasma (Leduc, 1984, page 158). This accumulation of cyanide against a concentration gradient cannot be attributed to an artefactual released of cyanide during the processing of the samples nor to the back conversion of thiocyanate into cyanide in the organism. It appears unlikely that cyanide would have been released from thiocyanate during the acidification process

used for its isolation since the mean plasma cyanide levels were constant over a wide range of mean plasma thiocyanate concentrations. No correlation was observed between individual plasma cyanide and thiocyanate values. We also tested for the release of cyanide in plasma samples spiked with cyanide, thiocyanate or cyanide and thiocyanate solutions but no cyanide was produced. Acidification of plasma samples containing hemoglobin could have also resulted in an artefactual release of cyanide (Vesey and Wilson, 1978). This possibility is however precluded since plasma samples showing any sign of hemolysis were discarded.

Blood thiocyanate may be back converted to cyanide in the organism. In mammals, injection of radio-labelled thiocyanate leads to exhalation of radio-labelled cyanide (Boxer and Rickards, 1952 a,b , Goldstein and Reiders, 1951). Cyanide was also present in the blood of fish exposed to waterborn thiocyanate (Hemming et al, 1985). However, the plasma concentrations of thiocyanate reported in their study to produce comparable plasma concentrations of cyanide were never attained in our experiments.

In rats, humans and dogs, between 93 and 99 % of the blood cyanide was shown to be strongly complexed to the erythrocytes since up to 6 washings with a saline solution did not dislodge any cyanide (Vesey and Wilson, 1978). Consequently, it appears unlikely that any significant

quantity of cyanide would have been released from the cells in the present study.

Another possible explanation for the bioconcentration of cyanide may be an active transport mechanism at the gill level. Epstein et al (1973, 1975) observed the inhibition of chloride transport at the gill level by thiocyanate, and Zadunaisky et al (1971) described how thiocyanate competes for the chloride transport sites in frog cornea. Recently, Hemming et al (1985) demonstrated that fish can concentrate thiocyanate against the concentration gradient established between water and the blood and that addition of chloride ions to the water decreased thiocyanate uptake. Owing to the anionic resemblance between CN^- and SCN^- the same mechanism might explain the bioconcentration of cyanide.

As appealing as this hypothesis might appear, it must be kept in mind that a minimal portion of the cyanide added to the test water exists as CN^- at pH 7.6 (Leduc, 1984, p.154). If an active transport mechanism for cyanide is existing it would have to be exceptionally efficient in order to maintain the plasma concentrations of cyanide in the reported range. Also, active transport of chloride is an ATP dependent mechanism most probably inhibited by cyanide which would make this hypothesis self-defeating.

Another possibility is that a portion of the cyanide load was complexed to some plasma component. At physiological pH of 7,9 (Hemming et al,1985) most of the cyanide present in

the blood is in the molecular form and is slightly liposoluble. In a partition test between water and benzene, up to 25% of hydrogen cyanide has been recovered in the benzene layer (Stephen and Stephen, 1963). Lipoproteins were shown to make up to 51% of the total blood protein load in goldfish, Carassius auratus, (Houston and Fenwick, 1964) and are a significant cyanide binding candidate. Some of the cyanide load present as cyanide ion could also possibly bind to some positively charged amino acid or metalloprotein carried in the plasma. Since extensive binding of thiocyanate to bovine plasma albumin has also been demonstrated (Chandra and McMenary, 1970), binding of cyanide to blood albumin could have contributed significantly to increase the apparent concentration of plasma cyanide.

If, at saturation, the complexation of cyanide is responsible for its bioconcentration, the difference in plasma cyanide concentration established between various treatment levels should reflect the cyanide level in the water. In this experiment, increasing the exposure concentration by 0,37 uM HCN produced a mean increase, although not significant, of 0,56 uM HCN in the plasma. This suggest that not all potential binding sites were occupied or that an active transport mechanism was present.

Cyanide (HCN) is a weak acid having a K_d of about $7,2 \times 10^{-10}$ (Leduc, 1984, page 154) so that at physiological blood pH most of cyanide should be present in its

undissociated form: HCN. Any increase in blood alkalinity would upset this equilibrium and produce a larger proportion of CN^-. Cyanide exposure could potentially induce a state of alkalosis by reducing the production of CO_2 through the inhibition of the oxidative metabolism or by reducing catalase activity (Chance, 1949). Accordingly, the proportion of CN^- would then be increased and more HCN could diffuse into the fish blood and explain its bioconcentration. No measurement of blood pH under cyanide exposure was found in the literature.

The pattern of cyanide bioaccumulation supports Raymond et al (1986) observations on the kinetics of liver cytochrome oxidase inhibition of rainbow trout exposed to similar sublethal cyanide concentrations. They found a 60-80 percent inhibition of cytochrome a_3 within the first 24 h of exposure and observed the level of inhibition to stay constant throughout the following 20 days. In this study, the plasma cyanide concentrations peaked within the first 48 h and remained constant throughout the test period. The absence of a dose-response relationship of cyanide bioaccumulation also occurred in Raymond et al (1986) work, as they could not demonstrate any difference in the levels of cytochrome a_3 oxidase inhibition at different HCN concentrations in the water.

Raymond et al (1986) have estimated the plasma cyanide concentration required to inhibit the liver cytochrome

oxidase to be of about 0,5 uM HCN. We can hardly compare our measured plasma concentration with their estimate since the effective cyanide concentration will vary between organs and does not necessarly equal the plasma concentration. In humans, ingestion of cyanide produces blood concentrations 3 to 10 times higher then in the tissues. The lowest levels were observed in muscle while the highest were established in the spleen (Ansell et al, 1970, Halstrom et al, 1945). In rats, the level of cytochrome a$_3$ inhibition achieved in the brain and the heart were shown to be independent of the administered dose even where the lowest was not lethal while the highest produced 100% mortality (Petterson and Cohen 1985).

The results of this study stress the necessity of investigating the compartimentalization of cyanide in fish blood as well as its speciation. Further investigations should also be concerned with its distribution at the tissues level under different exposure conditions.

THIOCYANATE ACCUMULATION

This study clearly confirmed that cyanide-exposed fish accumulate thiocyanate presumably originating from the biotransformation of cyanide into thiocyanate by rhodanese (Westley, 1981). Thiocyanate accumulates in plasma because it is a pseudohalogen and, in freshwater fish, competes with

other anions, especially chloride, for its reabsorbtion at the kidney level (Guyton, 1976, Hickman and Trump, 1969).

Background concentrations of plasma thiocyanate were observed and agree with values obtained in earlier investigations conducted in this laboratory by Raymond et al (1986), McGeachy (1984) as well as by others (Hemming et al, 1985). A background thiocyanate levels in control trout were expected and might even serve some biological function (s) as suggested by Solomonson (1981).

The patterns of thiocyanate accumulation observed during the first 15 d of cyanide exposure match closely the findings of both Raymond et al (1986) and McGeachy (1984) . They reported a constant increase that was not dose related. However, as in previous studies there was a tendency towards a relationship between the cyanide concentration in the water and the established thiocyanate levels in the plasma. However, this absence of a dose-effect relationship for thiocyanate agrees with our results of plasma concentrations of cyanide.

EFFECT OF SEASON ON CYANIDE AND THIOCYANATE ACCUMULATION.

The established plasma concentrations of both cyanide and thiocyanate differed with season. Titers of both compounds were drastically lower in winter than in summer.

There is some controversy on the interpretation as to how winter affects the metabolism of fish when kept under summer

like laboratory conditions of temperature and photoperiod. This arises most probably from the confusion in the literature on the difference between acclimation and acclimatization.

Acclimation is a process by which an organism adapts itself to a given set of physicochemical conditions. It is induced by environmental factors to which the fish responds in an effort to adjust its metabolic machinery so it can optimize its operation. Acclimatization on the other hand is "an adaptation phenomena which consist of a compensatory response to a complex array of varying factors and is anticipatory in essence" (Reynolds and Casterlin, 1979).

Evans et al (1962) have shown that rainbow trout acclimatized to outdoor winter conditions had a total metabolic rate higher under laboratory summer-like (16 ^{o}C;16 h light) then winter-like (8 ^{o}C; 8 h light) conditions. Since our experiment was of similar duration and conducted in the same season, we can reasonably assume that our fish had not yet compensated for the change in temperature and photoperiod and still had a higher metabolic rate then when they were brought in.

It has also been shown that cold-acclimated goldfish have a higher oxygen consumption then warm-acclimated fish (Karrungo and Prosser,1959). Beamish (1964) has shown that at a constant temperature the metabolic rate of brook trout (Salvelinus fontinalis) changes with season, being at a

minimum in March. It started to increase in late summer to reach a maximum in November/December in parallel with the seasonal gonadal development. The rainbow trout, which is a spring breeder (Scott and Crossman,1973) should then have its metabolic rate increasing until the breeding season is over. However, this relationship still has to be demonstrated in immature fish. Presumably, our fish would have had a higher metabolic rate during the winter experiment then during the late summer.

Under these conditions, irrigation of the gills should be higher during the winter and produce larger plasma concentrations of cyanide. We observed, however, the reverse since plasma concentrations of cyanide on day 15 were significantly lower during winter than in summer. The accumulation of thiocyanate also differed significantly between winter and summer in parallel to cyanide.

There are a few possible explanations for these observations. The first is based on the assumption that some of the measured cyanide was in fact bound to a plasma component. It is known that plasma protein concentrations decrease in brook trout during the winter months independently of their reproductive state and, presumably, of their nutritional state (Cunjak and Power,1986). Assuming binding of HCN with these proteins, their reduction could lower cyanide concentrations observed. It also appears plausible that cyanide elimination would proceed more rapidly

at an elevated metabolic rate thus contributing to a lowering of the plasma cyanide concentration. The third possibility is that the detoxification of cyanide into thiocyanate is enhanced during winter. However, plasma concentrations of thiocyanate were also depressed during the winter.

It is almost impossible to speculate on the efficiency of the cyanide detoxification mechanisms in the different seasons for the following reasons. First, the concentration of free cyanide ions available to the action of the rhodanase enzyme is unknown . Secondly, the reported concentrations of thiocyanate represent its free portion since the plasma sample was deproteinized before being analysed. Any seasonal modification in the proportion of the plasma constituent might suggest an inexistant seasonal variation in the activity of the rhodanase enzyme. Extensive binding of thiocyanate to bovine plasma albumin has also been demonstrated (Chandra and McMenary, 1970) Finally, we could not insure that a steady state was reached during the summer experiment.

Cyanide toxicity appears to be related to fish metabolic rate since higher temperature and activity increased resistance of fish against low lethal concentrations of cyanide (Koyacs and Leduc,1982a, McGeachy and Leduc,1988). Since at sublethal concentrations, both the plasma concentrations of cyanide and thiocyanate are depressed in

December, the decreased tolerance of the fish to its toxic action must arise from an increased sensitivity or in the availability of the toxicant rather then from an increased load. This most probably indicates that the seasonal modifications in cyanide toxicity cannot be explained by the usual metabolic rate toxicity relationship.

THIOCYANATE DEPURATION

In mammals, birds and in fish, clearance rate of thiocyanate from the plasma is a function of its concentration (Guyton, 1976, Hemming et al,1985). When the reabsorption capacity of the kidney is exceeded, thiocyanate begins to leak into the urine. However, thiocyanate depuration in fish may also be a function its excretion through the gills.

The second experiment of this study was initially designed to monitor the disappearance of thiocyanate and cyanide from the plasma of trout following a 30-day preexposure to sublethal concentrations of cyanide. Unfortunately, problems with the analytical apparatus precluded the analysis of the plasma cyanide.

It took 7 to 14 days to completely eliminate thiocyanate from the plasma. In rabbits injected with potassium thiocyanate, 90% of the dose was recovered in the urine in 5 days (Baumann et al, 1933). Clearance of thiocyanate was not shown to be dose related in this study ($0,05 > p > 0,10$), although a strong tendancy is observed toward faster

elimination for the 0,01 mg/L HCN preexposed fish. It is thus possible that the sampling schedule as well as the low number of sampling days precluded the demonstration of any dose-response relationship between the cyanide exposure concentration and the thiocyanate depuration rate. Determination of the type of thiocyanate clearance kinetic (first or second order), is also impossible for the same reasons. Even though the sampling schedule does not allow precise calculations of the usual kinetics parameters, the depuration rate can be estimated to be close to 0,02 uM/L SCN/day during the first 7 days following the arrest of the cyanide flow into the tanks.

The time required for thiocyanate depuration indicates that thiocyanate is, most probably, still reabsorbed at the kidney level since urinary excretion rate of thiocyanate in rainbow trout, following exposure to similar thiocyanate concentration (Hemming et al, 1985), would be sufficient to account mostly for its clearance from the plasma. Hemming et al (1985) showed that these plasma thiocyanate concentrations do not exceed the kidneys reabsorbtion capacity. However, since extensive binding of thiocyanate to bovine plasma albumin has been demonstrated (Chandra and McMenary, 1970), what looked to be a slower excretion of thiocyanate at 0,02 mg/L HCN might in fact reflect release of thiocyanate from the binding sites rather then a difference in the urinary excretion rate.

Moreover, if cyanide is truly bound to some complexing site, its release might provide a substrate for the rhodanese action and explain the apparent lower clearance rate of thiocyanate.

Since urinary excretion of thiocyanate could account for its depuration rate it appears to be a limited contribution of the gills under the present experimental conditions. This is consistent with the role of the gill chloride cells which, under freshwater conditions pump chloride ions into the fish. Gill contribution to thiocyanate excretion would then be limited to a leakage of thiocyanate through passive diffusion. However, under saltwater conditions, gill contribution might become significant since in that situation chloride ion excretion mechanisms would be in operation. This competition phenomena between thiocyanate and chloride (Hemming et al,1985) could account for the inability of cyanide preexposed rainbow trout to maintain their plasma chloride concentrations at control values when transferred to saltwater conditions. Leduc and Chan's (1975) study showed that cyanide preexposed rainbow trout took much longer to bring their plasma chloride concentration back to control levels when transferred to 18,7 ppt seawater. However, cyanide exposure might have induced this effect by impairing the ability of the fish to produce the physiological modifications required for normal function under saltwater conditions.

If we accept the assumption that the contribution of the gill to thiocyanate clearance was limited under our experimental conditions, no significant difference in the clearance rate should have been observed between the two treatment levels (Hemming et al, 1985) unless cyanide has an action of its own on the kidneys.

It was reported in earlier works (McGeachy, 1984, Raymond et al, 1986), that plasma thiocyanate concentrations becomes unpredictable after the first two weeks of cyanide exposure . In some of these studies plasma thiocyanate concentrations continued to rise well beyond 15 days of cyanide exposure while in others the values leveled. In other experiments the plasma concentrations of thiocyanate suddenly dropped. These authors tentatively attributed these varying responses to impairment of kidney function and an inability to reabsorb the thiocyanate ion. This hypothesis appears sustained by the observation that cyanide exposure induces necrotic figures in trout kidney (Dixon and Sprague, 1981). However both the stable thiocyanate concentrations observed up to day 30 as well as the low depuration values reported in this study do not support this hypothesis.

Discrepencies in these results may reflect the small sample size and the pooling of blood of many fish to obtain a sufficient volume.

THYROID FUNCTION

After 15 d of exposure to 0,02 mg/L HCN we observed a significant increase (13%) in the height of the thyroid follicle epithelial cells.

If cyanide exposure seriously decreased levels of thyroid hormones, the thyroid follicles would show signs of stimulation by the pituitary gland (TSH). This response has been demonstrated in humans after the ingestion of large amounts of cyanoglycosides or treated whith sodium thiocyanate (Wood, 1975, p. 203-206). In fish this response has not yet been demonstrated.

These symptoms can be evaluated through the use of a "thyroid index" (Sonstegard and Leatherland,1976). Of the three parameters utilised only the height of the epithelial cells appeared to be reliable in our case.

A severe depression of radioiodide uptake by the follicles has been observed in trout injected intraperritoneally with thiocyanate salt (Eales and Shostak, 1983). However, these authors could not demonstrate any effect on circulating levels of thyroidal hormones. They also showed the thyroid gland to maintain significant reserves of thyroidal hormones when exposed to thiocyanate.

Our results appears to contradict Eales and Shostak's (1983) statement that "KSCN is of no practical value in the present population of laboratory trout as an hypothyroid inducing agent" but the situation is different.

Firstly, our fish were exposed to the combined action of two chemicals; thiocyanate and cyanide. The latter may, for example, inhibit the thyroid peroxidase (Ohtaki et al, 1985) so that our observations cannot be attributed only to the action of thiocyanate. Secondly, these authors injected their fish intraperitoneally and the effective concentration attained is unknown. Finally, the iodide intake of our fish was limited and monitored through the plasma concentration of total iodide. Although the plasma concentrations of total iodide were not reported in Eales and Shostak's study (1983), it was probably much higher since their fish were fed a commercial diet manufactured by a company whose products contain extremely high concentrations of iodine (Gregory and Eales, 1974). A high iodine containing diet was shown to induce high concentrations of plasma iodide (Gregory and Eales, 1974). It is then possible, as Eales and Shostak (1983) suggested, that large plasma concentrations of iodide would have provided a sufficient concentration gradient for iodide to passively diffuse into the thyroid follicles and sustain normal synthesis of thyroxine.

Nevertheless, our observations are consistent with the classical model (hypothalamic-hypophyseal axis) of thyroid function control. Decreased iodide uptake by the thyroid gland would reduce production of thyroxine thus stimulating production of TSH from the pituitary. To this increased solicitation, the thyroid follicle would respond by

increasing its synthesis capacity which is noticed in the increased height of the epithelial cell of the follicles. Although limited, our results are the first demonstration of the possible goitrogenic effect of cyanide exposure to fish.

The results of this study indicate that cyanide exposure has no measurable effect on the plasma concentrations of total iodide. Since thiocyanate decreases iodide uptake by the thyroid gland (Eales and Shostak,1983) an increase in the plasma concentration could have been observed.

Plasma concentration of total iodide in the Salmo genus ranges from 0,01 to 5,97 ug/ml but are usually lower then 0,5 ug/ml (Gregory and Eales, 1975). Our mean value which was around 0,2 ug/ml iodide then falls at the lower end of the range.

The absence of an effect on the plasma concentration of iodide can be attributed to the following. The contribution of deiodination sources to the total iodide pool is minimal (Hunt and Eales, 1979) and minimal enterohepatic cycling of thyroid hormones metabolites occurs (Collicut and Eales,1974, Eales,1985, Eales and Sinclair,1974). Limited storage of iodide in fish muscles was shown to occur which, owing to the bulk of the tissues, could make it a significant pool in situation of altered iodide balance (Gregory and Eales, 1974). Assuming that the thyroidal uptake of iodide was reduced by thiocyanate, the thyroid follicles would react to this negative iodide balance and adjust their machinery

accordingly. However, the muscle storage, which depends on the plasma concentration of iodide, would absorb any extra iodide that could potentially be released by the follicles. Normal concentration of total plasma iodide would then be maintained.

If the latter explains the absence of an effect of cyanide on plasma iodide concentrations, longer exposure should depress plasma iodide concentrations especially with a limited dietary intake and only after the muscular reserves become depleted. Specific physiological conditions may speed up the process. For example, fish ovaries were shown to store large amount of iodide (Leloup and Fontaine,1960, Tarrant,1971). Lower plasma iodide concentrations were observed in mature then immature females, most probably because of a competion between the ovaries and blood plasma (Gregory and Eales, 1974). It can be suggested that if cyanide exposure affects the iodide balance, the effect might be enhanced in period of gonadal maturation.

CONCLUSION

We have shown in this study that cyanide bioaccumulate in the blood plasma of trout exposed to cyanide. Maximum concentrations were obtained within the first 48 h of exposure and stayed constant until the end of the experimental period. Intriguingly fish showed an ability to bioconcentrate cyanide. No dose response relationship could be established between the exposure and the plasma concentration.

We also confirmed the bioaccumulation of thiocyanate in fish plasma. No dose response relationship could be established between the cyanide exposure concentration and the plasma thiocyanate levels found.

We have also shown that cyanide and thiocyanate plasma concentrations established during the summer are larger then those in the winter.

This study has demonstrated that 7 to 14 days are required for complete elimination of thiocyanate from the plasma. This relatively long period of time suggests that kidney reabsorbtion capacity has not been exceeded.

Although limited, our results are the first demonstration of the possible goitrogenic effect of cyanide exposure to trout. The highest cyanide exposure concentration induced an statistically significant hypertrophy of the epithelial cells of the thyroid follicles.

BIBLIOGRAPHY

Ansell, M., and F.A.S. Lewis. 1970. A review of cyanide concentrations found in humans organs: A survey of literature concerning cyanide metabolism, 'normal', non-fatal, and fatal body cyanide levels. J. Forens. Med. 17:148-155.

Aysola, P., P.D. Anderson, and C.H. Langford. 1987. Wet ashing in biological samples in a microwave oven under pressure using poly(tetrafluoroethylene) vessels. Anal. Chem. 59: 1582-1583.

Baumann, E.J., Sprinson, D.B., and N. Metzberg. 1933. The relation of thyroid to the conversion of cyanides to thiocyanate. J. Biol. Chem. 102:773-782.

Beamish, F.W.H. 1964. Seasonal changes in the standard rate of oxygen consumption of fishes. Can. J. Zool. 42:189-194.

Bourdoux, P., F. Delange, F. Gerard, M. Mafita, M.A. Hanson, and A.E. Ermansi. 1978. Evidence that cassava ingestion increases thiocyanate formations: A possible etiologic factor in endemic goiter, J. Clin. Endocrinol. Metab. 46:613-621.

Boxer, G.E., and J.C. Rickards. 1952 a. Determination of traces of hydrogen cyanide in respiratory air. Arch. Biochem. Biophys. 39:287-291.

Boxer, G.E., and J.C. Rickards. 1952 b. Determinations of thiocyanate in body fluids. Arch. Biochem. Biophys. 39:292-300.

Chance, B. 1949. The reaction of catalase and cyanide. J. Biol. Chem. 179:1299.

Chandra, S.P., and R.H. McMenary. 1970. Thiocyanate binding with modified bovine albumins. Arch. Biochem. Biophys. 136:260-267

Cunjack, R.A., and G. Power. 1986. Seasonal changes in the physiology of brook trout Salvelinus fontinalis (M.) in a subartic river system. J. Fish. Biol. 129:279-288.

Collicut, J.M., and J.G. Eales. 1974. Excretion and enterohepatic cycling of 125 I-L-Thyroxine in channel catfish, Icatalurus punctatus Rafinesque. Gen. Comp. Endocrinol. 23: 390-402.

Dixon, D.G., and G. Leduc. 1981. Chronic cyanide poisoning of rainbow trout and its effects on growth, respiration, and liver histopathology. Arch. Environm. Contam. Toxicol. 10:117-131.

Dixon, D.G., and J.B. Sprague. 1981. Acclimation induced changes in toxicity of arsenic and cyanide to trout. J. Fish. Biol. 18:579-589.

Eales, J.G. 1985. The peripheral metabolism of thyroid hormones and regulation of thyroidal status in poikilotherms. Can. J. Zool. 63:1217-1231.

Eales, J.G., R.J. Omeljaniuk, and S. Shostak. 1983. Reverse T3 in rainbow trout, Salmo gairdneri. Gen. Comp. Endocrinol. 50:395-406.

Eales, J.G., and D.A.R. Sinclair. 1974. Enterohepatic cycling of thyroxine in starved and fed brook trout, Salvelinus fontinalis (Mitchill). Comp. Biochem. Physiol. A. 49: 661-672.

Eales, J.G., and S. Shostak. 1983. Influence of potassium thiocyanate on thyroid function of rainbow trout, Salmo gairdneri. Gen. Comp. Endocrinol. 51: 39-43.

Epstein, F.H., J. Maetz, and G. De Renzis. 1973. Active transport of chloride by the teleost gill: inhibition by thiocyanate. Am. J. Physiol. 224:1295-1299.

Epstein, F.H., P. Silva, J.N. Forest, and R.J. Solomon. 1975. Chloride transport and its inhibition by thiocyanate in gills of seawater teleosts. Comp. Biochem. Physiol A, Comp. Physiol. 52:681-683.

Evans, R.M., F.C. Purdie, and C.P. Hickman Jr., 1962. The effect of temperature and photoperiod on the respiratory metabolism of rainbow trout (Salmo gairdneri). Can. J. Zool. 40:107-118.

Faulkner, L.W., R.P. Levy, and J.R. Leonards. 1961. Simplified technique for determination of serum protein-bound iodide. Clin. Chem. 7:637.

Ganjeloo, A., G.E. Isom, R.L. Morgan, and J.L. Way. 1980. Fluorometric determination of cyanide in biological fluids with p-benzoquinone. Toxicol. Applied Pharmacol. 55: 103-107.

Goldstein, F., and F. Reiders. 1951. Formation of cyanide in dog and man following administration of thiocyanate. Am. J. Physiol. 167: 47-51.

Gregory, L.A., and J.G. Eales. 1974. Factors contributing to high levels of plasma iodide in brook trout, Salvelinus fontinalis (Mitchill). Can. J. Zool. 53: 267-277.

Guyton, A.C. 1976. Textbook of Medical Physiology. W.B. Saunders compagny, Toronto. 1194 p.

Halstrom, F., and K.O. Moller. 1945. The content of cyanide in human organs from cases of poisoning with cyanide taken by mouth. With a contribution to the toxicology of cyanides. Acta Pharmacol. 1:18-28.

Hemming, T.A., Thurston, R.V., E.K. Meyn, and R.K. Zajdel. 1985. Acute toxicity of thiocyanate to trout. Trans. Am. Fish. Soc. 101:620-625.

Hickman,C.P., and B.F. Trump. 1969. The kidney. In: Fish Physiology. edited by W.S. Hoar and D.J. Randall. Vol.1, p. 91-240, Academic Press, New York.

Higgs, D.A., V.H.M. Fagerlund. J.G. Eales, and J.R. Mcbride. 1982. Application of thyroid and steroid hormones as anabolic agent in fish culture. Comp. Biochem. Physiol. 73B:143-176.

Houston, A.H., and J.C. Fenwick. 1964. Thermoacclimatory variations in the plasma protein of the goldfish, Carassius auratus. Amer. Zool. 5:695.

Hunt, D.W.C., and J.G.Eales. 1979. Iodine balance in rainbow trout (Salmo gairdneri) and effect of testosterone propionate. J. Fish. Res. Board Can. 36: 282-285.

Karrungo, M.S., and C.L. Prosser. 1959. Physiological and biochemical adaptation of goldfish to cold and warm temperatures. II.Oxygen consumption of liver homogenate, oxygen consumption and oxidative phosphorylation of liver mitochondria. J. Cell. Comp. Physiol. 54:265-274.

Kovacs, T.G., and G. Leduc. 1982a. Acute toxicity of cyanide to rainbow trout (Salmo gairdneri) acclimated at different temperatures. Can. J. Fish. Aquat. Sci. 39:1426-1429.

Kovacs, T.G., and G. Leduc. 1982b. Sublethal toxicity of cyanide to rainbow trout (Salmo gairdneri) at different temperature. Can. J. Fish. Aquat. Sci. 39:1389-1395.

Kreuter, P.A., V. Varbarrov, W. Goodman, G. Olaya, and J.B. Steinberry. 1977. Interactions of protein deficiency, cyanide and thiocyanate and thyroid function in neonatal and adult rats. Am. J. Clin. Nutr. 31:282-289.

Kruzynski, G.M. 1972. Effect of dietary metoxychlor on brook trout Salvelinus fontinalis. M.Sc. Thesis. Sir George Williams University, Montreal. 131 p.

Lambert, J.L., J. Ramasamy, and J.V. Poukstelis. 1975. Stable reagent for colorimetric determination of cyanide by modified Konig reactions. Anal. Chem. 47: 916-918.

Leatherland, J.F. 1985. Studies of the correlation between stress-response, osmoregulation, and thyroid physiology in rainbow trout, Salmo gairdneri (Richardson). Comp. Biochem. Physiol. 80A:523-531.

Leduc, G. 1966a. Une bouteille à débit constant pour petits volumes de liquides. Nat. Can. (Que.) 93: 61-64.

Leduc, G. 1966b. Some physiological and biochemical responses of fish to chronic poisonning by cyanide. Ph.D. Thesis. Oregon state Univ., Corvallis, Or. 146p.

Leduc, G. 1984. Cyanides in water: toxicological significance. pp 152-224. In: Aquatic toxicology, Vol. 2. Edited by L.J. Weber. Raven Press, New York. 230 p.

Leduc, G., and K.S. Chan. 1975. The effects of chronic cyanide poisonning on the tolerance of rainbow trout to varying salinity. In: Water pollution research in Canada. Edited by Hutchison, T.C., pp. 118-125. Inst. Env. Studies., Univ. of Toronto.

Leloup, J., and M.M. Fontaine. 1960, Iodide metabolism in lower vertebrates. Ann. N.Y. Acad. Sci. 86:316-353.

McCraken, I.R., and G. Leduc. 1980. Allometric growth response of exercised rainbow trout to cyanide poisoning. Aquatic Toxicology, ASTM STP 707, J.G. Eaton, P.R. Parrish and A.C. Hendricks, Eds., American Society for Testing and Materials, pp 303-320.

McGeachy, S.M. 1984. Acute and sublethal toxicity of cyanide to exercised and non-exercised rainbow trout (Salmo gairdneri) at different time of the year. M.Sc. Thesis. Concordia Univ., Montreal, 71 p.

McGeachy, S.M., and G. Leduc. 1988. The influence of season and exercise on the lethal toxicity of cyanide to rainbow trout (Salmo gairdneri). Bull. Environ. Toxicol. in press.

Ohtaki, S., H. Nakagawa, S. Nakamura, S. Nakamura, and I. Yamazaki. 1985. Characterization of hog thyroid peroxidase. J. Biol. Chem. 260:441-448.

Petterson, J.C., and S.D. Cohen. 1985. Antagonism of cyanide poisoning by chlormoprazine and sodium thiosulfate. Toxicol. Appl. Pharmacol. 81:265-273.

Raymond, P., G. Leduc, and J.A. Kornblatt. 1986. Investigation sur la toxicodynamique du cyanure et sur sa biotransformation chez la truite arc-en-ciel (Salmo gairdneri). Can. J. Fish. Aquat. Sci. 43: 2017-2024.

Reynolds, W.W., and M.E. Casterlin. 1979. Behavioral thermoregulation and the final preferendum paradigm. Am. Zool. 19:211-224.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada Bulletin 184. 866 p.

Solomonson, L.P.. 1981. Cyanide as a metabolic inhibitor. In: Cyanide in biology, edited by B. Vennesland, E.E. Conn, C.J. Knowles, J. Westley, and F. Wissing. Academic press, Inc. Toronto. 548 p.

Sonstegard, R., and J.F. Leatherland. 1976. The epizootiology and pathogenesis of thyroid hyperplasia in coho salmon (Oncorhynchus kisutch) in lake Ontario. Cancer Res. 36:4467-4475.

Sprague, J.B. 1973. The ABC's of pollutant bioassay using fish. pp 6-30. In: Biological methods for the assesment of water quality. ASTM/STP/528. American society for testing and materials, Philadelphia, PA., 256p.

Stephen, H., and T. Stephen. 1964. Solubilities of inorganic compounds. Vol 2. Ternary systems part 1. Edited by Stephen,H., and T. Stephen. Mac Millan Co., New York. p 902.

Tarrant, R.M. 1971. Seasonnal variations in the accumulation and loss of 131-I by tissues of adult female channel catfish. Ictalurus punctatus (Rafinesque). Trans. Am. Fish. Soc. 100:237-246.

Toida, T., S. Tanabe, and T. Inamari. 1981. Fluorometric determination of cyanide and thiocyanate by Konig reaction. Chem. Pharm. Bull. 29: 3763-3764.

Turner, C.D., and J.T. Bagnara. 1976. General Endocrinology. W.B. Saunders Company. Philadelphia. 596p.

Vesey, C.J., and J. Wilson. 1978. Red cell cyanide. J. Pharm. Pharmacol. 30: 20-26.

Vogel, A.I. 1955. A textbook of quantitative inorganic analysis. Theory and practice. Longmans, Green and Co., London. 914 pages.

Westley, J. 1981. Cyanide and sulfane sulfur. In: Cyanide in biology, edited by B. Vennesland, E.E. Conn, C.J. Knowles, J. Westley, and F. Wissing, p.61-76. Academic press, Inc., Toronto.

Wood, J.L. 1975. Biochemistry. In: Chemistry and biochemistry of thiocyanic acid and its derivatives. Academic Press, Inc., London. 351 p.

Zadunaisky, J.A., M.A. Lande, and J. Hafner. 1971. Further studies on chloride transport in frog cornea. Am. J. Physiol. 221: 1832-1836.