THE EFFECT OF TEMPERATURE ON CYANIDE TOXICITY TO RAINBOW TROUT (*SALMO GAIRDNERI*)

PART I : ACUTE TOXICITY

PART II: SUB-LETHAL TOXICITY

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ABSTRACT

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The acute and sublethal toxicity of cyanide to thermally acclimated rainbow trout were measured at 6, 12 and 18°C.

The 96-hour LC50 at 6, 12 and 18°C were 0.028, 0.042 and 0.068 $mg.L^{-1}$ HCN, respectively. However, differences in the median survival time of trout at the three temperatures diminished with increasing cyanide concentrations, suggesting that the temperature effect on the acute toxicity of cyanide is concentration-dependent.

Cyanide significantly reduced wet weight growth rates at 0.015, 0.030 and 0.045 $mg.L^{-1}$ HCN levels at 6, 12 and 18°C, during 20-day exposures. Threshold concentrations reducing growth rates increased in a similar fashion with increasing temperatures as the maintenance ration and the median lethal concentrations. This can be related to biochemical adaptations of trout during thermal acclimation as well as to differences in detoxification processes reflecting standard metabolic rates.

Initial reductions of growth rates during the first 10 days of exposure were followed by lesser reductions or growth stimulation, depending on temperature and cyanide concentration. Monitoring liver glycogen and lactic acid levels of 0.015 $mg.L^{-1}$ HCN at each temperature revealed that cyanide initially reduces growth rates by forcing trout to utilize anaerobic pathways to

obtain energy. However the fish return to more efficient aerobic metabolism by about the tenth day of cyanide exposure when growth rates also rebound.

A cyanide concentration of 0.010 $mg.L^{-1}$ stimulated growth rates of trout at 12°C, whereas at 18°C, a 0.015 $mg.L^{-1}$ concentration stimulated fat synthesis. The reasons for these are unclear, since cyanide seriously reduced fat synthesis at all other concentrations and temperatures.

Previous exposure to cyanide markedly affected swimming performance of the fish tested at their acclimation temperatures, but in the absence of the toxicant. The effect also increased with decreasing temperatures.

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PART I: ACUTE TOXICITY

INTRODUCTION

Mining operations, metal finishing industries, chemical manufacturing plants and photographic processes often dispose their cyanide wastes into water. Because of this widespread use of cyanide, fish may encounter hydrocyanic acid over a wide range of water temperatures due to daily temperature fluctuations, seasons, latitude and thermal pollution. The purpose of this laboratory study was to evaluate the acute toxicity of cyanide to rainbow trout at three water temperatures: 6, 12 and 18°C.

Temperature stress, as a modifying factor of toxicity, has been given only little attention in establishing water quality criteria for toxic substances. These have recently been reviewed by Cairns et al (1975). No generalization can be made as to how temperature modifies toxicity, because the literature on this subject is rather scanty, inconclusive and sometimes contradictory.

Cyanide toxicity to fish has been studied under various experimental conditions, with many species of fish and the research on this subject has been reviewed by Doudoroff (1976). The available literature suggests lethal threshold concentrations of 0.05-0.07 $mg.L^{-1}$ HCN for salmonids tested between 15-17°C, (Karsten, 1934; Herbert and Merkens, 1952; Burdick et al, 1958; Brown, 1968) whereas the lethal threshold for warm water fishes (20-25°C) lies in the range of 0.10-0.15 $mg.L^{-1}$ HCN, (Burdick et al, 1958; Doudoroff, 1956; Doudoroff et al, 1966).

Temperature effects on cyanide toxicity to trout were first observed by Southgate et al (1932) and Alexander et al (1936). These authors found cyanide to be more toxic at higher temperatures.

Wuhram and Woker (1955) and Cairns and Scheier (1963), established also that higher temperatures accelerated the death from cyanide of the European minnow (Phoxinus phoxinus) and the bluegill (Lepomis macrochirus). On the other hand, researchers from Great Britain (Doudoroff, 1976) found that at slowly lethal concentrations the action of cyanide was more toxic to trout at low temperatures (3-4°C). In addition, Leduc (1977) suggested that temperatures, more than species differences, could explain apparent discrepancies in the impairment of growth and swimming performance at sub-lethal concentrations of cyanide, higher temperature reducing the toxic effect.

It seemed then, that the lethal action of cyanide at different temperatures could be concentration dependent. This laboratory project was therefore undertaken to determine the temperature effect on the acute toxicity of hydrocyanic acid to juvenile rainbow trout at slowly lethal concentrations, at 6, 12 and 18°C.

MATERIAL AND METHODS

Test Organisms

Rainbow trout (Salmo gairdneri) were used for this study and were purchased from La Pisciculture Mt. Sutton, Sutton, Quebec. Upon arrival at the Sir George Williams Campus, where this study was carried out, the fish were held in 200-litre fiberglass oval shaped tanks at a density of 200 fish per tank, supplied with a continuous water flow of 2.1 $L.min^{-1}$ at a temperature of 12 ± 1°C. The fish were fed daily, approximately 2% of their wet weight with Ewos Trout Chow size 3 and 4P.

Water Chemistry

The laboratory was supplied with City of Montreal water, which was dechlorinated, thermally regulated at 6, 12 and 18°C and delivered to the test apparatus through plastic (P.V.C.) piping.

The dissolved oxygen levels were monitored daily using the Winkler Method (Azide Modification, APHA, 1971), and were always above 80% saturation in the test tank. The pH of the water, also monitored daily, varied from 7.8 to 8.1 with an average value of 7.9. Other chemical parameters of the water during the experiments (December 1976 to July 1977) provided by the City of Montreal Public Works Department, Water and Sewage Division (average values) were as follows: Alkalinity, 87 $mg.L^{-1}$ as $CaCO_3$; Hardness, 127 $mg.L^{-1}$ as $CaCO_3$; CO_2, 0.47 $mg.L^{-1}$.

Test Tanks

The test tanks used consisted of 24 white polyethylene tanks (Rosedale Plastics, Montreal) measuring 68 cm long, 57.5 cm wide, and 42 cm deep. They were connected to a flow-through system provided with thermally controlled water at one of the three test temperatures (6, 12 and 18°C), at a flow of 1 $L.min^{-1}.tank^{-1}$, giving 99% replacement in 6 hours, as calculated after Sprague (1973). The entire 24-tank assembly was illuminated evenly by fluorescent lights, controlled by a time-switch to provide a 12-hour photoperiod (8:30-20:30).

Acclimation

After acclimation in the 200-litre holding tanks at 12°C, the fish to be tested at 6°C and 18°C were segregated and acclimated to the desired water temperatures by gradual adjustments of about one degree a day. The fish were then held for seven days at this temperature, prior to transfer into the test tanks, where they were held a

further two weeks at the test temperature. During this period, the fish were fed daily with Ewos Trout Chow up until 48 hours prior to the beginning of the tests, during which no food was given.

Experimental Design

The acute toxicity tests were carried out to determine the 96-hr LC50 of HCN to rainbow trout at three temperatures, 6, 12 and 18°C. The bioassays were carried out following standard methods recommended by Sprague (1973). The fish weighed from 9-18g with a mean weight of 12g; they were randomly chosen, acclimated as described above, and segregated for each test concentration and control group.

On the day the bioassays began, the tanks were cleaned and a calculated amount of cyanide stock solution was mixed into each tank to immediately produce the predicted concentrations. This was considered to be zero hour, and the desired concentrations were then maintained by metering cyanide stock solutions from Mariotte bottles (Leduc 1966). Cyanide was monitored in the test tanks twice daily, according to the method of Lambert et al (1975) and the cyanide flows were adjusted when necessary. The desired concentrations never varied by more than one percent of the predicted values.

The concentrations tested ($mg.L^{-1}$ HCN) ranged from 0.018 to 0.056 at 6°C, 0.032 to 0.087 at 12°C, and 0.042 to 0.087 at 18°C. These ranges were selected empirically such that the approximate 96-hr LC50 values, determined by preliminary screening bioassays would be close to the median of the eight concentrations tested which were selected according to APHA (1971) and included concentrations causing zero and 100 per cent mortality. Control groups were maintained at each temperature under identical conditions.

Observations on mortality were taken from zero hour on up to 96 hours, at logarithmically spaced intervals. However, additional incidental observations were made after 48 hours to obtain additional information on mortality. The criterion of death was absence of respiratory movements and lack of response to probing with a glass rod upon which the fish were removed, weighed and their fork length measured.

RESULTS

The results of the acute toxicity tests expressed as medium lethal concentration values (LC50), are presented in Table I. The 96-hour LC50 were determined from log-probit paper. The slope functions and 95% confidence intervals were calculated according to the nomographic methods of Litchfield and Wilcoxon (1949).

The resistance of juvenile rainbow trout to cyanide was markedly affected by temperature. The 96-hour LC50 values were significantly (i.e., $p < 0.05$) different at each of the test temperatures. In the range of cyanide concentrations tested (0.018–0.087 $mg.L^{-1}$ HCN), the cyanide level required to kill 50% of the trout in 96 hours was 2.4 times lower at 6° than at 18°C.

The median survival times (MST) were estimated as described by Sprague (1973) and the 95% confidence intervals were calculated according to Litchfield (1949). The MST values, presented in Table II, again show the influence of temperature on cyanide toxicity to trout by decreasing the MST with decreasing temperatures. The MST of rainbow trout exposed to 0.087 $mg.L^{-1}$ HCN at 18°C was 16 $\pm$ 3 hours, whereas at 12°C it was only 7 $\pm$ 1 hours. No mortality occurred after 96 hours at 12°C among fish exposed to 0.032 $mg.L^{-1}$ HCN, but at 6°C, 50% of the fish had died at the same concentration within 72 hours. The MST of trout at

TABLE I: Acute toxicity of hydrogen cyanide to rainbow trout acclimated and tested at different temperatures in flow-through system for 96 hours, showing the 96-hr LC50 with other test biological and chemical parameters.

	TEMPERATURE °C		
	6	12	18
96-hour LC50 and 95% confidence interval ($mg.L^{-1}$ HCN)	0.028 (0.024–0.035)	0.042 (0.038–0.046)	0.068 (0.064–0.072)
Slope function	1.30	1.30	1.12
Highest cyanide concentration with no mortality in 96 hours ($mg.L^{-1}$ HCN)	0.018	0.032	0.060
Lowest cyanide concentration causing 100% mortality within 96 hours ($mg.L^{-1}$ HCN)	0.37	0.053	0.087
Average weight of fish and range (g)	11.79 (9.05–15.98)	12.41 (8.29–16.02)	13.19 (9.63–17.21)
Average fork length of fish and range (mm)	102 (95–110)	103 (90–111)	106 (95–119)
Mean pH and range	8.06 (7.9–8.1)	8.10 (8.06–8.11)	7.82 (7.78–7.90)
Mean oxygen saturation and range (percent)	89.2 (88.2–90.1)	97.1 (95.2–98.5)	86.8 (85.7–88.3)

TABLE II: Median survival time and confidence intervals of rainbow trout exposed to various concentrations of hydrogen cyanide at different temperatures in flow-through systems.

Temperature °C	Cyanide Conc. ($mg.L^{-1}$ as HCN)	MST 1/ (hours)	95% Confidence interval
6	0.000	∝2/	-
	0.018	∝	-
	0.024	∝	-
	0.028	96	-
	0.032	72	59-95
	0.039	34	30-41
	0.042	30	25-36
	0.050	18	11-31
	0.060	13	12-17
12	0.000	∝	-
	0.032	∝	-
	0.037	∝	-
	0.040	∝	-
	0.045	70	53-93
	0.053	32	27-37
	0.065	14	12-17
	0.070	11	9-13
	0.087	7	6- 8
18	0.000	∝	-
	0.042	∝	-
	0.056	∝	-
	0.060	∝	-
	0.065	∝	-
	0.070	88	72
	0.073	48	25-58
	0.075	43	30-61
	0.087	16	13-19

1/ Median survival time

2/ The symbol ∝ indicates MST > 96 hours.

6°C exposed to 0.060 mg.L^{-1} was only 13 hours, whereas this concentration did not cause any mortality at 18°C.

It is also clear from Table II, that as the concentrations tested increase, the difference in the MST at each temperature became smaller. For example, while the MST of trout exposed to 0.070 mg.L^{-1} HCN at 12°C was eight times shorter than at 18°C, at a higher concentration of 0.087 mg.L^{-1} HCN, the survival difference was only 2.3 times shorter at 12 than 18°C.

Eye-fitted toxicity curves were constructed over cyanide concentrations ranging from 0.028 to 1.0 mg.L^{-1}, using data from this study as well as other comparable data from the literature (Ministry of Technology, 1968, Herbert and Merkens, 1952). Figure 1 shows that the curves intersect at a point near 0.1 mg.L^{-1} HCN, above which the temperature effect on the lethal action of cyanide to trout is reversed. In other words, at more rapidly lethal concentrations, cyanide becomes more toxic at 18°C than at 12° and 6°C.

DISCUSSION

Physiological Significance

In order to verify if the lethal response of rainbow trout to cyanide at different temperatures followed the Q_{10} law, the reciprocals of the 96-hr LC50 measured at 6, 12 and 18°C (see Table I) were calculated. The derived Q_{10} value was then compared with those of the maintenance ration (Kovacs, 1979) and the standard metabolic rate (Dickson and Kramer 1971) measured on the same species and at the same temperatures. It appears from Table III that when expressed by the Q_{10} law, the standard metabolism and food maintenance requirements respond

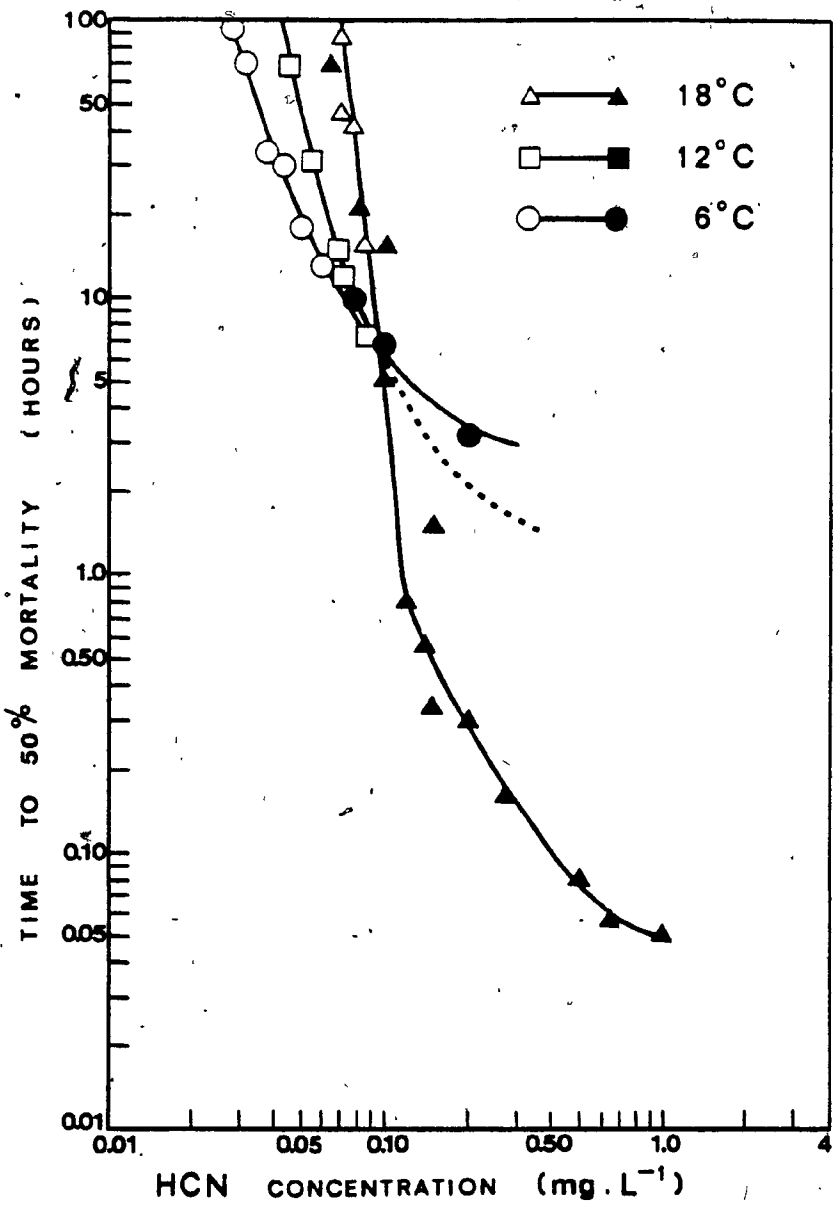

Figure 1: Toxicity curves of hydrogen cyanide to rainbow trout drawn from experimental data obtained in this study (open symbols) and from Herbert and Merkens (1952) (dark triangles) and from Ministry of Technology (1968) (dark circles).

TABLE III: Comparison of Q_{10} values of rainbow trout between the reciprocals of the 96-hr LC50 to cyanide, maintenance ration and oxygen consumption at different temperatures.

Temperature °C	Reciprocal of 96-hr LC50 ($1/mg.L^{-1}$ HCN)	Maintenance Ration (% weight.day^{-1}) 1/	Standard Metabolic rate ($mgO_2.kg^{-1}.hr^{-1}$) 2/
6	35.71	0.32	36.48
12	23.81	0.48	54.45
18	14.71	0.69	81.28
Q_{10} (6-18°C)	-2.1	2.0	2.0

1/ Measured by Kovacs (1979) under similar conditions as in this study

2/ Dickson and Kramer (1971)

similarly to temperature changes, whereas mortality rates at slowly lethal cyanide concentrations are equally but inversely affected. This comparison suggests that at concentration below 0.1 $mg.L^{-1}$ HCN the median toxicity threshold is linked, in opposite direction, to the standard metabolic rate, which increases continually up to a lethal level (Fry, 1967), showing greatest change at elevated temperatures (Brett, 1962).

Toxicological Significance

Temperature acclimated fish were about 2.4 times more tolerant to cyanide at 18°C than at 6°C, and the 96-hr LC50 values of 0.028 and 0.068 $mg.L^{-1}$ HCN determined in the present study at 6 and 18°C, respectively are very similar to the median threshold concentrations reported for similar temperatures by the Ministry of Technology (1968) and by Herbert and Merkens (1952). On the other hand, the 96-hr LC50 threshold value of 0.042 $mg.L^{-1}$ determined in this study, and 0.05 $mg.L^{-1}$ HCN as reported by Smith et al (1978) for rainbow trout at 10°C, is however significantly lower than the median threshold concentration of 0.09 $mg.L^{-1}$ HCN reported by the Ministry of Technology (1968) for trout tested at 12°C. Differences in the test procedures or water quality may account for some of these discrepancies, but temperature acclimation of fish and more accurate monitoring of actual cyanide concentrations in the test water rather than simply reporting calculated levels, can seriously change the results. In many of the earlier studies, fish were tested with little or no acclimation to the test temperatures. A two to three-week thermal acclimation period is essential for meaningful fish toxicity tests (Sprague, 1973).

The higher susceptibility of trout to cyanide at lower temperatures observed in this study contrasts sharply with the findings

of earlier workers Southgate et al (1932), Herbert and Merkens (1955) and Alexander et al (1938). These authors have reported that the toxic action of cyanide to trout increased with temperature. The main difference between this study and the others, lies in the range of concentrations tested. Whereas the earlier studies were carried out at rapidly lethal concentrations where fish died in less than 100 minutes, this study was carried out at concentrations causing 50% mortality in more than 420 minutes.

It appears from the above that the potency of cyanide at low and high temperatures is concentration dependent. This became clear from the examination of toxicity curves in Figure 1, which shows that the curves intersect at a point near 0.1 $mg.L^{-1}$ HCN, meaning that at this concentration, temperature would have no effect on the lethal action of cyanide. The concentration range where the toxicity curves become asymptotic to the concentration axis, called the reaction threshold by Warren (1971), represents rapidly lethal concentrations and here the reaction threshold is greater at lower temperatures. Below 0.1 $mg.L^{-1}$ HCN, the toxicity curves become asymptotic to the time axis, called the concentration threshold by Warren (1971), represent slowly lethal concentrations, and here the threshold is lower at lower temperatures. Matida (1960) pointed out that physiological and biochemical processes leading to death may be different in short term exposure to high concentrations of a toxicant from long term exposure to dilute solutions. This might explain why the temperature effect on cyanide toxicity to trout is concentration-dependent. At very high concentrations of hydrogen cyanide, death occurs due to the disruption in the activity of vital organs such as the heart and central nervous system and in this range of concentration, the toxicant is only limited in its speed of

action by the time it takes to get into the blood and circulate to the vital organs (Jones, 1964). Time to reach the reaction site (induction time) depends on the rate of uptake of the toxicant and its distribution to the tissues. At higher temperatures, the ventilation rate of fish increases (Heath, 1973) together with metabolic rate, thus accelerating toxicant uptake from the water at the gills and a faster distribution to the reaction site(s). Therefore the cyanide toxicity curve at 18°C should become asymptotic to the concentration axis in less time than at 12 or 6°C because cyanide reaches the site(s) of effect faster.

At lower concentrations, death from HCN may be due to the disruption of the oxidative processes because of the inhibition of the cytochrome oxidase system. In this range of concentrations, lower temperatures speed up mortality. Since only two-thirds of the oxidative processes are cyanide-sensitive (Jones, 1964), it is possible that due to different biochemical makeup of trout acclimated to different temperatures, cyanide toxicity may be altered because of important changes in the biochemical composition at the cellular level and/or metabolic pathways (Hochachka and Somero, 1973). At higher temperatures, oxidative processes might not be dependent on the cytochrome oxidase, or cytochrome oxidase itself might not be as easily inhibited by cyanide. Ekberg (1958) found that gills of the goldfish (Carassius auratus) showed higher resistance to cyanide poisoning at 30 than at 10°C, as measured by oxygen consumption. Ekberg (1962) attributed this to a qualitative alteration of the cytochrome oxidase, found in the mitochondrial membrane which is susceptible to structural changes by temperature causing possible stereochemical alterations in the enzyme. Caldwell and Vernberg (1966) have indeed found that membrane lipids in gill mitochondria of fishes becomes increasingly unsaturated at low temperatures.

At concentrations where the fish are not immediately overwhelmed by cyanide, detoxification and excretion mechanisms may also play a part in determining the effect of temperature on toxicity. In fact, Doudoroff (1976) believes that cyanide is less toxic at higher temperatures, mostly due to faster elimination of the poison from the body through detoxification and excretion. It is possible that at 6°C, due to the low metabolic rate of the animal, commencement of detoxification might be too slow to be of any value to the fish.

Overall, temperature definitely modifies cyanide toxicity to rainbow trout, but the effect is concentration-dependent. This differential effect of temperature at fast and slowly lethal concentrations also occurs with other toxicants. Hodson and Sprague (1975) found with Atlantic salmon (Salmo salar) that lower acclimation temperatures (3°C) led to a longer survival time in zinc solutions than at acclimation temperatures of 11 and 19°C. Lower lethal threshold concentrations were however determined at lower temperatures when concentrations tested were low enough to carry on the tests for 14 days.

Similarly, Brown et al (1967) tested the acute toxicity of phenol on rainbow trout at temperatures ranging from 6.3 to 18.1°C and found that at higher concentrations the reaction time decreased as the temperature increased, whereas at lower concentrations, the toxicity increased as the temperature decreased. With ammonia, Lloyd and Herbert (1962) and Lloyd (1961), found that the lethal threshold concentrations were not influenced by temperature, but Brown (1968) using higher concentrations, found the 48-hour LC50 of ammonia for trout to decrease linearly with an increase in temperature from 8 to 20°C.

Smith and Heath (1979) used 5 species of fish to test the acute toxicity of cyanide, chromate, copper and zinc at three

temperatures. While the results were not always uniform, low concentrations generally led to greater survival at high temperatures, while the reverse was true at higher concentrations. Rainbow trout showed the reverse of this trend with copper and chromate, and surprisingly the 24-hour LC50 of cyanide to trout was not influenced by temperature. However, in the case of cyanide, the authors based their conclusions on scanty data.

Some toxicants such as chlorine and hydrogen sulfide are more toxic at higher temperatures regardless of the concentrations tested, (Thatcher et al, 1976; Adelman and Smith, 1972). It seems therefore that the concentration dependence of temperature modification of toxicity does not always apply, but depends on whether or not the mechanisms leading to death at high concentrations differ from those operating at lower levels, as may be the case for cyanide.

The prediction of acceptable concentrations of chemicals for aquatic organisms has and will remain the utmost goal of water pollution research. This constant need to estimate safe levels is enhanced by the ever increasing number of new products each year, for which short term acute toxicity data must often be substituted to the slowly acquired and costly chronic data. In lieu of other rapid and acurate screening methods to measure toxicity, the lethal test is and will remain the main source of information for the aquatic toxicologist on which to base a scientific judgement on the hazard of a toxicant in nature. The notion of application factor, or a safe dilution of the LC50, has long been considered, but must remain biologically valid (Mount, 1977).

In the present study, we have shown a concentration dependence of the effects of temperature on cyanide toxicity to trout. Dilution can

increase toxicity at low temperature, and this phenomenon should therefore be kept in mind whenever application factors are derived solely from LC50 data.

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PART II: SUB-LETHAL TOXICITY

Temperature acts as a controlling factor on the metabolic rate of fish influencing the animal's activity in terms of development, growth and swimming performance, (Fry, 1971). Since fish in temperate zones must function at different temperatures due to the seasons, the need to consider temperature as a modifying factor of toxicity to fish is important. While some work has been done to evaluate the effect of temperature on the acute toxicity of many compounds, virtually no information exists if temperature alters the sub-lethal effects of toxicants to fish.

In Part I of this thesis, it was established that temperature alters the lethal action of cyanide, the alteration being concentration-dependent. In the concentration range of 0.018-0.087 $mg.L^{-1}$ HCN, low temperatures increased the toxicity of cyanide. Whether temperature has an effect on the sub-lethal toxicity of cyanide has not yet been studied.

The effect of chronic cyanide poisoning on various physiological responses of fish has recently received much attention and was reviewed by Leduc, (1977). Cyanide has been shown to seriously reduce the growth of juvenile rainbow trout exposed to concentrations as low as 0.01 and 0.02 $mg.L^{-1}$ HCN in flow-through aquaria at a temperature of 10.5°C, (Dixon, 1975; Speyer, 1975; McCracken, 1978). Leduc, (1966b) however, observed lesser effects of cyanide on growth of coho salmon (Oncorhynchus kisutch) and cichlids (Cichlasoma bimaculatum Linn.) tested at 16 and 25°C, respectively.

Swimming capacity of fish is temperature-dependent (Warren, 1971, p. 106), and has been long recognized as a sensitive indicator of

chronic poisoning. Neil, (1957) showed that cyanide concentrations as low as 0.01 and 0.05 mg.L^{-1} as CN^- reduced the swimming stamina of brook trout by as much as 98% after 29 days of exposure at 8°C. Broderius, (1970) and Speyer, (1975) observed that cyanide concentrations of 0.01 at 15°C and 0.02 mg.L as HCN at 11°C decreased the swimming ability of coho salmon and rainbow trout by about 50%. In contrast, Leduc, (1966b) demonstrated that at 25°C the maximum sustained swimming speed of cichlids was reduced by only 30% after a 24-day exposure to 0.04 mg.L^{-1} HCN.

While these various studies of the effects of cyanide on growth and swimming performance are difficult to compare, because of different experimental procedures, it is important to note that tests carried out at higher temperatures reduced the toxicity of cyanide. This factor more than species differences may explain the apparent discrepancies between various authors.

This project was undertaken to determine the effect of temperature on sub-lethal toxicity of cyanide to rainbow trout, as measured by growth and swimming performance at 6, 12 and 18°C. Furthermore, in an attempt to explain the different effect of cyanide at the three temperatures tested, liver metabolites of glycogen and lactic acid were monitored during sub-lethal tests.

MATERIAL AND METHODS

The rainbow trout (Salmo gairdneii) used for these studies, were purchased from La Pisciculture Mt. Sutton, Sutton, Québec. They were transported and held at the Sir George Williams Water Pollution Research Laboratory under the same conditions as described in Part I.

Two 21-day growth experiments were performed. The first one was designed to determine the food maintenance requirements of rainbow trout at 6, 12 and 18°C. The second was planned to measure the effects of cyanide on growth at 6, 12 and 18°C, at feeding rates five times the maintenance requirements.

In both growth experiments, 25 fish about 20g were randomly distributed to four test tanks at each of the three temperatures and acclimated as previously described (Kovacs, 1979). In the first growth experiment, the fish were fed to satiation level once a day during acclimation, whereas in the second, they were fed 1.6, 2.5 and 3.3 percent of wet body weight per day at 6, 12 and 18°C, respectively during this period.

Before the start of the experiments, the fish were not fed for 24 hours. They were then lightly anesthetized with 20 $mg.L^{-1}$ MS222 (tricaine methane sulphonate, Sandoz Corp.) individually branded using the liquid nitrogen method of Mighell (1969), blotted dry, weighed to the nearest hundredth of a gram, and their fork lengths measured to the nearest millimeter.

Twenty fish were replaced in each of the test tanks, but five randomly chosen individuals from each tank were sacrificed, oven dried for 7 days at 70°C, and stored to provide pre-experimental values of fat and water content. Fat content was measured by ether extraction with a Laboratory Goldfisch Fat Extractor (Lab Can Co. Model 35003), using a 2.000g sample subjected to a 4-hour reflux distillation.

In the first experiment, the fish were kept on four different feeding levels at each of the three temperatures, as given in Table I.

TABLE I: Ration levels fed to juvenile rainbow trout at 6, 12 and 18°C, used to determine maintenance ration over a 20-day period.

Temperature °C	Ration Level (% Wet Body Weight Day^{-1})			
6	0.0	0.8	1.5	2.5
12	0.0	1.2	2.5	4.0
18	0.0	1.5	3.5	4.5

They were fed half their rations, twice a day. Before each feeding, the tanks were cleaned to ensure that all the food was eaten.

Trout were weighed on the tenth day of the experiment, and the feeding levels adjusted as necessary. At the termination of the experiment, they were weighed, sacrificed and oven dried at 70°C for seven days again to determine water and fat content.

The second growth experiment was performed in a similar way. On the day following the branding and weighing, the metering of the cyanide solutions were initiated from Mariotte bottles (Leduc, 1966a) to establish the concentrations as given in Table II.

TABLE II: Concentrations of hydrogen cyanide that juvenile rainbow trout were exposed to for 20 days, at 6, 12 and 18°C.

Temperature °C	HCN Concentration (mg.L^{-1})			
6	0.000	0.005	0.010	0.015
12	0.000	0.010	0.020	0.030
18	0.000	0.015	0.030	0.045

Cyanide concentrations were determined daily by the method of Lambert et al (1975), but never varied by more than 5% of the predicted values. The concentrations were chosen to test levels just below lethality as determined from acute toxicity tests, and to provide a reasonably wide range between the control and the highest concentration.

The fish were exposed to cyanide for 20 days, during which they were fed five times the maintenance ration determined in the first experiment. They were fed twice a day, and the tanks cleaned by siphoning before each feeding.

The fish were weighed on the tenth day, and the feeding levels adjusted accordingly. At the end of the 20-day exposure period, 15 fish from each group were weighed, measured, sacrificed, oven dried at 70°C for seven days, and their water and fat content determined as already described. The five remaining fish from each group were tested for swimming stamina, as described below.

Swimming Stamina Tests

Fish of approximately the same length and weight were chosen and the following procedure was used.

One fish was inserted in the swimming chamber described by Kruzynski, (1972) which had been partially filled with cyanide-free water of the same temperature as the fish had been held in. The lid was bolted on, and the chamber was filled completely. The current was then turned up to 15 cm. sec^{-1} for three minutes, then to 30 cm. sec^{-1} for three minutes, finally to 45 cm. sec^{-1} for one minute. This gave the fish some time to get accustomed, and most fish adjusted well and faced the current immediately. Those fish that could not even maintain themselves against the current during the acclimation period were

ascribed a zero swimming time ability.

After the acclimation period, the current was raised up to 54 cm. sec^{-1} and held there for 30 minutes or fatigue time of the fish, whichever came first. A trout was judged to be fatigued when it could no longer hold its position against the current and drifted back against a screen in the chamber. It was then given a single electric shock and the water velocity was stopped for a few seconds so that the fish could come away from the screen. The current was then turned up again to 54 cm. sec^{-1}, and the next time the fish failed was the time considered to fatigue.

The fish was then removed, weighed, measured and discarded.

Liver Metabolites

Glycogen and lactic acid were monitored intermittently in livers of rainbow trout averaging 28g, over a 20-day period, at 6, 12 and 18°C, in the presence of 0.015 $mg.L^{-1}$ HCN and in controls.

Twenty-five trout per tank were acclimated as described previously, and fed twice a day with the described diet at the same feeding level as used in the second growth experiment. The trout were sampled on four occasions: on day zero, just before cyanide exposure, and 5, 10 and 20 days after initial cyanide exposure. The control and cyanide exposed groups were sampled simultaneously. Routine daily experimental maintenance was similar to that described in the second growth experiment.

At each sampling date, five fish were removed from the exposure tanks. The fish were immediately killed with a blow on the head, weighed and measured. Each liver was immediately excised, cut in half and each half weighed to the nearest ten-thousandth of a gram, frozen in liquid

nitrogen and stored in a freezer at -20°C. Livers from the five individual fish were pooled at each sampling time. Thus, half the pooled livers were used for glycogen determinations and the other half were used for lactic acid determinations.

The liver samples designated for glycogen determinations were removed from the freezer the next day and were digested in 30% KOH and precipitated with 95% alcohol, according to the method of Good et al, (1933). Glycogen concentrations were determined by the phenol-sulphuric acid colorimetric method of Montgomery, (1957) using a Bausch and Lamb Spectronic 70 spectrophotometer.

Liver samples stored for determinations of lactic acid were removed from the freezer within a week after storage. They were crushed to a powder form in a mortar filled with liquid nitrogen. Sufficiently chilled 8% (w/v) perchloric acid was then immediately added to the liver powder, so that the ratio of the final total liquid volume of the sample to its original weight was 4:1, (Bücher et al, 1963). This sample was homogenized for 2 minutes with a microblade homogenizer (Sorval, Omni-Mixer Microhomogenizer) in a 10ml volume cup. The tissue homogenate was then cold centrifuged at 1000g for 15 minutes. The supernatent was decanted and stored at 2-5°C before a 0.2ml aliquot was analyzed for lactic acid, determined by the lactic dehydrogenase colorimetric method (Sigma Chemical Co., Kit No. 826-UV, 1968), using a Bausch and Lamb Spectronic 70 spectrophotometer.

RESULTS

Maintenance Requirement

Preliminary experiments were carried out to determine the food

maintenance requirement of juvenile rainbow trout, that is the ration level required to just maintain body weight in the absence of cyanide at 6, 12 and 18°C. The growth of the trout over the 20-day period at different rations was calculated as the percent gain from the difference of the sum of individual wet weights taken at 0 and 20 days. These data are presented in Table III, together with the regression line equations calculated from these data and the derived values of food maintenance requirements at each temperature.

The maintenance level for wet weight of rainbow trout at 18°C is 2.2 times greater than the maintenance ration at 6°C, whereas it is 1.5 times greater at 12°C than at 6°C. These maintenance rations served as the guideline on which ration levels were chosen for the experiment on the effect of cyanide on the growth of rainbow trout at different temperatures.

The effect of temperature on the growth of juvenile rainbow trout varied with different feeding levels. At rations of 1.5% and lower, the trout grew significantly better at 6°C than at 18°C. This relationship changed at the next ration, since the growth of trout was favoured by a temperature of 12°C over a temperature of 6°C.

The effect of increasing rations on the body composition of trout, over a 20-day period at each temperature tested, can be compared in Table IV. Increasing rations at all temperatures seems to increase the fat content of the fish and decrease the moisture content. The fat-free dry matter content of trout does not seem to be much affected by ration level at either temperature.

Effect of Cyanide on Growth, Behaviour and Survival

During the acclimation period, the fish readily adapted to

TABLE III: Wet weight gains of juvenile rainbow trout at different rations and temperatures in 20 days; also shown are the equations of growth curves and the derived maintenance requirement values at different temperatures.

Ration % body wt. day^{-1}	Mean Wet Weight Gain (percent) 1/		
	6°C	12°C	18°C
0.0	-8.14 (3.27)	-10.33 (2.35)	-13.21 (1.96)
0.8	12.31 (3.37)		
1.2		14.29 (5.32)	
1.5	28.19 (5.39)		15.51 (4.48)
2.6	29.12 (5.67)	38.05 (6.99)	
3.5			51.13 (7.14)
4.0		60.13(12.71)	
4.5			65.49(13.04)
Equation of Regression Line	Y = -7.81 + 24.25 x 2/	Y = 8.37 + 17.61 x	Y = 12.07 + 17.60 x
Correlation Coefficient	0.99942	0.99716	0.99895
Maintenance Ration (% body wt. day^{-1})	0.32	0.48	0.69

1/ Figures in parentheses are standard deviation values.

2/ Equation calculated without using % gain value at highest ration.

TABLE IV: Body composition[1/] of juvenile rainbow trout (based on wet weight) at the beginning and at the end of a 20-day period at different rations and temperatures.

Temperature (°C)	Ration (% body wt. day^{-1})	Water (%)	Fat (%)	Fat-free dry matter (%)
6	Pre-experimental	79.6	1.9	18.5
	0.0	81.4	1.5	17.2
	0.8	78.5	2.2	19.4
	1.5	78.9	3.0	18.1
	2.5	78.9	3.0	18.1
12	Pre-experimental	78.0	2.0	20.0
	0.0	80.8	1.2	18.0
	1.2	78.8	2.7	18.5
	2.5	77.3	4.0	18.7
	4.0	76.2	5.0	18.8
18	Pre-experimental	78.6	2.4	19.0
	0.0	79.1	2.0	18.9
	1.5	77.0	3.6	19.5
	3.5	75.1	4.9	19.9
	4.5	75.0	5.4	19.6

1/ Average values based on pooled samples.

the test conditions and their behaviour was not affected by the toxicant except at cyanide concentrations of 0.015 mg.L^{-1} HCN at 6°C and 0.030 mg.L^{-1} HCN at 12°C. In these tanks, the fish exhibited rapid, erratic swimming activity and loss of appetite evidenced by the much longer period of time required to consume their entire ration. These behavioural abnormalities occurred only during the first ten days of exposure, and there were no apparent effects later on.

The initial number and mean wet weights of the fish in all test groups, are given in Table V. All fish survived the test conditions except for fish that died due to accidents as also reported in Table V.

Body Composition

The effect of cyanide exposure on body composition of rainbow trout at each temperature, is presented in Table VI. Control fish at different temperatures exhibited different body compositions, probably due to differences in temperature as well as ration levels. Fish held at 6 and 12°C exhibited higher water content than those at 18°C. This difference was mostly compensated for by fat.

Cyanide had a very marked effect on water and fat content, the effect varying with temperature and concentration. At 6°C, cyanide caused an increased water content in fish and a corresponding decrease in the percent fat at all concentrations tested. A decrease in the percent fat-free dry matter occurred only at the highest concentration tested.

At 12°C, cyanide caused a higher moisture content only at 0.030 mg.L^{-1}. Fat decreased slightly at 0.020 mg.L^{-1} HCN, but this was compensated for by a slight increase in the fat-free dry matter not, by water. A drop in percent fat at 0.030 mg.L^{-1} HCN level however, corresponded to the increase water content of the fish at this

TABLE V: Cyanide concentrations, rations, initial mean wet weights and their standard deviations and incidence of mortality during the 20-day growth experiments carried out at different temperatures.

Temperature (°C)	Ration (% body wt. day^{-1})	Cyanide Concentration ($mg.L^{-1}$ as HCN)	Initial Mean Wet Wt. (g) 1/	Mortality
6	1.5	0.000	17.43(0.94)	0
		0.005	15.70(0.88)	0
		0.010	16.61(0.92)	0
		0.015	16.97(0.99)	1 2/
12	2.5	0.000	19.34(0.94)	0
		0.010	17.16(0.91)	0
		0.020	18.29(0.87)	0
		0.030	17.81(0.84)	0
18	3.3	0.000	19.02(0.85)	0
		0.015	19.79(0.97)	0
		0.030	19.02(0.93)	0
		0.045	19.21(0.86)	4 3/

1/ Figures in parentheses are standard deviation values.

2/ One fish jumped out of the tank over night.

3/ Four fish died of cyanide poisoning when the dilution water was accidentally shut off for about 60 minutes.

TABLE VI: Body composition[1/] of juvenile rainbow trout (based on wet weight), at the beginning and at the end of a 20-day period, exposed to various levels of cyanide at different temperatures.

Temperature (°C)	HCN ($mg.L^{-1}$)	Water (%)	Fat (%)	Fat-free dry matter (%)
6	Pre-experimental	80.4	1.6	18.0
	0.000	79.1	2.6	18.4
	0.005	78.9	2.1	18.0
	0.010	79.9	2.1	18.0
	0.015	81.4	1.7	17.1
12	Pre-experimental	79.5	1.7	18.8
	0.000	77.5	4.0	18.5
	0.010	77.5	3.7	18.9
	0.020	77.7	3.3	19.0
	0.030	80.2	2.2	19.1
18	Pre-experimental	77.7	2.8	19.5
	0.000	74.9	4.6	20.5
	0.015	74.4	4.8	20.8
	0.030	73.3	4.4	20.8
	0.045	75.7	3.7	20.6

1/ Average values based on pooled samples.

concentration.

Trout tested at 18°C had their moisture levels affected only at 0.045 mg.L^{-1} HCN by an increment corresponding to the decreased fat of the fish.

These results indicate that cyanide changes the body composition of trout mostly by replacing fat with water at all temperatures. Temperature in turn influenced the level of cyanide at which the effect was manifested.

Growth Calculation

Growth, in wet and dry weight, was expressed as the means of specific growth rates (MSGR) of individual fish in percent gain per day, calculated from Brown's (1957, p. 365) equation:

$$MSGR = 100 \frac{\ln YT - \ln Yt}{T-t}$$

where lnYT and lnYt are the natural logarithms of the weight of fish at times T and t days. The mean specific growth rates of the cyanide toxified groups were statistically compared to control groups by means of a Student's T-test and the levels of significance presented in the appropriate tables.

Wet Weight

The effects of cyanide on the growth in wet weight of juvenile rainbow trout during the periods 0-10, 10-20 and 0-20 days, are presented in Table VII. Control fish grew better at 18 than at 12 than at 6°C, clearly showing that when rainbow trout are fed five times their maintenance ration, the fish acclimated to the higher temperatures are favoured with respect to growth. Most of the reduced growth due to cyanide exposure occurred during the first ten days, although not in identical fashion at the three temperatures.

TABLE VII: Mean specific growth rates (MSGR)[1/] of rainbow trout exposed to various concentrations of cyanide during a 20-day period at different temperatures.

Temperature (°C)	HCN $mg.L^{-1}$	MSGR: 0-10 days ($\% day^{-1}$)	MSGR: 10-20 days ($\% day^{-1}$)	MSGR: 0-20 days ($\% day^{-1}$)
6	0.000	1.10 (0.34)	1.23 (0.30)	1.16 (0.18)
	0.005	1.07 (0.39)	1.33 (0.38)	1.19 (0.26)
	0.010	1.02 (0.62)	1.26 (0.64)	1.14 (0.46)
	0.015	0.50* (0.63)	1.31 (0.55)	0.91* (0.53)
12	0.000	1.59 (0.46)	1.76 (0.53)	1.67 (0.39)
	0.010	1.71 (0.45)	1.83 (0.29)	1.77 (0.26)
	0.020	1.13* (0.71)	1.93 (0.58)	1.53 (0.53)
	0.030	0.39* (0.89)	1.26* (0.95)	0.82* (0.82)
18	0.000	1.99 (0.37)	2.00 (0.29)	1.99 (0.27)
	0.015	1.99 (0.36)	1.96 (0.38)	1.98 (0.28)
	0.030	1.85 (0.32)	1.98 (0.51)	1.91 (0.33)
	0.045	1.00* (0.87)	1.34* (0.67)	1.17* (0.72)

1/ Figures in parentheses are standard deviation values.

* $p < 0.05$

At 6°C, 0.015 mg.L^{-1} HCN reduced growth by 55% in the first ten days, whereas during the second ten-day period, trout at this concentration actually grew slightly faster than controls, resulting in only a 22% reduction in wet weight growth rate over the entire test period. The two other concentrations had virtually no effect at this temperature.

At 12°C, when compared to controls, trout exposed to 0.020 and 0.030 mg.L^{-1} HCN during the first ten days, showed reductions in wet weight growth rates of 29 and 75%, respectively. On the other hand, a 0.010 mg.L^{-1} HCN slightly stimulated growth. During the following period, the same fish exhibited a different pattern of growth. Trout grew slightly better at 0.010 and 0.020 mg.L^{-1} HCN than controls, whereas at 0.030 mg.L^{-1} HCN, growth was only reduced by 28%. As a result of the changing growth rates, over the entire 20-day period, 0.020 and 0.030 mg.L^{-1} HCN reduced growth in wet weight by 9 and 51%, respectively, with only the latter being significant ($p < 0.05$).

At 18°C, during the first ten days, trout experienced a 50% reduction in wet weight gain at 0.045 mg.L^{-1} HCN, but were not affected by any other concentrations. This pattern remained unchanged, but during the following ten days, these fish accelerated their growth, thus resulting in an overall 41% reduction in wet weight gain during the 20-day growth period. Thus, 0.045 mg.L^{-1} HCN was the only effective level at 18°C.

Dry Weight

Growth of fish is best represented by dry weight gain, which represents true tissue deposition. The mean specific growth rates in dry weight over the 20-day period was presented in Table VIII. At all

TABLE VIII: Mean specific grow rate (MSGR) based on dry weights and absolute fat gain by rainbow trout during a 20-day exposure to various cyanide concentrations at different temperatures

Temperature (°C)	HCN ($mg.L^{-1}$)	MSGR: 0-20 days ($\% \ day^{-1}$) 1/	Absolute Fat Gain: 0-20 days (g)
6	0.000	1.49 (0.18)	0.295
	0.005	1.35 (0.30)	0.177
	0.010	1.22* (0.48)	0.168
	0.015	0.71* (1.67)	0.071
12	0.000	2.12 (0.43)	0.714
	0.010	2.19 (0.35)	0.571
	0.020	1.93 (0.50)	0.489
	0.030	0.63* (0.81)	0.147
18	0.000	2.61 (0.27)	0.770
	0.015	2.61 (0.36)	0.854
	0.030	2.53 (0.33)	0.676
	0.045	1.60* (0.72)	0.372

1/ Figures in parentheses are standard deviation values.

* $p < 0.05$

temperatures the dry weight gains of control fish were higher than those of wet weight, but followed the same relationship with respect to temperature. However, at concentrations of cyanide where reduction in growth expressed as wet weight gain were found to be significant at 6 and 12°C (0.015 and 0.030 $mg.L^{-1}$ HCN, respectively), the mean specific growth rates in dry weight were found to be much lower than for wet weight. For example, the mean specific growth rate in dry weight over a 20-day period at 0.015 $mg.L^{-1}$ HCN and 6°C was 0.71% per day, whereas on a wet weight basis, it was 0.91% per day. This suggests that growth expressed on a wet weight basis is not the best indicator because growth can be masked by disturbance of the water of the fish. By contrast, at 18°C, even at 0.045 $mg.L^{-1}$ HCN, the percent gain in dry weight per day was greater than for wet weight.

At 6°C, a concentration as low as 0.010 $mg.L^{-1}$ HCN produced a significant reduction of 52% in dry weight gain, an effect which was visible on wet weight gain only at 0.015 $mg.L^{-1}$ HCN.

At 12°C, a concentration of 0.030 $mg.L^{-1}$ HCN reduced dry weight growth rate by 70% when compared to control fish. By contrast, 0.010 $mg.L^{-1}$ HCN stimulated dry weight gain, and a 0.020 $mg.L^{-1}$ HCN level only reduced dry weight gain by 9%.

At 18°C, only the highest concentration significantly affected dry weight gain, reducing it by 39%.

Fat

Fat gains were calculated by subtracting the pooled fat content of fish at day 0 from the pooled fat content of fish on day 20 and were divided by the number of fish to derive average absolute fat gains by fish in grams.

Absolute fat gains by control and toxified fish at each temperature are also presented in Table VIII. Among the control fish, trout at 18°C gained more fat than at 12 or 6°C, although the difference between 12 and 18°C was minimal. At 6 and 12°C, even the lowest cyanide concentrations caused a significant reduction in fat gain whereas at 18°C only much higher concentrations were required to produce similar effects.

Fat gains in fish tested at 6°C were reduced by 40, 43 and 76% when compared to fat gain of control fish at 0.005, 0.010 and 0.015 $mg.L^{-1}$ HCN, respectively. At 12°C, concentrations of 0.010, 0.020 and 0.030 $mg.L^{-1}$ HCN reduced the absolute fat gain by 20, 31 and 79% when compared to control.

At 18°C, two different responses were noted. The lowest concentration (0.015 $mg.L^{-1}$ HCN) increased the fat gain of trout by 11%, but concentrations of 0.03 and 0.045 $mg.L^{-1}$ HCN decreased it by 12 and 52%.

Thresholds

The overall effect of cyanide on growing juvenile rainbow trout during the 20-day experimental period, expressed in terms of wet weight, dry weight and fat gains is illustrated in Figure 1 by eye-fitted curves. From these curves, an attempt was made to estimate the threshold levels of cyanide concentrations for each growth parameter and at each of the three test temperatures. This was achieved by taking, wherever possible, the inflection point of the growth curve as being the threshold cyanide concentration. In those instances where reductions occurred at the lowest concentration tested, the threshold values are given as below that value. The threshold values thus obtained are

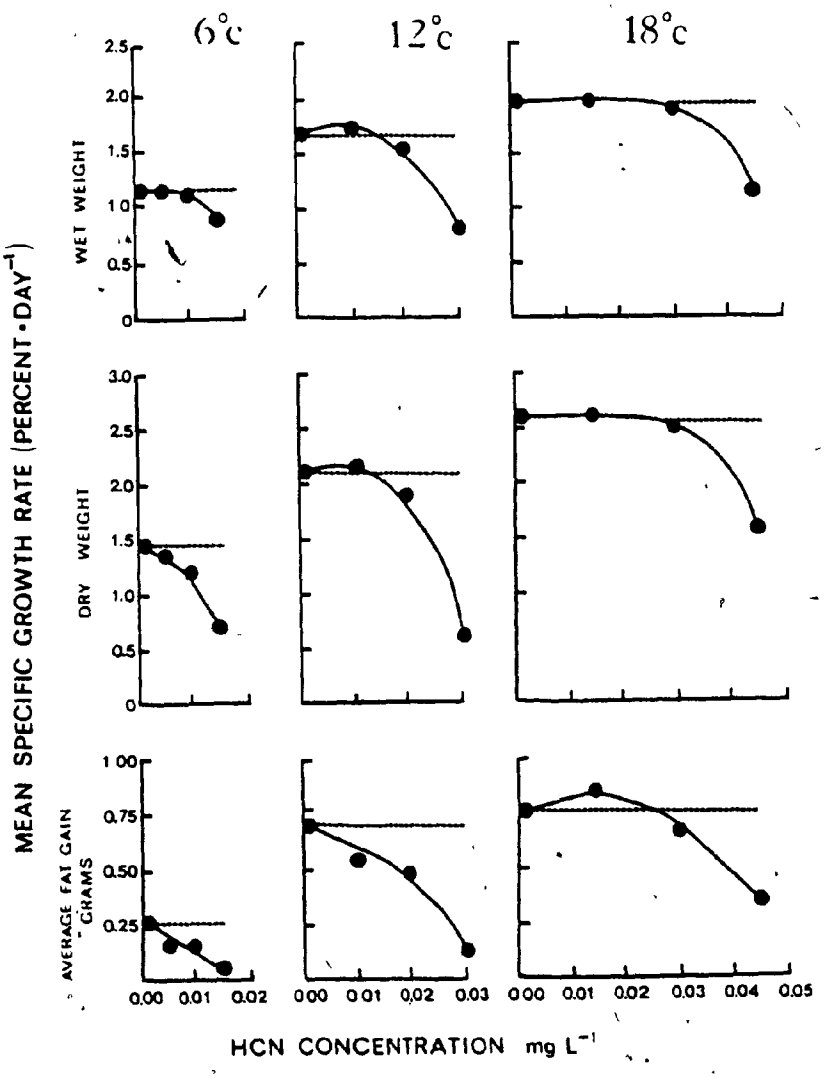

Figure 1: The relationship between wet weight, dry weight and fat gain of juvenile rainbow trout and the concentrations of cyanide to which they were exposed during 20-day experiments under flow-through conditions of various temperatures.

TABLE IX: Threshold concentrations of cyanide ($mg.L^{-1}$ HCN) that produced no effect on the growth of rainbow trout during a 20-day exposure in flow-through aquaria at different temperatures.

Temperature (°C)	Wet Weight	Dry Weight	Fat
6	0.01	<0.005	<0.005
12	0.015	0.010	<0.01
18	0.030	0.030	0.025

presented in Table IX. The threshold values increase with temperature for all three parameters. For wet weight, in the range of 6 to 18°C, the EC is increased by a factor of 3, whereas for dry weight and fat gain, it is increased by a factor greater than 4. In all cases, the threshold for dry weight and fat gain were lower than for wet weight.

Swimming Performance

At the end of the growth experiment, five fish of approximately the same size were chosen from each of the twelve test groups for swimming stamina experiments at the same water temperature that the fish had been held, but without cyanide. The distance travelled by each fish was calculated by multiplying swimming time by the velocity ($0.54 m.sec^{-1}$) against which the fish was swimming. The results are graphically presented in Figure 2 by the calculated regression lines of mean log distance travelled versus cyanide concentrations at 6, 12 and 18°C.

There were extensive individual variations in swimming ability, but, it was possible to demonstrate some significant relations between temperature and cyanide. For any given temperature, the mean log swimming distance of rainbow trout decreased linearly with increasing cyanide concentrations with the slope of the regression lines increasing with decreasing temperature. The three slopes were significantly different ($p < 0.05$) from each other when compared by analysis of covariance. This shows that the cyanide effect on the swimming ability of rainbow trout is greater at the lower temperature.

Temperature also influenced the swimming ability of control fish, the effect being most pronounced between 6 and 12°C, but only statistically significantly different ($p < 0.02$) between 6 and 18°C.

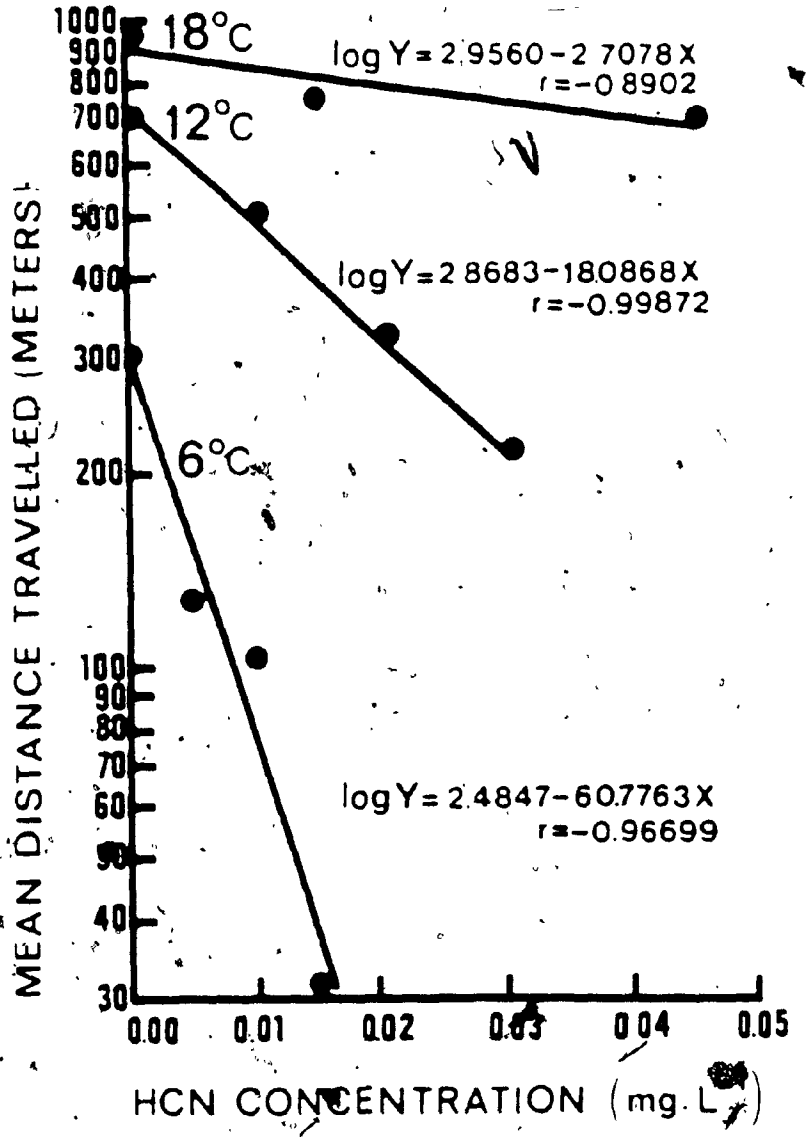

Figure 2: The relationships (regression equations) between mean distance travelled by juvenile rainbow trout and the concentrations of cyanide to which they were exposed for 20 days at various temperatures.

Changes in glycogen and lactic acid levels in control and cyanide exposed trout over a 20-day period at 6, 12 and 18°C, are shown in Table X.

At day 0, before the treatment began, the values for both glycogen and lactic acid were fairly uniform between control and treated groups for any given temperature. Later, in the control groups, glycogen tended to increase with time at 6 and 12°C, but not at 18°C. Lactic acid on the other hand decreased slightly at 6 and 12°C, but was stable at 18°C. On the average, glycogen levels were higher at 12°C than at 6 or 18°C, whereas lactic acid levels increased from 6 to 18°C.

In the cyanide-treated group, glycogen levels dropped drastically after five days exposure at 6°C, but started to reach normal levels by the twentieth day. At 12°C, glycogen concentration decreased around the tenth day, but again returned to a near normal level by day 20. At 18°C, the drop in glycogen was noticed only after 20 days of exposure, thus suggesting a delayed effect of cyanide at higher temperatures. The extent of reduction was however less than at 6°C. Comparing average values of glycogen between control and treated groups, it appears that glycogen levels were decreased by cyanide at 6 and 12°C, but not at 18°C. The decrease was greatest at 6°C.

At 6°C, cyanide produced changes in lactic acid as the inverse of glycogen, as it sharply rose on day five, but returned to normal by day ten, suggesting a switch to anaerobic metabolism. At 12°C and 18°C, liver lactic acid concentrations were similar in control as in cyanide-exposed fish, and lower glycogen levels were not reflected by higher lactic acid levels.

TABLE X: Liver glycogen and lactic acid levels of juvenile rainbow trout during exposure to 0.015 $mg.L^{-1}$ HCN at different temperatures.

Day of Exposure	Liver Glycogen Levels ($mg.g^{-1}$)					
	Control			Treated		
	6°C	12°C	18°C	6°C	12°C	18°C
0	57	69	56	52	63	55
5	47	72	54	12	66	62
10	64	79	54	16	48	61
20	70	83	56	47	67	47
Average	60	76	55	32	61	54
Day of Exposure	**Liver Lactic Acid Levels ($mg.100g^{-1}$)**					
	Control			Treated		
	6°C	12°C	18°C	6°C	12°C	18°C
0	22	26	27	19	28	27
5	15	28	30	34	27	25
10	16	21	26	17	22	30
20	18	18	21	19	21	25
Average	18	23	26	22	24	27

DISCUSSION

This study revealed that the toxicity of cyanide to rainbow trout at the sub-lethal level remained more potent at lower temperatures as at the slowly lethal level. The effective concentrations (EC's) of cyanide on growth, as well as on swimming ability, proved to be lower when the fish were acclimated to lower temperatures. However, the toxic action of cyanide at the sub-lethal level did not manifest itself in identical fashion at 6, 12 and 18°C. While a certain growth rebound, due to cyanide, occurred at all temperatures tested, the extent of this response was concentration and temperature-dependent. Furthermore, throughout the 20-day experimental period, wet and dry weight gains higher than in controls, occurred at 12°C at 0.01 $mg.L^{-1}$ HCN, whereas 0.015 $mg.L^{-1}$ HCN stimulated fat gain at 18°C.

The inhibition of cytochrome oxidase by cyanide, blocking the oxidative chain, stimulates anaerobic metabolism and the accumulation of reduced coenzymes leading to an unbalanced redox state and diminishing energy pools (ATP) within the cell. Thus, cyanide, by upsetting aerobic metabolism, leads to a decreased efficiency of energy utilization compounded by increases in the maintenance requirements of trout, (McCracken, 1978). Therefore, less energy is obtained by trout from their food consumed, and a greater proportion of this is required to support life processes, leaving less energy available for growth. It would appear that the survival of trout at sub-lethal concentrations of cyanide would depend on their ability to derive energy from anaerobic metabolism, to increase the efficiency of the aerobic enzyme system and to detoxify cyanide. Furthermore, Isom et al (1975) found that in mice,

chronic cyanide poisoning leads to catabolism of carbohydrates by the pentose phosphate cycle, apparently in an attempt by cells to maintain a balanced redox state and energy pool.

The different responses of trout to cyanide at different temperatures must reflect temperature-related changes in the physiological and biochemical state of the fish. Metabolic changes in poikilotherms, during temperature compensation, has received much attention. Hochachka (1967) observed that in trout, cold (4°C) adaptation favors extramitochondrial over mitochondrial metabolism in both liver and muscle, thus increased glycolysis, gluconeogenesis, glycogen synthesis, lipogenesis and pentose monophosphate shunt participation. On the other hand, the activity of the Krebs cycle in the tissues of the cold-acclimated fish may decrease, increase, or remain unchanged, depending on the species and the tissues, (Hochachka and Somero, 1971). These metabolic reorganizations during cold temperature acclimation produce biophysical and biochemical restructuring of cellular and tissue components leading to compensatory adjustments in the metabolic rate of fish, (Hochachka and Somero, 1971). Since the survival and performance of trout in cyanide depends on its ability to alter its metabolic processes, changes in the biology of the fish during temperature acclimation will certainly influence this.

Growth EC

The effective concentration (EC) is the minimal concentration of a poison causing an effect. Basically, the EC is a sub-lethal threshold concentration which in this growth study revealed two important phenomena. First, the EC values increased with increasing temperatures. Second, the EC for dry weight gain was lower than for wet weight at 6°C and 12°C, and

the EC's for fat deposition were lower than for either wet or dry weight growth rates at the three temperatures tested.

The effect of temperature on EC is illustrated in Figure 3, where the EC for wet weight growth rate reduction and the 96-hr LC50 values determined by Kovacs (1979) were plotted against temperature. The two curves are essentially parallel and this pattern in the increase of toxicity thresholds with increasing temperature closely resemble the changes of the standard metabolic rate of trout, with the greatest change occurring between 12 and 18°C. Dean (1969) found that in cold (5°C) adapted rainbow trout, acetate-flow through the Krebs cycle is held relatively constant between 5 and 11.5°C, due to the highly competitive lipogenesis pathway. In warm (18°C) acclimated trout, the substrate is channeled mostly into the Krebs cycle. This kind of metabolic reorganization due to temperature may explain the greater toxicity of cyanide at lower temperatures, as well as small differences between 6 and 12°C. If at low temperature, the Krebs cycle activity is already functioning at low capacity, the effect of cyanide on the oxidative enzymes of the system would be more deleterious than at higher temperatures.

Also, Ekberg (1962) and Webb (1963) suggested that the greater sensitivity of cyanide to some poikilotherms at lower temperature may reflect some qualitative changes of the cytochrome oxidase, rendering it more sensitive to cyanide. These changes appear to be linked to the nature of the mitochondrial membrane phospholipids, (Sidell et al, 1973) due to alterations in the lipid microenvironment of the enzyme, (Shaklee et al, 1977). Caldwell and Vernberg (1971) have determined that the lipids in fish gill mitochondria become increasingly unsaturated at low environmental temperatures. The relation between such a change and

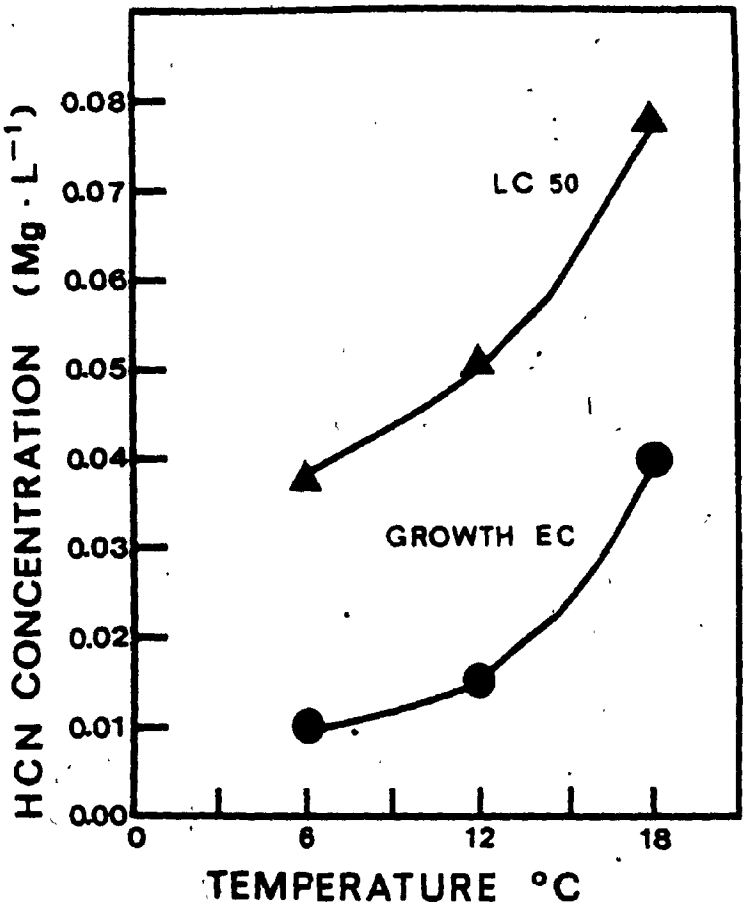

Figure 3: The pattern of lethal and sub-lethal cyanide threshold concentrations with increasing temperatures.

cytochrome oxidase sensitivity to cyanide is unclear. Yet in this study, an exposure of trout to 0.015 $mg.L^{-1}$ HCN stimulated glycolytic activity only at 6°C and to a small extent at 12°C (see Table X). At 18°C, this concentration of cyanide was not sufficient to produce such a change in tissue metabolism presumably because cytochrome oxidase was not as sensitive to HCN at this temperature. Glycolytic activity was measured by lower liver glycogen and higher lactic acid levels. Such symptoms are classical signs of anaerobic metabolism and have been used by Burton et al (1972) to pinpoint the cause of death by zinc poisoning of rainbow trout as anaerobiosis.

Webb (1963) also stated that the effect of temperature on cyanide toxicity could depend on the behaviour of the cyanide-resistant fraction of respiration which account for an important fraction of the cell's energy supply. It is possible that during the metabolic adaptations of thermal acclimation, this cyanide resistant respiration was enhanced at higher temperatures. This question would require extensive research and was beyond the scope of this study.

While the greater toxicity of cyanide at lower temperatures may be explained at the cellular and biochemical levels, physiological responses must also be considered. Hyperthyroidism in mammals has been related to chronic cyanide poisoning due to reduced thyroxine formation, (Bourdoux et al, 1978) but not yet demonstrated in fish, although Leduc (1966b) suggested it to explain various deleterious effects of cyanide on growing cichlids. Thyroxine promotes growth in trout, (Narayasingh and Eales, 1975), but its need for growth is greater at lower (7°C) rather than higher (15-19°C) temperatures, (Leatherland et al, 1977). It would therefore seem that any damage to the thyroid gland by cyanide (Chan,

1971) would have a greater effect on the growth of trout at 6° rather than at 12° or 18°C.

In addition to growth, thyroxine plays an important role in osmoregulation which represents 20-27% of the total metabolic cost of a trout with greater energy expenditure at 5°C than at 15°C (Rao, 1968). Chan (1971) established that cyanide impaired the osmoregulatory capacity of trout. These findings further corroborate the results of this study as water contents of control fish were higher at low temperatures, and further enhanced by exposure to cyanide, (see Table VI). These higher increased water contents masked the true weight gains of cyanide-exposed fish so when dry weight gains were used to assess growth, lower concentrations of cyanide were found to reduce growth. This was also observed by Dixon (1975), Speyer (1975) and McCracken (1978).

The greater effect of cyanide on dry weight gain also reflects the reduced fat synthesis with the lowest EC values at all temperatures. The impact of cyanide on fat synthesis was greatest at the lower temperatures, surprisingly since cold-adapted trout are geared towards fat synthesis, (Hochachka and Hayes, 1962). However, fat synthesis is also ration-dependent (see Table IV) and the smaller ration fed at 6°C than at 12 and 18°C, because of lesser maintenance ration could possibly explain the greater effect of cyanide at the lowest temperature. Furthermore, a reduced assimilation efficiency would upset the carbohydrate metabolism and all intermediate precursors to fat synthesis. A reduction in assimilation efficiency could be equivalent to a reduced ration (Webb and Brett, 1972) and, in the experiment on the effect of ration on growth, we showed that one of the best indicators of the amount of ration consumed by the fish was its fat content. With cyanide-exposed trout (see Table IV), reduced growth rates corresponded to lower fat

contents. Cyanide thus appears to have affected the assimilation of food with its obvious impact on fat deposition. Such an effect would be magnified at lower temperatures since Brocksen and Bugge (1974) have shown that rainbow trout assimilated only 71.8% of their food at 5°C, whereas at 17°C, this figure was 77.9%.

Reduced lipogenesis has also been measured by Dixon (1975) and Speyer (1975) in cyanide-exposed trout, reflecting high energy demands which cannot be met by the fish forced into utilizing less efficient energy producing anaerobic processes. These observations are also in agreement with the findings of Shapiro et al (1957) who also observed reduced fat synthesis (esterification of fatty acids) in the adipose tissue of cyanide-exposed organisms.

On the other hand, accelerated fat deposition was observed at 18°C and 0.015 mg.L^{-1} HCN in this study as well as by McCracken (1978). This question will be discussed in the next section.

Growth Rebound and Stimulation

The effects of cyanide on growth were most prominent during the first ten days of exposure at all three temperatures. During the second half of the experiments, cyanide-exposed fish grew better than during the first half and in some cases better than the controls, (see Table VII). Furthermore, actual growth stimulation during the entire 20-day test period occurred in terms of wet and dry weight at 0.01 mg.L^{-1} HCN level at 12°C and in terms of fat gain at 0.015 mg.L^{-1} HCN and 18°C.

The ability of trout to rebound from an initial growth depression due to cyanide poisoning seems to depend on the degree of the original depression, cyanide concentration and/or temperature, a phenomenon also measured by Leduc (1966b), Dixon (1975) and Speyer (1975).

This growth rebounding phenomenon also appears related to the liver glycogen and lactic acid levels measured in a separate experiment. At 6°C, 0.015 $mg.L^{-1}$ HCN caused a marked reduction in growth rate of the fish during the first ten days of exposure and at the same time, a reduction of liver glycogen and an increase of liver lactic acid within five days (see Tables VII and X). Liver glycogen and lactic acid levels began returning to normal by day ten, at the same time the growth rebound phenomenon was observed.

The nature and explanation of this response is not fully understood, yet some indirect evidence is available. On exposure to cyanide, trout are immediately forced to obtain some energy from anaerobic processes, depending on cyanide concentration and temperature. This initial impact may be alleviated by the production of more cytochrome oxidase to reduce dependence from anaerobic metabolism followed by a slow return to normal growth. Finally, detoxification of cyanide would leave the fish with excess cytochrome oxidase insuring a better energy utilization and even slight stimulation in growth.

We have already shown that on exposure to cyanide, trout temporarily shift to anaerobic metabolism, the effect being well marked at 6°C, less at 12°C and not noticeable at 18°C. There is no direct evidence of cyanide-induced production of cytochrome oxidase, but respiration data of cyanide poisoned trout are most interesting. Dixon (1975) measured the routine metabolic rate of rainbow trout in flow-through respirometers (without cyanide) at the ends of 18-day exposure periods to 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN. During the six-day measurements, the mean respiration rates of the cyanide-exposed fish which were initially lower, peaked by day four and finally levelled off higher than the controls. The decreasing respiration rate of the control

probably reflected acclimation and changes from a feeding to a non-feeding regime, but the higher respiration rate of the cyanide-exposed fish could not be clearly explained. Assuming higher cyanide-induced cytochrome oxidase levels in poisoned fish, and cytochrome oxidase inhibition being reversible, one may speculate that the fish could perform a higher routine metabolic rate mediated through higher levels of cytochrome oxidase, once removed from cyanide inhibition. It is also interesting to note in Dixon's (1975) study that respiration of the trout were inversely related to the degree of growth inhibition in the previous 18-day exposure to cyanide, i.e., to greater reduction in growth corresponded higher oxygen consumption. The level of oxidative enzymes in cyanide-exposed fish have not been measured, but it has been shown that guinea pigs adapted to high altitude (4200 m), where oxygen levels are low, thus simulating cyanide poisoning, have higher succin-oxidase and cytochrome oxidase levels than controls kept at sea level, (Tappan et al,1957).

Cyanide can be detoxified through the presence of rhodanase (thiosulfate sulfurtransferase) which has been identified in many animals including fish, (Sido and Koj,1972; Schievelbein et al,1969). The enzyme transfers a sulfur to cyanide ion to produce thyocyanate which, although less toxic, can over long periods of time be harmful to the thyroid gland in mammals through interference with thyroxine production, (Bourdoux et al,1978). No parallel studies seem to have been carried out with fish, but Achard and Binet (1934) have noted that thiosulfate also prolonged the survival of carp (Cyprinus carpio) subjected to lethal concentrations of cyanide. This suggests a detoxification mechanism for cyanide in fish similar as in mammals.

For the growth rebounding to occur, increased cytochrome oxidase followed by detoxification of cyanide seems essential to explain the observed temperature and cyanide conentration dependence of the phenomenon.

At 6°C, the rebounding of growth was much more pronounced than at 12 or 18°C, possibly due to increased activity and quantity of cytochrome oxidase, a response observed in cold acclimated goldfish by Caldwell (1969) and Freed (1965) as well as in the green sunfish (Lepomis cyanellus) by Shaklee et al (1977). It seems that in fish, acclimation to low water temperatures involves increased production of cytochrome oxidase and other electron transport enzymes, possibly to meet depressing metabolic conditions. Fish seem to be geared towards increasing cytochrome oxidase levels at low temperatures as required. Thus cyanide, by inhibiting this enzyme, would stimulate further cytochrome oxidase production easier in cold-adapted trout than in trout acclimated to higher temperatures. Therefore, trout at 6°C would return to normal metabolism sooner than trout at 12 and 18°C. As detoxification mechanisms start to nullify cyanide, the extra cytochrome oxidase could lead to better food conversion efficiency and eventually better growth than control fish, as was observed at 6°C.

At 12°C and 0.01 $mg.L^{-1}$ HCN, cyanide stimulated growth throughout the experimental period. This growth stimulation was not due to greater fat deposition. Presumably, cyanide did not cause any deleterious effects, but still stimulated the compensatory mechanisms discussed above. At 0.02 $mg.L^{-1}$ HCN, the response of trout growth was similar as at lower concentrations at 6°C, that is an initial depression, followed by better growth than controls in the second half. The explanation is the same as

for 6°C tested trout, except that a higher concentration of cyanide was necessary to produce the same effect. At 12°C a 0.03 $mg.L^{-1}$ HCN was sufficiently high enough to cause growth reduction as well as disturbance of the compensatory mechanisms. At 18°C, the production of cytochrome oxidase being less active but the enzyme perhaps more resistant to cyanide, only a 0.045 $mg.L^{-1}$ HCN level caused significant growth reduction, with little change in growth between the first and second period of the experiment. Similarly little changes in liver glycogen and lactic acid levels were observed at 0.015 $mg.L^{-1}$ HCN and 18°C.

Overall, low cyanide concentrations (less than 0.015 $mg.L^{-1}$ HCN) initially depress growth at low temperatures followed by compensatory mechanisms, whereas at higher temperatures actually stimulate some growth processess. Concentrations above 0.015 $mg.L^{-1}$ HCN seem to have a more lasting effect at all temperatures, possibly because they are too high at 6°C, whereas at higher temperatures the production of more cytochrome oxidase is more difficult in fish.

Growth rebounding, similar in nature to this study, were observed by Dixon (1975) and Speyer (1975) at 12.5°C and 11°C, respectively. On the other hand, Leduc (1966b) working with cichlids at 25°C, found low concentrations of cyanide (0.01-0.02 $mg.L^{-1}$ HCN) initially stimulated growth, but later reduced it by the end of the 36-day period. This different response could be due to the different species tested and/or the duration of the experiment. At higher concentrations of cyanide (0.06-0.10 $mg.L^{-1}$ HCN), Leduc (1966b) found cichlid growth rate reduced at the beginning, but better than control by the end of the test period. Apparently better growth in cyanide solutions resulted because the fish, fed unrestricted rations, consumed more food.

With coho salmon (Oncorhynchus kisutch), Leduc (1966b) found that cyanide (0.02-0.08 $mg.L^{-1}$) initially reduced growth at 16°C, but stimulated it during the second 12-day experimental period. This response is similar to those observed on rainbow trout in this study, and by Dixon (1975) and Speyer (1975), but Leduc (1966b) explained better growth by salmon in cyanide solution resulting from better food conversion efficiency as the fish became less active, thereby reducing their metabolic cost.

At a concentration of 0.015 $mg.L^{-1}$ HCN at 18°C cyanide slightly stimulated fat deposition. Such an increase is difficult to explain since no change in liver metabolite levels suggest little metabolic compensation and since fat synthesis was otherwise found to be the most sensitive indicator of cyanide poisoning. However, McCracken (1978) also observed 5g rainbow trout to gain more fat than controls when swimming against a current of 12 $cm.sec^{-1}$ and Szabo et al (1973) noted little effect of cyanide on triglyceride production by human placenta.

Several other workers have also noted growth stimulation to occur due to cyanide poisoning. At low cyanide concentrations of 0.01 $mg.L^{-1}$ HCN, Negilsky (1973) found growth rates and production of young chinook salmon (Oncorhynchus tswawystscha) held in an artifical stream at a current of 24 $cm.sec^{-1}$ to increase. The growth rates of small 5g rainbow trout swimming at 12 $cm.sec^{-1}$ in annular growth chambers and exposed to 0.01 $mg.L^{-1}$ HCN at 10°C, was also stimulated when compared to controls, fat deposition making the difference, (McCracken, 1978). Swimming against a current increases the metabolic rate of salmonids, possibly leading to accelerated detoxification of cyanide and increased cytochrome oxidase production.

The impairment of swimming stamina of fish has been recognized as one of the most sensitive indicators of environmental pollution, (Sprague, 1971). The swimming performance of salmonids can be severely hampered by exposure to low concentrations of cyanide, even long after removal from cyanide solutions, (Neil, 1957; Leduc, 1966b; Broderius, 1970; Speyer, 1975).

In this study, control fish acclimated and tested at 18°C swam 2.8 times further in 30 minutes than trout acclimated and tested at 6°C, when subjected to a current of 4.2 lengths sec^{-1}, which Brett (1964) considers the point of transition between steady (prolonged) and burst swimming. Prolonged swimming depends on aerobic and anaerobic metabolism (Farlinger and Beamish, 1977), relying both on the capacity of the liver to maintain reserves of glycogen and on the delivery of metabolites and oxygen by the blood to the muscles, (Brett, 1964). The rate of these metabolic processes are temperature-dependent and will directly influence swimming stamina which is reduced at lower temperatures as also observed by Glova and McInernery, (1977). Swimming fatigue, as measured in this study, thus results from the accumulation of lactate from anaerobic catabolism of carbohydrates, (Black, 1958). Delays of fatigue depend on aerobic metabolism functioning at its maximum capacity and on the ability of organs and physiological processes to be in perfect condition.

After a 20-day exposure to sub-lethal levels of cyanide, the swimming ability of rainbow trout was reduced at all concentrations previously tested. While cyanide-exposed fish appeared normal, and as already seen, had by day-20 all returned to normal or near normal growth

rate, such a response to a non-cumulative poison like cyanide suggests biochemical disturbance and perhaps tissue damage. These would only be manifested when the fish are stressed by a factor such as swimming bursts. Dixon (1975) observed liver necrobiosis in cyanide-exposed trout and we noted biochemical changes occurring in trout during adaptation to cyanide, as seen in growth rebounding phenomenon. Also, Dixon (1975) has shown the routine metabolic rate of trout to be lower at 0.01, 0.02 and 0.03 $mg.L^{-1}$ HCN concentrations even after 18 days of exposure. Such changes could easily explain the observed effect of cyanide on swimming performance.

Lower temperatures further compounded this condition, as at 6°C, a concentration as low as 0.015 $mg.L^{-1}$ HCN reduced swimming stamina by 87%, while 0.03 and 0.045 $mg.L^{-1}$ HCN reduced swimming stamina by only 71 and 25% at 12 and 18°C, respectively.

Swimming ability of fish depend on their scope of activity, defined by Fry (1971) as the difference between active and standard metabolism. The scope for activity, is the smallest at low temperatures, (Fry, 1971). Furthermore, the cytochrome oxidase system of trout acclimated to lower temperatures seems to be less efficient, with the ability of the fish for aerobic metabolism and thus prolonged swimming greatly reduced. This was manifested by better swimming stamina of control fish at higher temperatures, and could be one reason for increased production of this enzyme by cold adapted fish. Moreover, since fatigue is due to oxygen debt and lactate burden, the aerobic scope of a poikilotherm could also depend on the removal of lactate. The lactate formed during anaerobic activity is temperature independent in reptiles, but its rate of removal increases directly with temperature,

(Gordon, 1977). If lactate excretion is also greater in trout at 18°C than at 12° or 6°C, due to higher metabolic rate, this could also explain the greater effect of cyanide on their swimming ability at lower temperatures.

Concluding Remarks

In temperate zones, fish must survive a wide range of temperatures due to the four seasons. During acclimatization to the changes in temperature regime, compensatory mechanisms occur which alter the biology of the animal. In this study, we saw that acclimation to various temperatures affected the toxic action of cyanide on at least two physiological functions of rainbow trout, growth and swimming performance. The threshold or no effect of cyanide increases from 6° to 18°C.

Cyanide toxicity to trout was also shown to be modified by the size and activity of the fish. McCracken (1978) found growth of large fish (19g) more sensitive to cyanide concentrations than growth of small fish (7g). McCracken (1978) further established that increasing the swimming speed of trout also reduced cyanide toxicity. Negilsky (1973) actually observed increased salmon production at low cyanide concentrations when the fish were swimming against a current in experimental channels.

The unifying element in temperature, activity and size of fish, that alter cyanide toxicity, is a variation in the metabolic rate. The metabolic rate may be defined as the sum of the catabolic reactions which yield energy the organism utilizes, (Fry, 1971). Increases in energy yielding processes (i.e., metabolic rate), would hinder the action of cyanide which through histotoxic anoxia reduces

the total energy reserves and the cell's ability to utilize these reserves. High temperatures, activity and smaller body weight, increase the metabolic rate of animals, (Schmidt-Nielsen, 1975), and would thus be expected to reduce cyanide toxicity.

Increased metabolic rates could also lead to increased detoxification and excretory processes. At low cyanide concentrations, the toxicant levels in the fish is not expected to vary greatly, regardless of uptake rates. Under such conditions, increased detoxification and excretory mechanisms would lessen the effect of cyanide. At higher temperatures, this dampening of cyanide toxicity is further aided by the biological state of the trout due to changes involved during acclimation.

At low water temperatures (4-5°C), under metabolically depressing conditions, fish are under some stress to maintain their life processes. This is evidenced by greater water content of fish, less food availability in nature, greater specific dynamic action, weaker assimilation and food conversion efficiency, (Warren and Davis, 1967). Under such conditions, the addition of another stressor such as cyanide would have serious effect on fish production and even long term survival. This study indicates that at 6°C, even a concentration as low as 0.005 $mg.L^{-1}$ HCN caused marked reduction in fat synthesis and swimming performance. Fish depend on greater fat synthesis at low temperatures and utilize this energy reserve during migration and reproduction. Reductions in the swimming performance of rainbow trout imply that fish may be hindered in their attempt to migrate, maintain their position in a current, attempt to escape predators, or catch prey.

In Canadian waters, fish spend a significant amount of time at low water temperatures. Yet, government regulations for assessing

the toxicity of various industrial effluents, require that bioassays be carried out at 15°C, (Water Pollution Control Directorate, 1971; 1972). This study suggests the need for evaluating toxicity to fish at low temperatures for a more realistic appraisal of our water pollution problems.

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