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THIOCYANATE TOXICITY TO *DAPHNIA MAGNA* ; MODIFIED BY PH AND TEMPERATURE

Shelley J. Watson

A Thesis

in

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of

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ABSTRACT

THIOCYANATE TOXICITY TO *DAPHNIA MAGNA* : MODIFIED BY PH AND TEMPERATURE

SHELLEY J. WATSON

The toxicity of thiocyanate to *Daphnia magna* was investigated under varying conditions of pH (5, 6, and 7) and temperature (8, 12, and 16°C). Water hardness was kept constant at 75 mg/L. Results indicate that at all pH levels an increase in temperature from 8 to 16°C increased toxicity. A rise in temperature from 8 to 16°C at pH 7 showed the greatest increase in toxicity (10 fold). At pH 5 a similar increase in temperature resulted in a 5.5 fold increase in toxicity. At all temperatures tested an increase in pH from 5 to 7 decreased toxicity. The most toxic combination was found at pH 5 at 16°C, the least toxic combination pH 7, 8°C. There are two main conclusions to be found based on these data. One, if the water body concentration reaches the currently proposed standard for thiocyanate levels in effluent it will be too high to safely maintain *Daphnia magna* populations. Two, the parameters used, pH and temperature are important modifying factors of thiocyanate toxicity.

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My sincere thanks is extended to Dr. Ed Maly for his patience, guidance and positive outlook. Without his trust and endorsement, this thesis could never have been accomplished.

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I would like to thank my parents for their constant support and confidence. A special thank-you to Stephen Prescott for his encouragement and supportive enthusiasm. He made the bad times a little less so.

DEDICATION

This thesis is dedicated to my fiance Stephen Prescott

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INTRODUCTION

BACKGROUND

The resolution of thiocyanate toxicity is imperative in today's everexpanding industrial society. As increasing amounts of thiocyanate are released into the environment (Devuyst 1982), the lack of standards to protect the aquatic ecosystem becomes increasingly obvious.

The Ontario Ministry of the Environment (1985), has recently recommended the following guideline: "In areas where receiving waters contain less than 200 mg/L hardness, a mine-mill effluent should not contain more than 150 mg/L thiocyanate. In areas where receiving waters contain more than 200 mg/L hardness, the allowable levels of thiocyanate in mine-mill effluent should be determined on a case by case basis."

This statement has direct and important consequences to the mining industry. With a recent development (INCO Metals Co. 1982) there has been increasing pressure to change the method of cyanidation reclamation. The long established method for reclamation is alkaline chlorination (Huiatt et al. 1983). The new INCO sulfur dioxide air process, although less costly,

allows effluent to contain elevated thiocyanate levels (INCO Metals Co. 1982).

Thiocyanate toxicity data found in the literature is scant and ambiguous (for reviews see Doudoroff 1976, Towill et al. 1978, and Huiatt et al. 1983). Doudoroff (1976), in his literature review on thiocyanate toxicity documented a wide range of toxic thresholds, ranging from 59 mg/L in mosquito fish to 1800 mg/L in rainbow trout (Salmo gairdneri). From these data Doudoroff (1976) was unable to conclude more than "thiocyanate is somewhat toxic". The APHA et al. (1980) did not consider thiocyanate toxic, except in the form of cyanogen chloride. The USEPA (1980) did not include a criterion for thiocyanate.

Recent studies by Heming et al. (1985), Speyer and Raymond (1984), and Parker (1983) have shown that thiocyanate has deleterious effects on juvenile rainbow trout. Speyer and Raymond (1984) established the LC50 for potassium thiocyanate in rainbow trout as between 178 and 263 mg/L, depending on the temperature and pH of the test water. They also found that increased hardness decreased toxicity. Parker (1983) found that pH had no effect on thiocyanate toxicity, while water hardness increased toxicity. The discrepancies in results by Parker (1983) and Speyer and Raymond (1984) may be partially due to the large difference in the ionic content of their test

water. For the following tests it was decided to use a water standard from the same source as Speyer and Raymond (1984).

Parkhurst et al. (1979) and Anderson (1946) have reported thiocyanate toxicity to Daphnia magna in a 48 hr LC50 as 57.4 mg/L, and an immobilization concentration of 11.3 mg/L respectively. Although water conditions were not uniform in these two studies, their results suggest that Daphnia magna are more sensitive to thiocyanate than are rainbow trout.

DAPHNIA MAGNA AS A TEST ORGANISM

Thiocyanate toxicity has been established with fish (Speyer and Raymond 1984, Parker 1983, Heming et al. 1985). It would be useful to determine the sensitivity of Daphnia magna to thiocyanate under similar conditions. Daphnia has proved to be a sensitive species in detecting deleterious effects of chemicals on aquatic organisms (Leeuwangh 1978, Canton and Adema, 1978). Although Daphnia magna distribution is more limited than some other North American daphnids (Ward and Whipple 1959), when compared to other daphnid species, Daphnia magna did not differ significantly in its sensitivity to xenobiotics (Canton and Adema 1978). Winner and Farrell (1976) also found no differences when comparing D.magna , D.pulex , D.parvula , and D.ambigua in their sensitivities to copper

Daphnia was chosen as the test organism because a 96 hr bioassay represents a large proportion of their lifespan (60 days at 20 °C). Their short parthogenic reproductive cycle, with numerous offspring also allowed the use of many daphnids per experiment (Attar and Maly 1982).

Daphnia magna was specifically chosen since its larger size and ease of laboratory maintenance make it an ideal test model.

The response of Daphnia magna to lake acidification associated with acid rain has been studied by Sprules (1975), Roff and Kwiatkowski (1977), Yan and Strus (1980), Confer et al. (1983). Confer et al. (1983) found that Daphnia magna were only found in water bodies with a pH between 5.0 and 7.2.

The natural occurrence of Daphnia magna is also associated with water temperature. Macissac et al.(1985) found Daphnia magna only where the mean water temperature of the warmest month was lower than 22.2 °C. This correlates with the work done by Kersting (1978) who found the highest value for feeding and filtering rates to be at 22 °C, with a negative growth rate found above that temperature. He also found that the highest rate of 'growth efficiency' was at 10 °C. 'Growth efficiency' is defined as the ability of a population to maintain itself under a certain set of pre-defined conditions (Kersting 1978). This definition does not preclude the possibility that Daphnia magna can tolerate brief exposure to temperature at east as low as 0 °C, and as high as 39 °C, but it is between 6 and 22 °C that growth, reproduction, and other functions are within a normal range (MacArthur and Baille 1959)

MODIFYING FACTORS

PH and temperature were chosen as the abiotic 'modifying factors'- i.e.'any characteristic of the organism or the surrounding waters that affects toxicity of a pollutant' (Rand and Petrocelli 1985). Temperature has been well documented in the literature for its major role in modifying toxicity (Rand and Petrocelli 1985 and Cairns et al. 1975). Temperature, especially with ectothermic organisms, plays a significant role in the environment and physiological conditions. Although pH has been less documented as modifying toxicity, it also plays an important role in the environment, and subsequently the physiology of ectothermic organisms, including Daphnia, mainly due to ion speciation of the toxicant (Rand and Petrocelli 1985, Dixon and Sprague 1981, and Howarth and Sprague 1978).

From past research on the modifying factors of pH and temperature, it is apparent that there is no general pattern of interaction between temperature and pH in their effect on toxicity and the interaction may be synergistic, additive or antagonistic. It is the combination of model species, toxicant and surrounding environment, which determines the modifying effect. Both pH and temperature can increase, decrease or have no

obvious effect on toxicity. The following research review will illustrate this point.

PH and TOXICITY

The EIFAC (1973b) found that with an increase in pH of Ø.3 units (7.Ø to 7.3), the concentration of the un-ionized (NH_3) ammonia doubled, therefore increasing toxicity to salmonids.

Broderius et al.(1977) investigated the relative toxicity of dissolved sulfide forms on fathead minnows (Pimephales promelas). An increase in pH from 6.5 to 8.5 gave rise to a linear decrease in molecular H S LC5Ø and a logarithmic decrease in dissolved sulfide LC5Ø.

Russo et al.(1981) found the acute toxicity of nitrate on rainbow trout varies with pH. When pH increases, toxicity in terms of NO_2^- decreases but toxicity in terms of HNO_2 increases.

Bender (1969) showed that the hydrolysis of Malathion was dependant on an alkaline pH. The by-product of this hydrolysis, diethyl fumarate, was found to be more toxic than Malathion to the fathead minnow.

Zectran (Mexacarbate) was shown to be up to 38 times more toxic at pH 9.5 than at pH 7 in coho salmon (Oncorhynchus kisutch), fathead minnows, and bluegills (Lepomis macrochirus) (Mauck et al. 1977).

Broderius et al. (1977) and Doudoroff et al. (1966) studied cyanide as a toxicant. The molecular hydrogen cyanide (HCN) is more toxic than the CN^- ion and an increase in pH decreases its presence, especially above a pH of 8.5. The dissociation of this complex can be seen with nickelocyanide: an increase in pH from 7.5 to 7.8 decreases toxicity tenfold (Broderius et al. 1977). This is because the toxic molecule is not nickelocyanide, but the free cyanide, and the relative amounts of each are governed by pH.

The effect of pH on the toxicity of antimycin to carp (Cyprinus carpio), green sunfish (Lepomis cyanellus), and bluegills was investigated by Marking (1975). He found the toxicity of antimycin decreased gradually from 6.5 to 8.5 and abruptly from 8.5 to 9.5.

Sano (1976) investigated the role of pH on the acute toxicity of sulfite to guppies (Poecilia reticulata). He found that with increasing pH, toxicity of sulfite decreased because the HSO_3^- ion is more toxic than the $SO_3^=$ ion.

An increase in pH decreased the toxicity of 2,4-Dichlorophenol to the fathead minnow (Holcombe et al. 1980). This is because the lower levels of the undissociated form at the higher pH were less toxic.

Woodward (1976) tested the toxicity of the herbicides Dinoseb and Picloram to cutthroat (Salmo

clarki) and lake trout (Salvelinus namaycush). He found that increasing pH from 6.5 to 8.5 decreased the toxicity of Dinoseb by a factor of 43 and Picloram by a factor of 0.5

Cusimano and Brakke (1986) explored the effects of pH on the toxicity of cadmium, copper and zinc to steelhead trout (Salmo gairdneri). They showed that the LC50's decreased with increasing pH between 4.7 to 7.0.

It was shown by Woodward (1976) that pH had no effect on the toxicity of Rotenone and the three esters of 2-4 Dichorophenoxyacetic acid.

The EIFAC (1973b) showed that an increase in pH from 6.5 to 8.5 did not affect the toxicity of cresols, xylenols and phenol to rainbow trout.

TEMPERATURE and TOXICITY

Woodward (1976) found increasing temperature in reased the toxicity of the herbicides Dinoseb and Picloram to cutthroat and lake trout.

Hokanson and Smith (1971) investigated the toxicity of linear alkylate sulfonate (LAS) to the bluegill, finding that increased temperature increased toxicity.

Adelman and Smith (1972) found the toxicity of

H_2S to goldfish (Carassius auratus) was strongly influenced by temperature. At 6.5 °C LC50 was 530 ug/L while at 25 °C it was 44 ug/L.

The EIFAC (1973b) noted that since the un-ionized fraction of ammonia is poisonous, and this portion increases with increasing temperature, the toxicity of ammonia increases with increasing temperature. This only holds true above 10 °C however. It was also found that at 3 °C the LC50 is about half that at 10 °C for rainbow trout and chinook salmon (Oncorhynchus tshawytscha).

Johnson (1968) found that a temperature rise increased the toxicity of Endrin to coho salmon, but DDT's toxicity was decreased with increasing temperature when tested on rainbow trout and bluegills.

Sanders and Cope (1966) tested several pesticides on two species of cladocerans. DDT, Methoxychlor, Chlordane, Aldrin, Endrin, Parathion and Malathion were found to be more toxic at lower temperatures. Dieldrin, Baytex and Diazinon were found to be more toxic at higher temperatures.

Macek et al (1969) also tested the effect of temperature on the susceptibility of bluegills and rainbow trout to selected pesticides. Rainbow trout were tested at 1.6, 7.2 and 12.7 °C, bluegills were tested at 12.7, 18.3 and 23.8 °C. for a 96-hr LC50. Chlordane, Dieldrin, Endrin, Malathione and Aldrin all increased in toxicity with

increasing temperature. The exception was Methoxychlor and DDT which decreased in toxicity with inceased temperature.

Brown et al. (1967) found that phenols are more toxic to rainbow trout at lower temperatures (6 °C as opposed to 18 °C) by up to a factor of 2.

Cairns et al. (1964) found that even though naphthenic acids became more toxic to snails as temperature decreased, toxicity remained the same for bluegills with decreasing temperature.

Mauck et al. (1977) studied the effect of temperature on the toxicity of Mexarcarbate (Zectran) to coho salmon, brown trout (Salmo trutta), fathead minnows, bluegills and yellow perch (Perca flavescens). They found that temperature did not effect toxicity.

OBJECTIVES OF THESIS

The objective of this thesis is three fold; firstly to establish, under laboratory conditions, the toxicity of thiocyanate to Daphnia magna . Secondly to examine the modifying effects of pH and temperature on the toxicity of thiocyanate and if possible to develop a mathematical model as an aid in the establishment of water quality criteria. Thirdly, to compare the toxicity of thiocyanate as established in the following experiments with its toxicity in other organisms, as indicated in the literature.

METHODS AND MATERIALS

A Daphnia magna culture was purchased from Wards Mississauga, Ontario. The initial culture was reared in a 15 L aquarium. It is from this seed culture that four large, 60 L, polyethylene culture tanks were started and replenished as necessary (see Fig 1). Daphnia were fed a mixture of Ward's Daphnia food, consisting of fishmeal and cerophyll powder, plus living algae, predominantly Chlorella vulgaris and Scenedesmus , with minor amounts of Anfiestrodesmus , Cryptomonus and other flagellates, and colonial sheathed blue green algae (Pat Chow-Fraser, Personal communication). Water used for rearing Daphnia was dechlorinated Montreal City water (Table 1). All tanks were constantly aerated.

The cultures were maintained at 18.5 C (+ 1°C) under a photoperiod of 12 h light/dark regime. The cultures were cleaned out periodically by opening a valve at the bottom and draining out seventy-five percent of the water plus the organic wastes.

A static bioassay was employed. The exposure chambers were 100 ml propylethylene beakers, supported on a floating platform and immersed in a constant temperature bath (Fig 2). The assembly held 108 beakers (Fig.3). Five daphnids (<12h) were placed in each beaker and starved

FIG 1 Culture tanks were filled with dechlorinated Montreal City Water. Water was aerated constantly, with a standard temperature of 18°C (± 1°C).

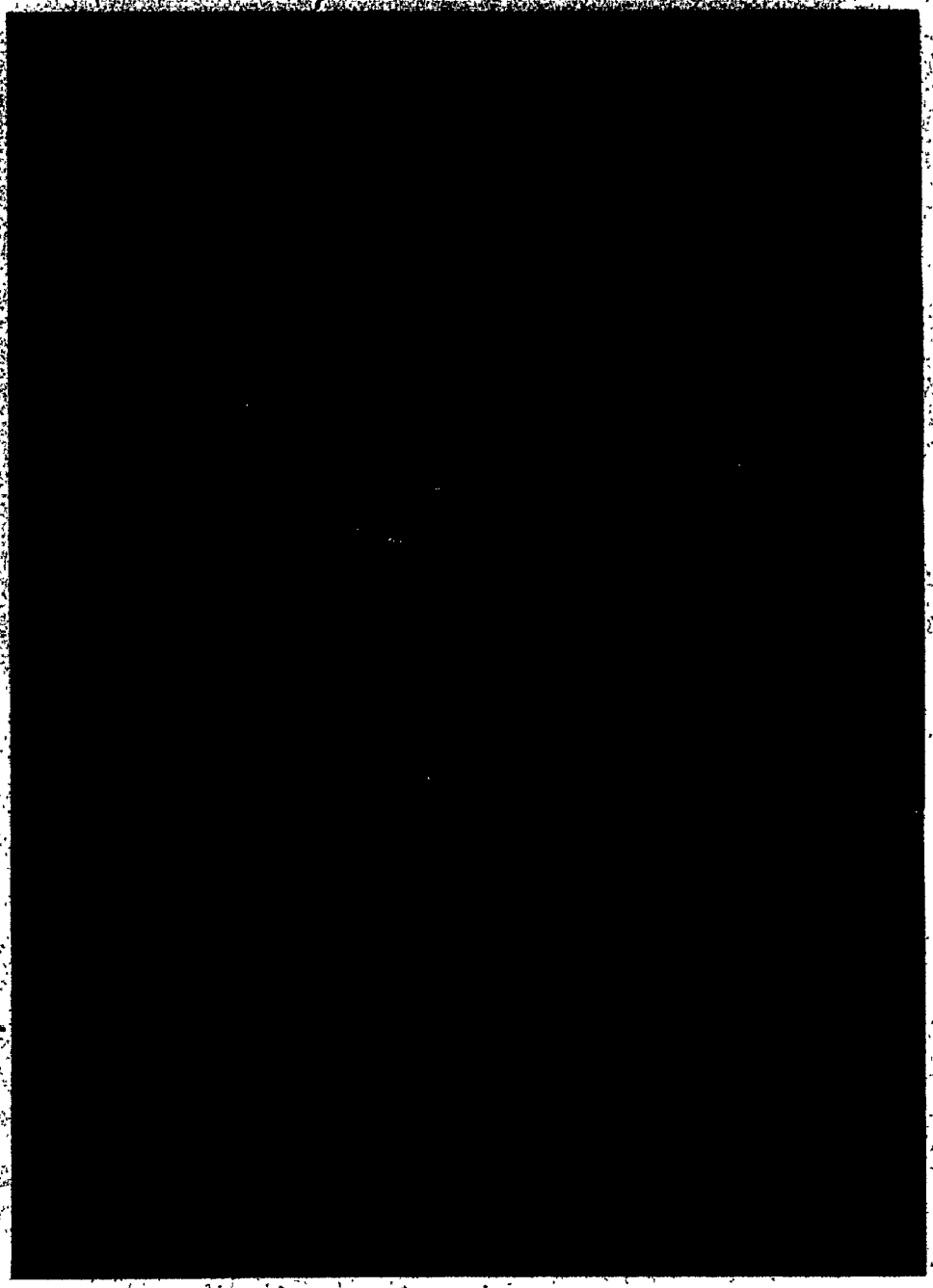


Figure 1.

TABLE 1 AVERAGED MONTHLY CHARACTERISTICS OF DECHORINATED MONTREAL CITY WATER AS MEASURED IN THE LABORATORY

Hardness	127.0	(± 4.5)	mg/L as CaCO
pH	7.3	(± 0.2)	
DO	10.6	(± 0.3)	mg/L @ 12 °C

FIG 2 Experimental apparatus consisted of 108 beakers supported on a floating platform, immersed in a constant temperature regulated bath, with continous recordings.

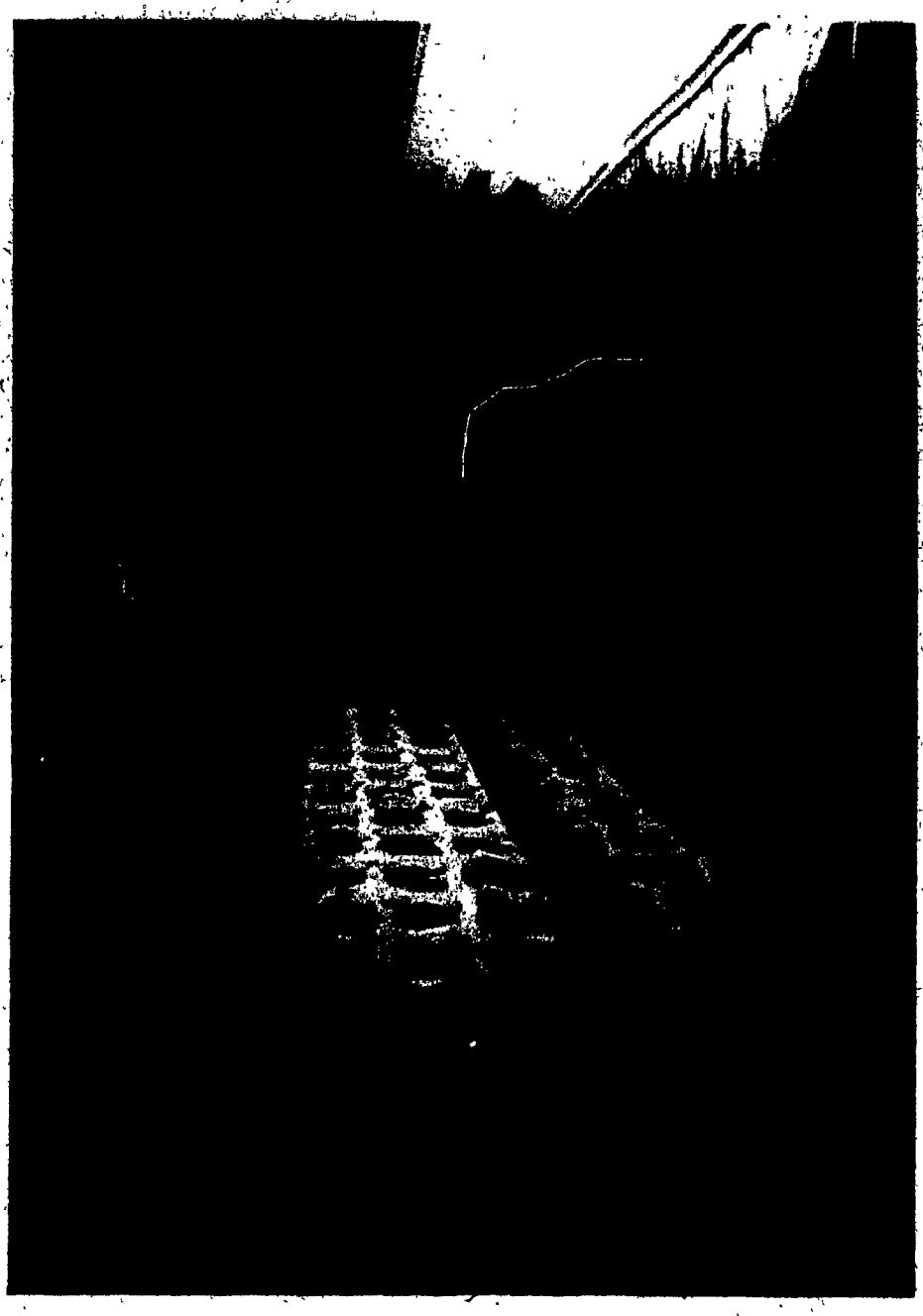


Figure 2.

FIG 3 Schematic representation of experimental apparatus. Inflow was regulated by combining two temperatures. Test chamber was 100 ml beaker.

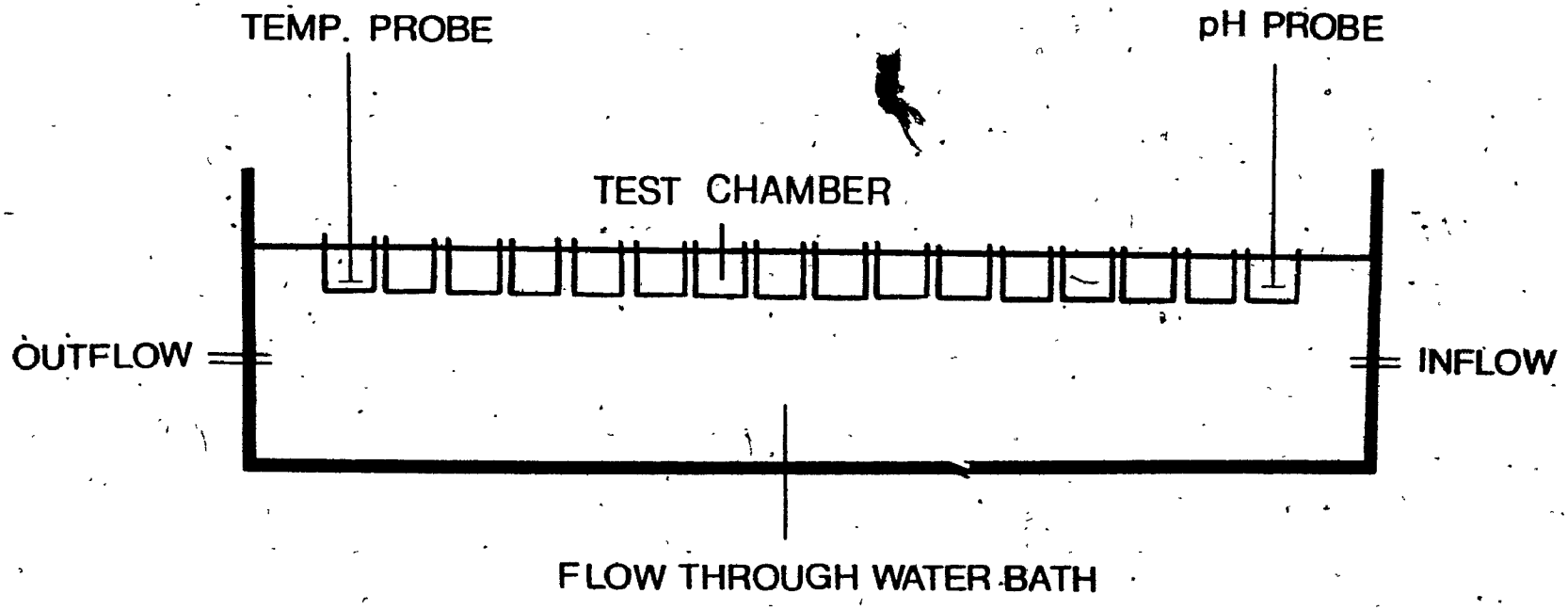

TEMP. PROBE
pH PROBE
TEST CHAMBER
OUTFLOW
INFLOW
FLOW THROUGH WATER BATH

Figure 3.

during the test period. Twelve hours before the start of an experiment, gravid females were removed from the culture tanks and put into 100 ml propylethylene beakers containing the standard water (Table 2). After this 12 h period the adults were removed and the juveniles (≤12 hrs) were collected and randomly placed into beakers containing the test solutions. The beakers were placed in the water bath, and allowed to equilibriate to the appropriate temperature. The equilibrium time was dependant on temperature, for 8 °C it was about 60 minutes, 12 °C was about 30 min and 16 °C was about 10 min.

Thiocyanate solutions were made up using Fisher certified grade potassium thiocyanate and were added to the standard water which had the characteristics described in Table 2.

The pH was adjusted through addition of reagent grade H_2SO_4 and NaOH. This solution was then aerated and allowed to equilibriate for 12 hours. The pH was readjusted before the Daphnia were introduced and subsequently checked and adjusted daily. Temperature was monitored and recorded continuously. Dissolved oxygen was also measured daily. Hardness, conductivity, and thiocyanate concentrations of test solution were recorded at start and end of experiments. Seven concentrations were used in each test condition, three replicates with fifteen daphnids per replicate. So, for example; condition pH 5

Table 2 CHARACTERISTICS OF STANDARD WATER

Alkalinity	68.75	mg/L
Hardness	75.00	mg/L
Conductivity	250	umhos
Chloride	12.25	mg/L
Nitrogen	40.25	mg/L
Sulphate	22.5	mg/L
Carbonate	80.5	mg/L
Sodium	47.5	mg/L
Calcium	4.13	mg/L
Manganese	<.0025	mg/L
Magnesium	8.78	mg/L
Potassium	3.00	mg/L
Copper	<.0025	mg/L
Zinc	<.0025	mg/L
Iron	<.013	mg/L

and 8 °C was run on three separate dates which included 7 concentrations with three beakers per concentration, five daphnids per beaker on each date (Table 3). Three experiments were run simultaneously to the thiocyanate toxicity bioassays. These were; experiment #1, a control, where the pH and temperature parameters were identical to those used in the thiocyanate treatment however no thiocyanate was added. There were no deaths observed in the controls (Table 4). Experiment #2 (table 4) to ensure time sensitivity, was run simultaneous to all experimental replicates in order to ensure that the chemical sensitivity of thiocyanate to Daphnia magna did not change through time. This was run at 12 °C for the bioassay conditions of 16 °C and 8 °C but 16 °C for the 12 °C conditions. The results of these experiments were required to fall within the confidence interval of the initial replicate to ensure that the sensitivity over time had not changed (Table 4). Experiment #3 was established to ensure that temperature shock brought about by rapid decline from 18 to 8 °C had no significant mitigating deleterious effect. The daphnia were raised in the same fashion until they became gravid. At this point they were also put into 100 ml propylethylene beckers which had been allowed to equilibriate to the temperature of the specific experiment i.e. 8 °C. This decrease in temperature increased the length of time it took to bear daphnids. The comparable length of time at 8

TABLE 3 TABULATION OF EXPERIMENTS

REF #	CONDITION	EXP#1	EXP#2	EXP#3
1	pH 5/8 C x7*	YES	YES	YES
2	pH 5/12 C x7	YES	YES	NO
3	pH 5/16 C x7	YES	YES	NO
4	pH 6/8 C x7	YES	YES	NO
5	pH 6/12 C x7	YES	YES	NO
6	pH 6/16 C x7	YES	YES	NO
7	pH 7/8 C x7	YES	YES	NO
8	pH 7/12 C x7	YES	YES	NO
9	pH 7/16 C x7	YES	YES	NO
10	pH 5/8 C x7	YES	YES	NO
11	pH 5/12 C x7	YES	YES	NO
12	pH 5/16 C x7	YES	YES	NO
13	pH 6/8 C x7	YES	YES	NO
14	pH 6/12 C x7	YES	YES	NO
15	pH 6/16 C x7	YES	YES	NO
16	pH 7/8 C x7	YES	YES	NO
17	pH 7/12 C x7	YES	YES	NO
18	pH 7/16 C x7	YES	YES	NO
19	pH 5/8 C x7	YES	YES	NO
20	pH 5/12 C x7	YES	YES	NO
21	pH 5/16 C x7	YES	YES	NO
22	pH 6/8 C x7	YES	YES	NO
23	pH 6/12 C x7	YES	YES	NO
24	pH 6/16 C x7	YES	YES	NO
25	pH 7/8 C x7	YES	YES	NO
26	pH 7/12 C x7	YES	YES	NO
27	pH 7/16 C x7	YES	YES	NO

* 7 different concentrations used; .1um SCN^-/.006mg/L SCN^-, 1.0um SCN^-/.06 mg/L SCN^-, 10um SCN^-/0.6 mg/L SCN^-,.10mm SCN^-/6.0 mg/L SCN^-,.51mm SCN^-/30.0 mg/L SCN^-,.73mm SCN^-/45.0 mg/L SCN^-,1.0mm SCN^-/60 mg/L SCN^-.

TABLE 4 TABULATION OF EXPERIMENTAL RESULTS

ref #	condition	EXP#1 (Control)	EXP#2 (Time Control)		EXP#3 (acclimated)
	Ph/Temp°C		pH/Temp°C	LC50	LC50
1	5/8	no deaths	5/12	1.892	1.711
2	5/12	no deaths	5/16	0.691	-----
3	5/16	no deaths	5/12	1.784	-----
4	6/8	no deaths	6/12	9.922	-----
5	6/12	no deaths	6/16	1.247	-----
6	6/16	no deaths	6/12	7.956	-----
7	7/8	no deaths	7/12	17.238	-----
8	7/12	no deaths	7/16	3.757	-----
9	7/16	no deaths	7/12	20.922	-----
10	5/8	no deaths	5/12	2.061	-----
11	5/12	no deaths	5/16	0.554	-----
12	5/16	no deaths	5/12	1.784	-----
13	6/8	no deaths	6/12	9.237	-----
14	6/12	no deaths	6/16	1.622	-----
15	6/16	no deaths	6/12	10.168	-----
16	7/8	no deaths	7/12	17.983	-----
17	7/12	no deaths	7/16	3.224	-----
18	7/16	no deaths	7/12	19.481	-----
19	5/8	no deaths	5/12	1.907	-----
20	5/12	no deaths	5/16	0.691	-----
21	5/16	no deaths	5/12	1.892	-----
22	6/8	no deaths	6/12	7.956	-----
23	6/12	no deaths	6/16	1.477	-----
24	6/16	no deaths	6/12	8.403	-----
25	7/8	no deaths	7/12	17.238	-----
26	7/12	no deaths	7/16	2.950	-----
27	7/16	no deaths	7/12	20.296	-----

°C was 37.43 h as opposed to 12 h at 18 °C. One could not use the 12 hour developmental period since temperature and metabolic rate in Daphnia are linearly correlated (Schindler 1973). The literature suggested an increase of 37.43 hours would compensate for this decrease in temperature. The daphnids were then placed into their test solutions which were made up using the same method but had already been equilibriated to 8 °C. The test was then conducted as before. This experiment was necessary to investigate the possibility that metabolism does not decrease linearly with temperature (Schindler 1973), but in fact shifts, creating new metabolic pathways. This shift was not found (Table 4). All bioassays were run on a 12 h light/dark photoperiod.

Chemical determinations of the thiocyanate concentrations were performed using a colorimetric method, the reagent being ferric nitrate-perchloric acid (Charlot 1964).

Determination of hardness was carried out using the EDTA method as described by the Standard Methods for the Examination of Water and Wastewater 13 Ed.

PH was measured with a pH meter produced by Concordia University Science Technical Center (STC), the probe was manufactured by Fisher Scientific (Catalogue number 13-639-90).

Temperature was measured with a digital H2O/AIR

probe manufactured by Concordia University STC and was continuously recorded with the Linear Instrument flat bed recorder model #255/MM.

Conductivity was measured by the radiometer model #CDM2d.

Daphnids were visually examined at twelve hour intervals during the 96-h period, but recordings of death were only made at 96 h. This approach was decided upon because of SDS syndrome related to thiocyante toxicity. It has been documented by Potts and Fryer (1979) that manipulation of daphnia with a 5.5 mm Pasteur pipette, is of sufficient agitation to release ions resulting in death; therefore manipulations were kept to a minimum.

Mortality was determined by the lack of movement of the second antennule and internal organs in a five second period.

The 96 h LC50 values with their corresponding 95% confidence intervals were calculated using the Trimmed Spearman-Karber method (Hamilton et al. 1977). Further treatment of the data included analysis of co-variance. A 5% level of probability was the criterion for statistical significance.

RESULTS

Thiocyanate toxicity bioassay data were illustrated by creating dose mortality curves (Figs. 4-9), using the Trimmed Spearman Karber Method (10∝) because of the characteristics of the data; the skewedness as well as the nonlinear progression (Stephen 1977). The resulting LC50s ranged from 0.554 to 33.467 mg/L, dependent on pH and temperature. The most toxic combination was at pH 5 and 16° C temperature, the least toxic combination being pH 7 and 8° C temperature (Table 5).

The LC50 values were then plotted to find the correlation of pH and/or temperature with SCN toxicity (Figs. 10&11). The effect of pH at different temperatures on thiocyanate toxicity (Fig. 10) illustrates that at a given pH, thiocyanate toxicity increases with rising temperature. The most extreme example is illustrated at pH 7, where the LC50 decreases from values of 33.467, 30.228, 32.510 mg/L SCN^- at 8 °C to values of 2.790, 3.618 and 3.224 mg/L SCN^- at 16 °C. The linear regression equation for pH 7 and temperatures of 8, 12, and 16 °C is Y = 61.52 + (-3.61x). The toxicity increased from 4.796, 2.940, and 2.837 to 0.666, 0.554 and 0.666 mg/L SCN^- at pH 5 with the same increase in temperature. The linear regression for this relationship is Y = 6.36 + (-.362x). The effect of

FIG 4-9 Concentration mortality curves illustrating the effects of pH and temperature on thiocyanate toxicity.

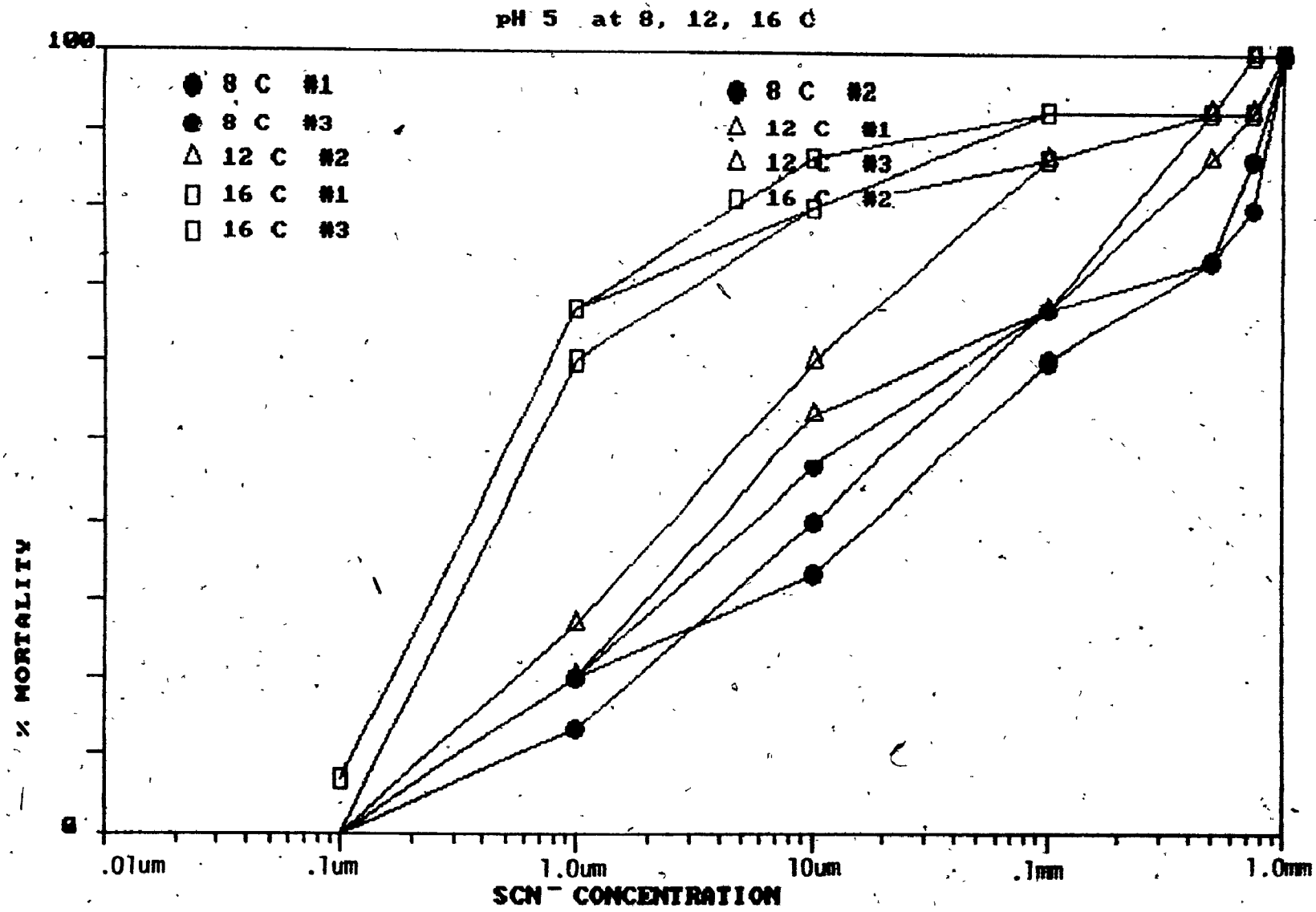

pH 5 at 8, 12, 16 C
100
0
% MORTALITY
8 C #1
8 C #3
12 C #2
16 C #1
16 C #3
8 C #2
12 C #1
12 C #3
16 C #2
.01um
.1um
1.0um
10um
.1mm
1.0mm
SCN⁻ CONCENTRATION

Figure 4.

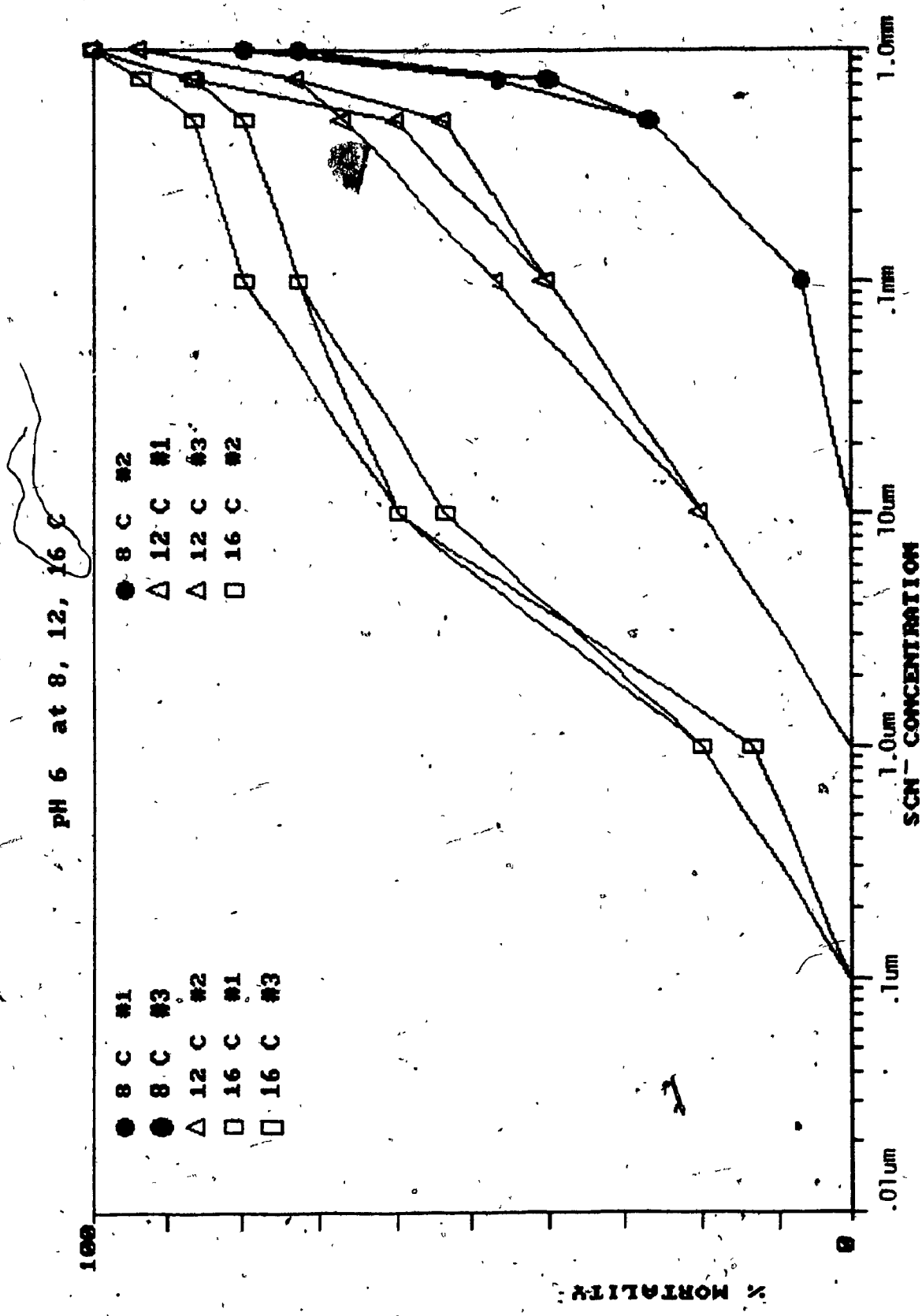

pH 6 at 8, 12, 16 C
8 C #1
8 C #3
12 C #2
16 C #1
16 C #3
8 C #2
12 C #1
12 C #3
16 C #2
% MORTALITY
100
0
.01um
.1um
1.0um
10um
.1mm
1.0mm
SCN- CONCENTRATION

Figure 5.

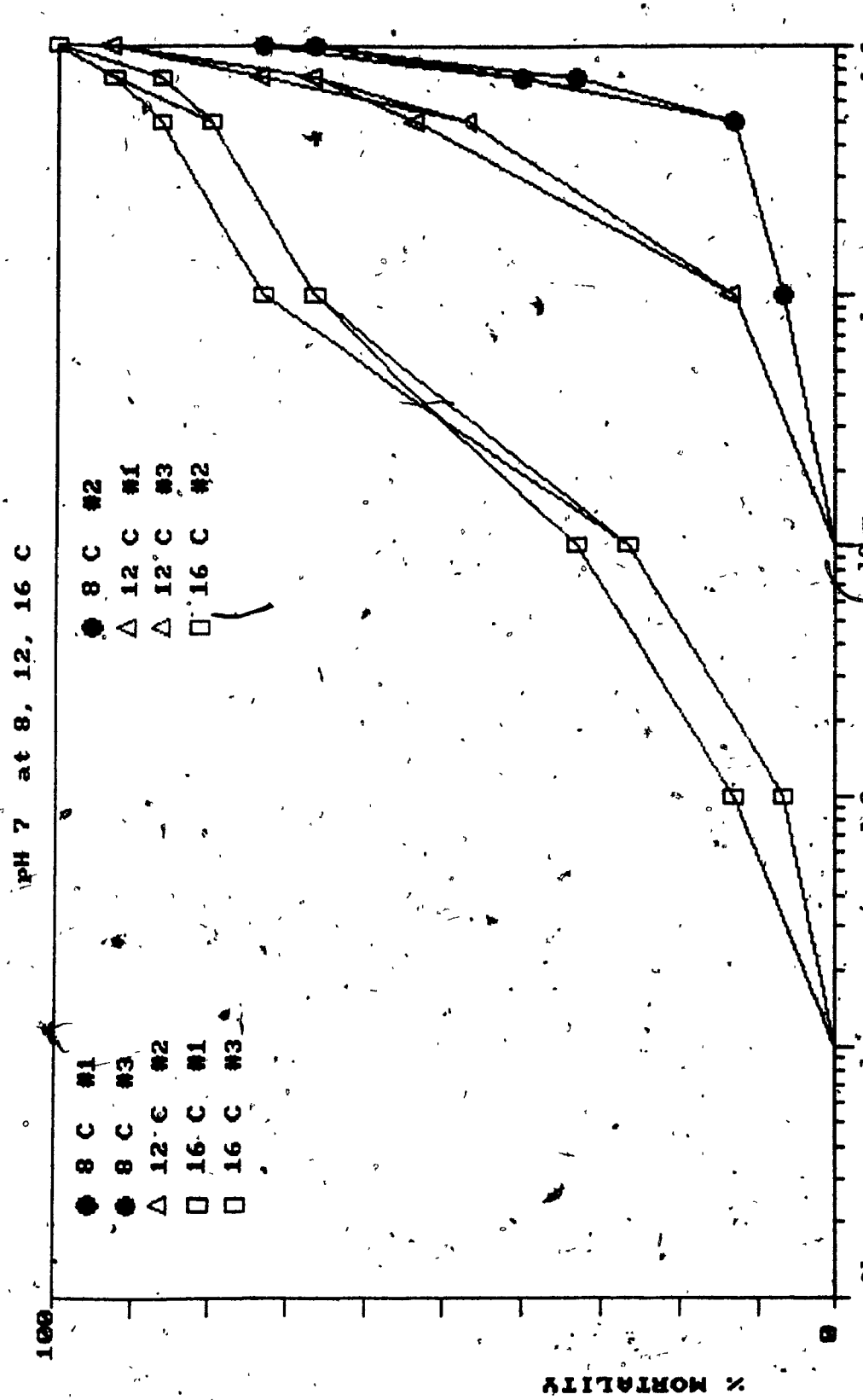

% MORTALITY
100
0
.01um
.1um
1.0um
10um
.1mm
1.0mm
SCN⁻ CONCENTRATION
pH 7 at 8, 12, 16 C
8 C #1
8 C #3
12 C #2
16 C #1
16 C #3
8 C #2
12 C #1
12 C #3
16 C #2

Figure 6.

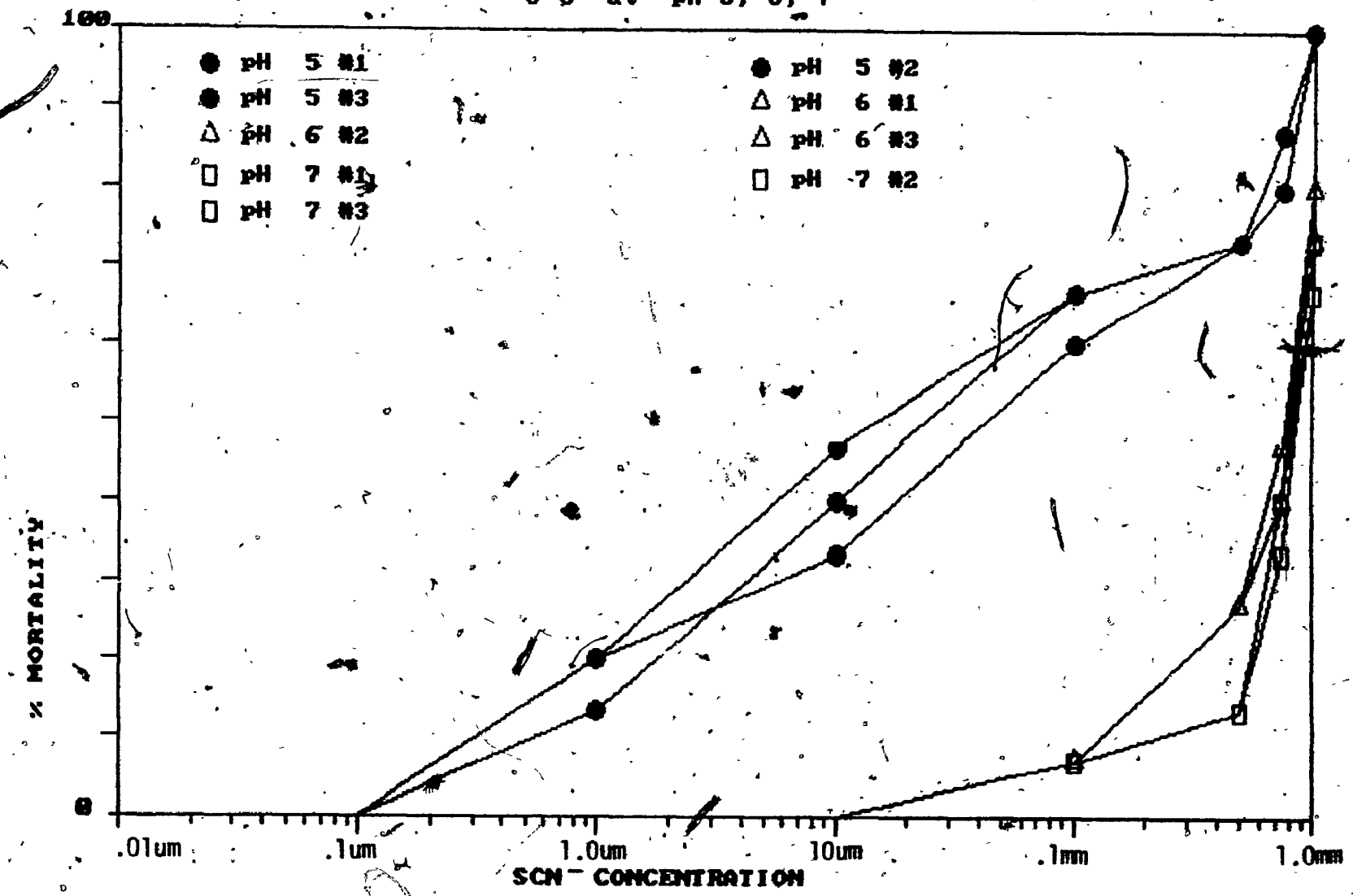

8 C at pH 5, 6, 7
100
0
% MORTALITY
pH 5 #1
pH 5 #3
pH 6 #2
pH 7 #1
pH 7 #3
pH 5 #2
pH 6 #1
pH 6 #3
pH 7 #2
.01um
.1um
1.0um
10um
.1mm
1.0mm
SCN⁻ CONCENTRATION

Figure 7.

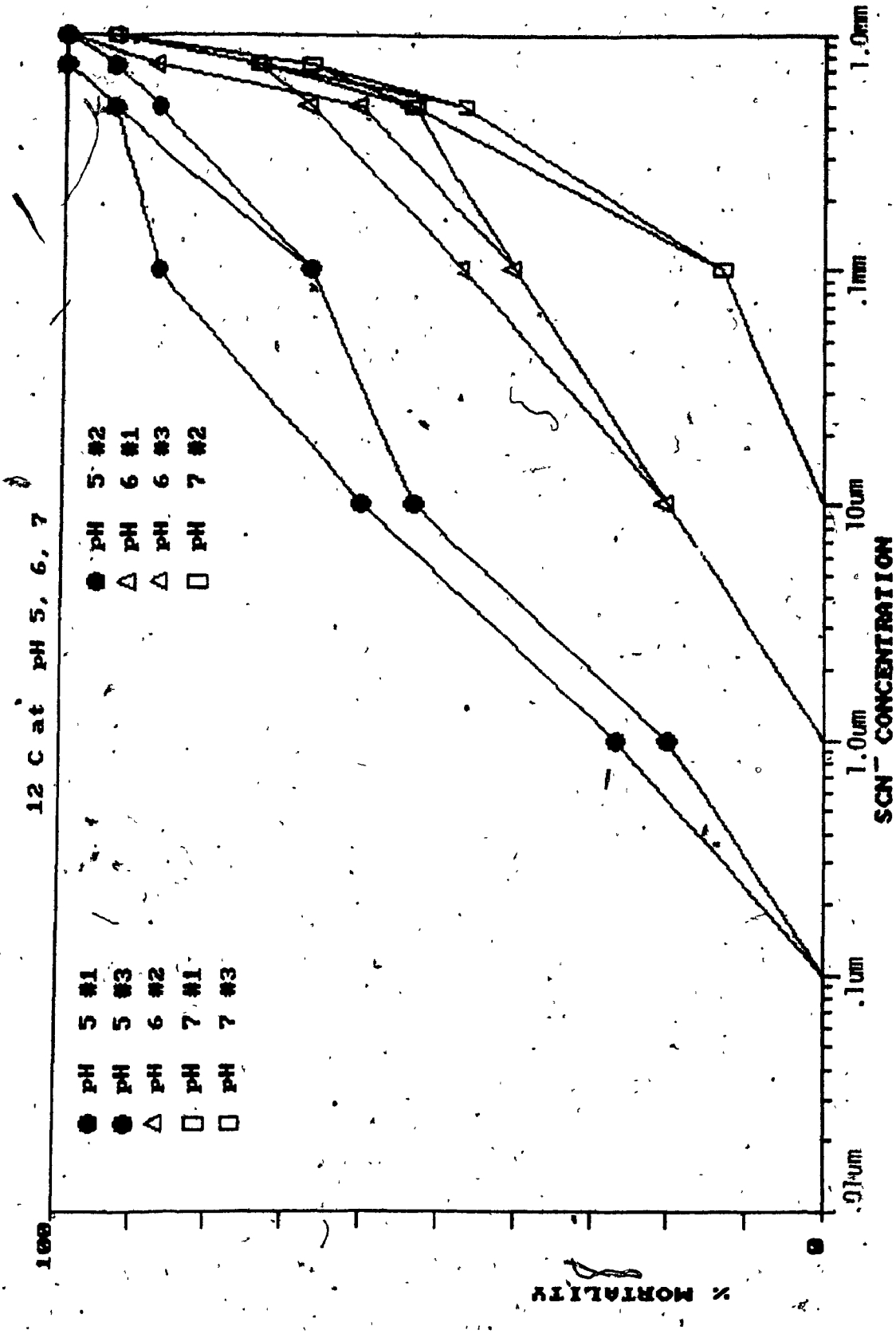

12 C at pH 5, 6, 7
% MORTALITY
100
0
.01um
.1um
1.0um
10um
.1mm
1.0mm
SCN⁻ CONCENTRATION
pH 5 #1
pH 5 #3
pH 6 #2
pH 7 #1
pH 7 #3
pH 5 #2
pH 6 #1
pH 6 #3
pH 7 #2

Figure 8.

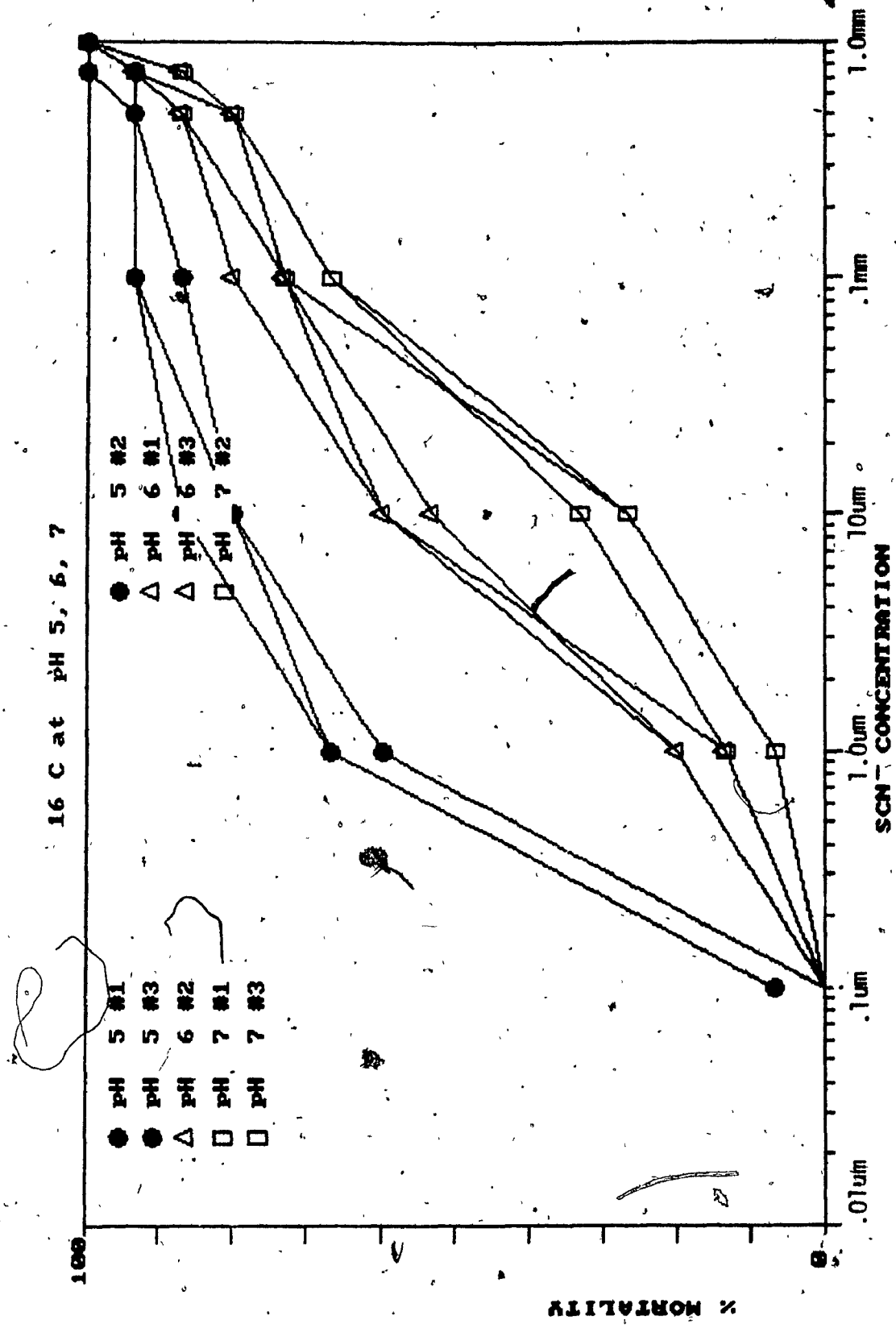

16 C at pH 5, 6, 7
pH 5 #1
pH 5 #3
pH 6 #2
pH 7 #1
pH 7 #3
pH 5 #2
pH 6 #1
pH 6 #3
pH 7 #2
100
0
% MORTALITY
.01um
.1um
1.0um
10um
.1mm
1.0mm
SCN⁻ CONCENTRATION

Figure 9.

TABLE 5 Results from bioassays

pH	Temp°C	#of org.	96-h LC50 (mg/L SCN)	Av.values of bioassay	95% C.I. (mg/LSCN)	Y (Eqn #1) (+95% CI mg/LSCN)
5	8	15	4.796		1.585-14.537	2.878 + 1.56
5	8	15	4.940	3.524	1.028- 8.396	L1 1.318
5	8	15	2.837	(+1.102)	0.921- 8.731	L2 4.438
5	12	15	1.907		0.634- 5.753	1.264 + 0.99
5	12	15	1.784	1.897	0.604- 5.286	L1 -0.247
5	12	15	2.000	(+0.108)	0.646- 6.213	L2 2.254
5	16	15	0.666		0.233- 1.859	-0.349 + 1.25
5	16	15	0.554	0.629	0.233- 1.292	L1-1.599
5	16	15	0.666	(+0.065)	0.263- 1.674	L2 0.901
6	8	15	15.374		10.351-22.832	16.849 + 1.49
6	8	15	13.968	14.574	9.646-20.320	L1 15.359
6	8	15	14.380	(+0.723)	9.938-20.810	L2 18.340
6	12	15	8.403		3.492-20.224	10.199 + 0.63
6	12	15	11.718	10.096	4.874-28.154	L1 10.17
6	12	15	10.168	(+1.659)	4.258-24.285	L2 10.83
6	16	15	1.791		0.592- 5.364	3.548 + 1.20
6	16	15	0.995	1.421	0.371- 2.649	L1 2.569
6	16	15	1.477	(+0.401)	0.496- 4.365	L2 39.069
7	8	15	33.467		23.860-46.937	30.819 + 8.25
7	8	15	30.288	32.088	22.030-41.633	L1 22.596
7	8	15	32.510	(+1.631)	23.149-45.657	L2 39.069
7	12	15	20.041		12.389-31.287	19.133 + 1.32
7	12	15	18.443	19.322	11.810-28.794	L1 17.813
7	12	15	19.481	(+0.811)	12.504-30.360	L2 20.453
7	16	15	2.790		1.142- 6.799	7.446 + 1.76
7	16	15	3.618	3.211	1.429- 9.155	L1 5.686
7	16	15	3.224	(+0.414)	1.160- 8.946	L2 9.206

Mean values and ranges for all bioassays: water temperature 8°C (7.5-8.5°C), 12°C (11.5-12.5°C), 16°C (15.5-16.5°C); pH 5 (4.97-5.10), pH 6 (5.92-6.03), pH 7 (6.93-7.03); dissolved oxygen 90% (84-100%); hardness 75 mg/L as $CaCO_3$ (72-77mg/L); conductivity 384 umhos (250-511 u mhos); thiocyanate concentrations .01 mg/L (.006-.014), 0.1 mg/L (.092-.112), 1.0 mg/L (0.974-1.099), 10.0 mg/L (9.320-9.974), 50.0 mg/L (49.568-50.966), 75.0 mg/L (74.681-75.421), 100 mg/L (99.101-100.121).

FIG 10 The effect of pH at different temperatures on thiocyanate toxicity.

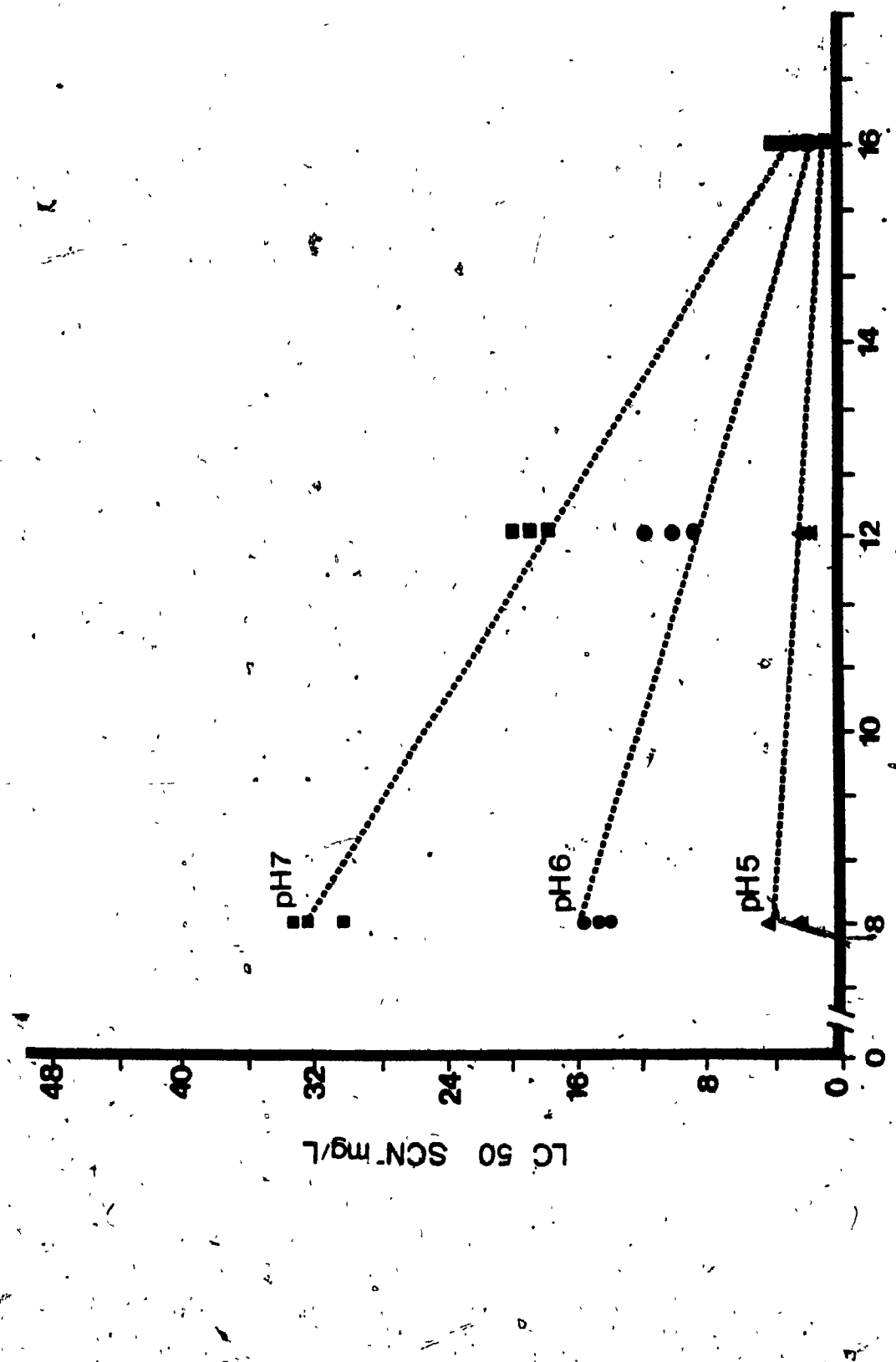

LC 50 SCN⁻ mg/L
48
40
32
24
16
8
0
0
8
10
12
14
16
TEMPERATURE (CELSIUS)
pH7
pH6
pH5

Figure 11.

FIG 11 The effect of temperature at different pH on thiocyanate toxicity.

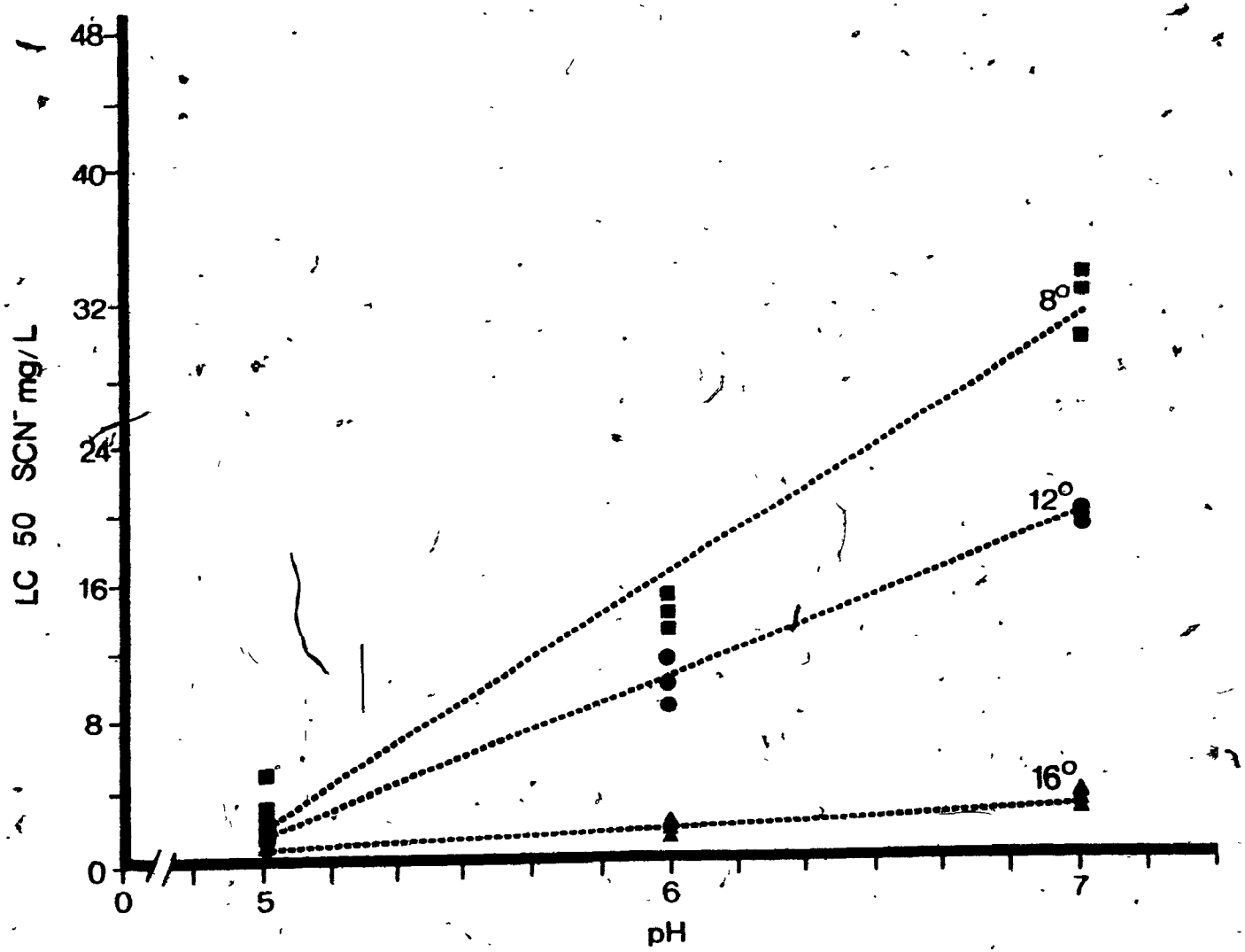


Figure 10.

temperature at different pH on thiocyanate toxicity (Fig. 11) also illustrates that, at a given temperature, thiocyanate toxicity decreases with rising pH. This is most extreme at 8 °C, where an increase in pH from 5 to 7 decreases thiocyanate toxicity; 4.796, 2.837, 2.837 to 33.467, 30.288 and 32.510 mg/L SCN^-. The linear regression for this relationship is Y = -68.96 + (14 .28)x. A similar increase in pH had the least modifying effect on thiocyante toxicity at 16 °C; 0.666, 0.554, 0.664 to 2.790, 3.618 and 3.224 mg/L SCN^-. The linear regression relationship here was found to be Y = -5.993 + (1.291x).

Results of the three experiments run simultaneously to the thiocyanate toxicity bioassays are tabulated in table 4.

Analysis of covariance (Table 6) indicated a highly significant ($p<.001$) interaction of pH and temperature in regards to thiocyanate toxicity. Through analysis of covariance, the relationship (pH, temperature, interaction and thiocyanate toxicity) is given by the following equation, Equation 1;

$$Y = -133.3837 + 27.5812\,(a) + 7.8710\,(b) + 1.658\,(c)$$

where Y = thiocyanate toxicity

a = pH

b = temperature

c = (pH x temperature) or interaction factor

TABLE 6 TABULATION OF ANALYSIS OF CO-VARIANCE RESULTS

FACTOR	F	d.f.	P
TEMPERATURE	459.50	1,23	p<.001
pH	537.13	1,23	p<.001
INTERACTION	230.50	1,23	p<.001

This equation illustrates the relationship between pH, temperature, interaction factor and thiocyanate. The descriptive equation (equation #1) as derived from multiple regression has inbuilt error. This error is minimal when one or more of the variables is equal to its mean and the error increases as the variable moves further from the mean. This is illustrated by the examples in Table 5. At pH 6 12°C, the predictive equation value Y, is virtually identical to the mean of the LC50's and the confidence interval is small. As the variable moves further from the mean, the Y value becomes less accurate and the confidence interval larger; pH 6, 8 and 16°C Y=16.849 + 1.49 and Y=3.548 +1.20 respectively. As the LC50 is a number with corresponding confidence limits, this should be the same with multiple regression. The numbers from the descriptive equation (Table 5) illustrate the importance of not using the equation beyond the experimental parameters but also obtaining sufficient points of data so as to use variables close to the mean.

The acclimation experiment performed at pH 5, 8°C shows a deviation from the unacclimated results (Table 4). Although only a single trial was performed, we can speculate as to the consequences of the results. The following assumptions have been adopted; 1) The same percent decrease that occurred at pH 5, 8°C would occur at pH 6 and 7, 8°C i.e. 3.524:1.711 is a 48.5 percent

reduction. Therefore values at pH 6 and 7 would be reduced from 14.574 to 6.995 and 32.088 to 15.402 respectively. 2) As the change in toxicity at 12 °C (a difference of 6 °C from the breeding temperature of 18 °C) would be considerably less and that at 16 °C (a difference of 2 °C from the breeding temperature of 18 °C) there would be a very small relative difference in toxicity change.

The change in relationships that the acclimation experiment creates can best be illustrated through figures 10 and 11. Fig. 10 - the effect of pH at different temperatures on thiocyanate toxicity, could alter the graph in such a way that at pH 5 the differences or range in value 8-16 °C would decrease from 4.386 to 1.157 mg/L. If this scenerio was to occur at pH 6 and 7 also, the 8 °C line would become closer and possibly less distinct from the 12 °C line. All three relationships 8-12-16 °C would be closer together. This change would decrease the large range i.e. 28.877 - 12.191 mg/L between 8 - 16 °C at pH 7.

In regards to fig. 11- the effect of temperature at different pH on thiocyanate toxicity, the acclimation experiment might also alter the shape of the relationships; at pH 5,6 and 7. This would involve going from a linear to a more curvilinear or parabolic relationship. This would then suggest that increased toxicity in terms of differences in LC50's would be more

enhanced between 12-16 °C than between 8-12 °C.

The changes, assumptions and questions the acclimation experiment raises illustrates that further studies into this area of research would be very useful in understanding the full effect of thiocyanate toxicity in relationship to pH, temperature and Daphnia magna .

The data were then used to calculate dose mortality frequencies (Fig. 12). These were calculated by the convention used by Levine (1983) in which the histogram has a 'concentration of SCN^-' on the x-axis and '% mortality' on the y-axis. The % mortality is calculated by subtracting the preceeding concentration's mortality to obtain the frequency. The mean, mode, and median were then calculated for each distribution (Table 7). Comparisons of these measures of central tendency reveal skewness. A shift of the mean to the left of the median and mode indicates that the frequency is skewed to the left. A shift of the mean to the right, in like manner illustrates that it is skewed to the right.

The frequency values for the replicates were then averaged to facilitate the depiction of trends. These averaged values were again plotted as a bar diagram to illustrate the trends as either change in temperature at a constant pH or change in pH at a constant temperature (figs. 13-14). These trends can help describe the effect of thiocyanate toxicity. Their corresponding mean, median

FIG 12 Concentration mortality frequencies of non-averaged data.

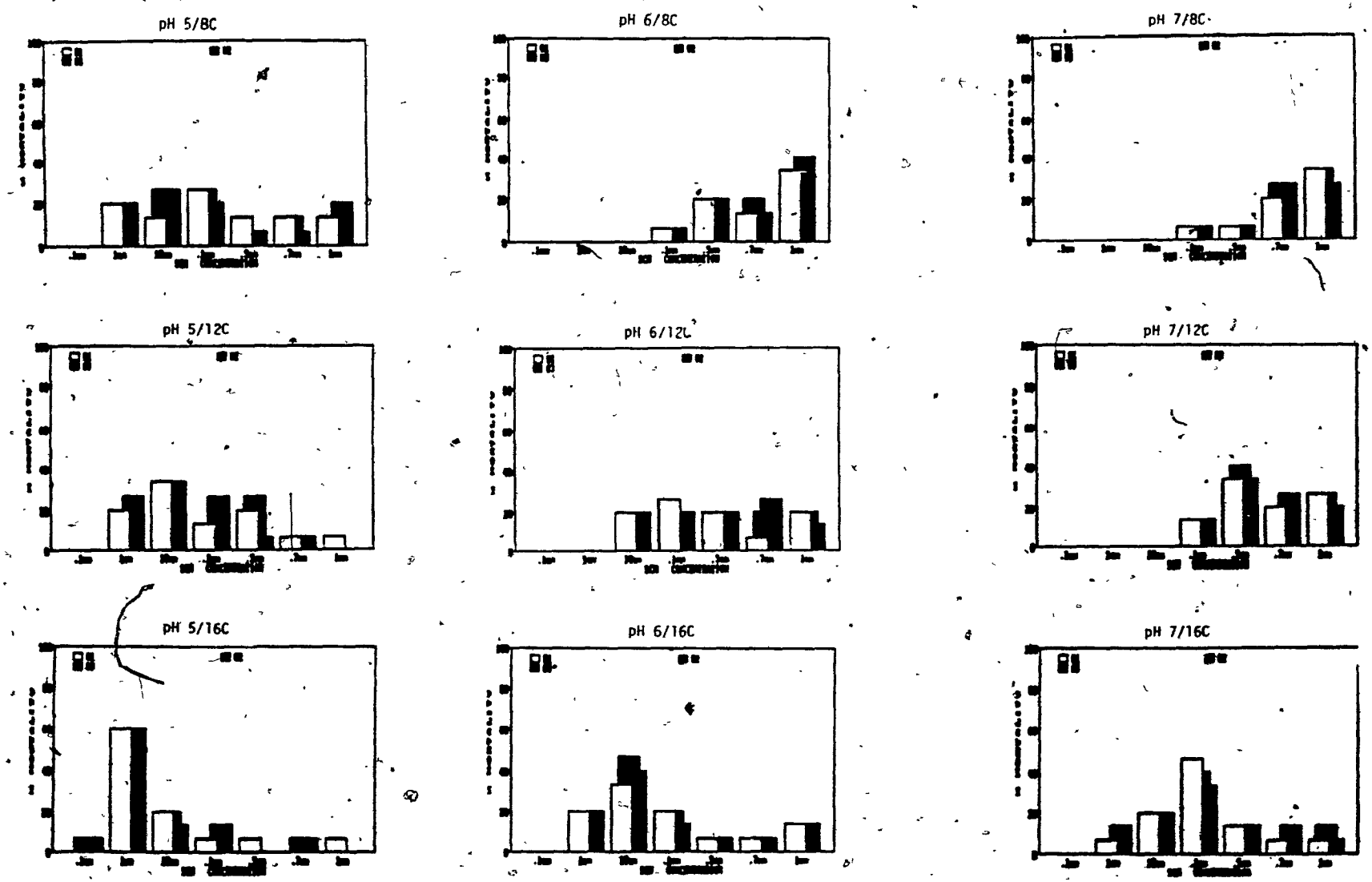

pH 5/8C
pH 6/8C
pH 7/8C
pH 5/12C
pH 6/12C
pH 7/12C
pH 5/16C
pH 6/16C
pH 7/16C

Figure 12.

TABLE 7 CONCENTRATION MORTALITY FREQUENCY ANALYSIS OF NON AVERAGED DATA

CONDITION	X	MEDIAN	MODE	RESULT
pH 5/8 °C	32.8	10.0	10.0	+ve skewed
	29.6	10.0	1.0, 10.0	+ve skewed
	30.6	10.0	1.0	+ve skewed
pH 5/12 °C	23.3	1.0	1.0	+ve skewed
	20.2	1.0	1.0	+ve skewed
	11.4	0.1	0.1	+ve skewed
pH 5/16 °C	10.9	0.1	0.1	+ve skewed
	5.9	0.1	0.1	+ve skewed
	6.5	0.1	0.1	+ve skewed
pH 6/8 °C	73.6	75.0	100.0	-ve skewed
	73.7	75.0	100.0	-ve skewed
	75.8	87.5	100.0	-ve skewed
pH 6/12 °C	40.6	30.0	10.0	+ve skewed
	47.0	50.0	1.0,10.,75,100.	-ve skewed
	45.5	50.0	75.0	-ve skewed
pH 6/16 °C	24.0	1.0	1.0	+ve skewed
	17.4	1.0	1.0	+ve skewed
	23.4	1.0	1.0	+ve skewed
pH 7/8 °C	78.5	87.5	100.0	-ve skewed
	78.1	75.0	100.0	-ve skewed
	76.0	87.5	75.0,100.0	-ve skewed
pH 7/12 °C	63.9	62.5	50.0	+ve skewed
	60.85	50.0	50.0	+ve skewed
	62.1	62.5	50.0	-ve skewed
pH 7/16 °C	23.2	10.0	10.0	+ve skewed
	29.2	10.0	10.0	+ve skewed
	26.8	10.0	10.0	+ve skewed

FIG 13-14 Concentration mortality frequencies of averaged data.

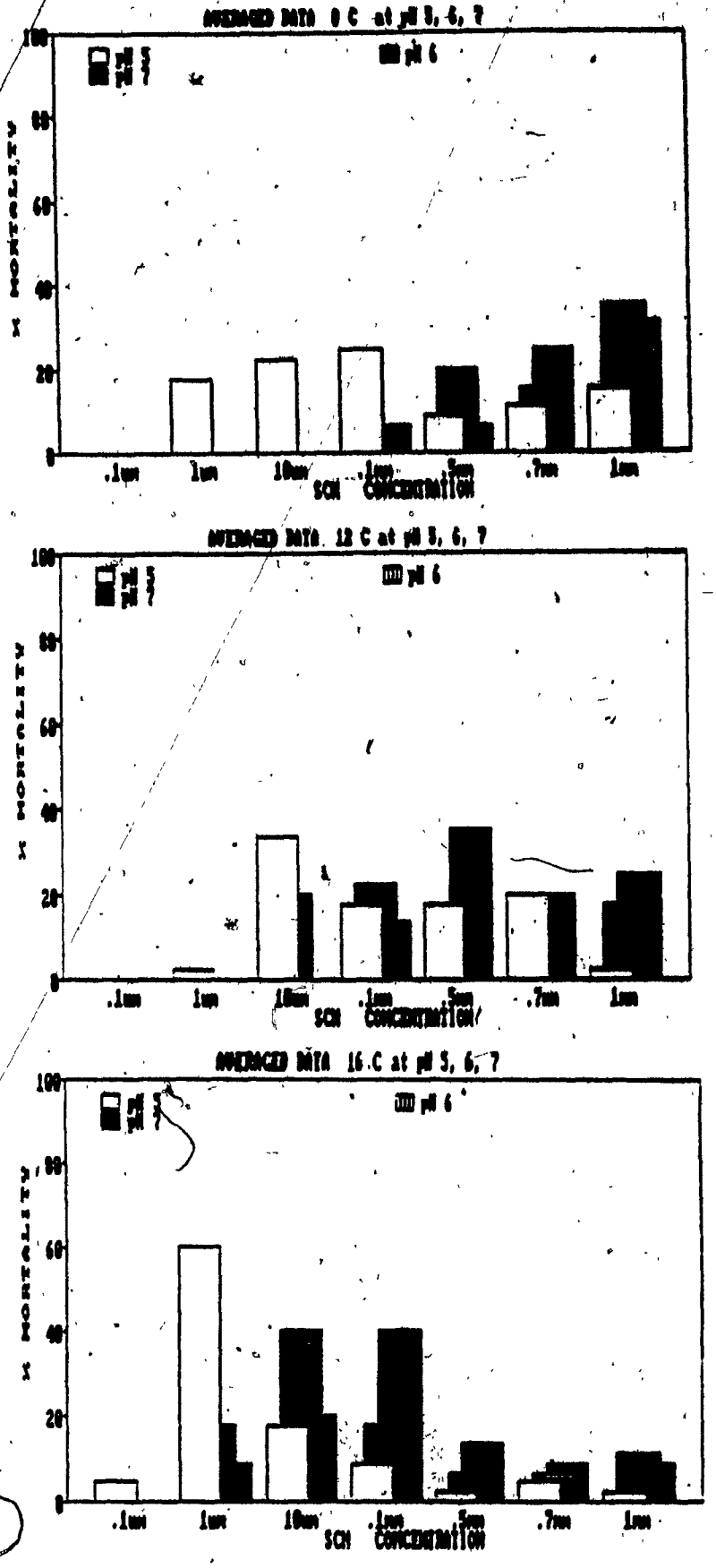

AVERAGED DATA 8 C at pH 5, 6, 7
pH 6
% MORTALITY
SCH CONCENTRATION
.1mm
1mm
10mm
.1mm
5mm
.7mm
1mm
AVERAGED DATA 12 C at pH 5, 6, 7
pH 6
% MORTALITY
SCH CONCENTRATION
AVERAGED DATA 16 C at pH 5, 6, 7
pH 6
% MORTALITY
SCH CONCENTRATION

Figure 13.

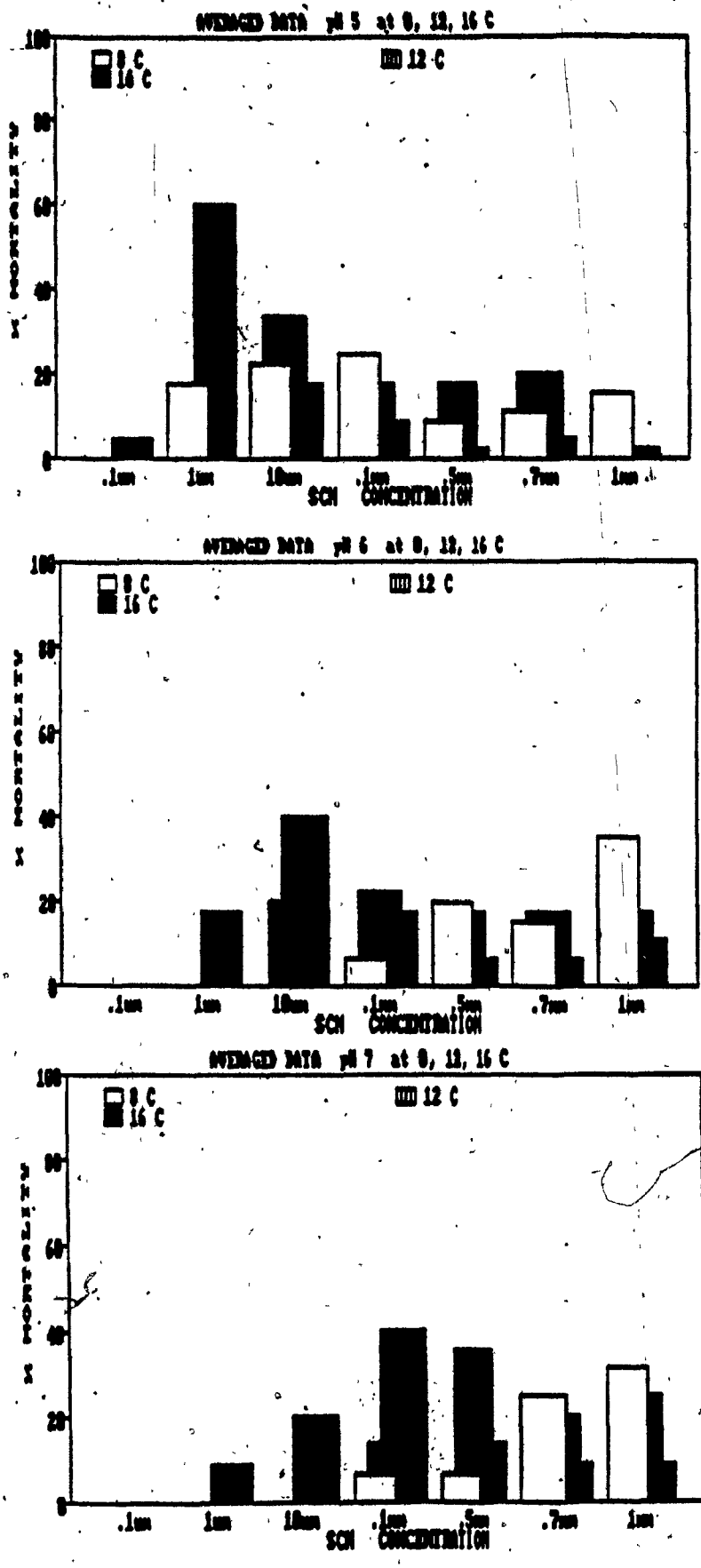

AVERAGED DATA pH 5 at 8, 12, 16 C
8 C
16 C
12 C
% MORTALITY
SCN CONCENTRATION
.1mM
1mM
10mM
.5mM
5mM
.7mM
1mM
AVERAGED DATA pH 6 at 8, 12, 16 C
8 C
16 C
12 C
% MORTALITY
SCN CONCENTRATION
.1mM
1mM
10mM
.5mM
5mM
.7mM
1mM
AVERAGED DATA pH 7 at 8, 12, 16 C
8 C
16 C
12 C
% MORTALITY
SCN CONCENTRATION
.1mM
1mM
10mM
.5mM
5mM
.7mM
1mM

Figure 14.

and mode can be found in Table 8.

TABLE 8 CONCENTRATION MORTALITY FREQUENCY ANALYSIS OF AVERAGED DATA.

CONDITION	X	MEDIAN	MODE	RESULT
pH 5/8 °C	31.00	10.00	10.00	+ve skewed
pH 5/12 °C	18.28	1.0	1.0	+ve skewed
pH 5/16 °C	7.76	0.1	0.1	+ve skewed
pH 6/8 °C	74.38	79.16	100.00	-ve skewed
pH 6/12 °C	44.36	43.33	10.0	+ve skewed
pH 6/16 °C	21.60	1.0	10.0	+ve skewed
pH 7/8 °C	77.53	83.33	100.00	-ve skewed
pH 7/12 °C	62.28	58.30	50.00	+ve skewed
pH 7/16 °C	26.40	10.00	10.0	+ve skewed

DISCUSSION

MODIFYING FACTORS

The results obtained from this study illustrate the toxicity of thiocyanate to Daphnia magna under specific conditions. The LC50 values ranged from 0.554 to 33.467 mg/L thiocyanate, dependent on pH and temperature. Modifying factors, as mentioned earlier, are defined as 'any characteristic of the organism or the surrounding water that affects the toxicity of a pollutant' (Rand and Petrocelli 1985). Under this definition, pH and temperature have proven to be abiotic modifying factors of thiocyanate toxicity. Fry (1971), suggested that physiochemical interactions with the organism through alteration of the environment be defined as a masking factors. A masking factor is an identity which modifies the operation of a second identity on the organism. The interactive effects within the organism may include actions on the physiological and the regulatory systems. This research was restricted to observing the changed response. Since I did not investigate the mechanism of thiocyanate toxicity, I cannot infer if pH and temperature are masking factors.

The effect of pH and temperature on

thiocyanate toxicity can be illustrated by the Figs. 10 and 11. They illustrate the changes of LC50s of thiocyanate in mg/L with temperature and pH. In examining the effect of temperature at different pH on thiocyanate toxicity (Fig 11) at 16 °C, an increase in pH from 5 to 7 decreases toxicity 5 fold, and at 8 and 12 °C, a similiar increase in pH decreases toxicity 10 fold. The effect of pH at different temperatures on thiocyanate toxicity (Fig 10) illustrates that an increase in temperature from 8 to 16 °C, increases thiocyanate toxicity at pH 5 5.5 fold; at pH 6 and 7 a 10 fold increase in toxicity was observed. Statistics show the importance not only of temperature and pH but also the significant interaction between pH and temperature on determining the toxicity of thiocyanate (Table 6).

These results are indicative of the ability of pH and temperature to modify toxicity by changes to both the organism and/or its environment, i.e. speciation of the ions available to the organism, solubility of the substances, oxygen concentration, stress to the organism, metabolism, suspended organic material, dissolved salts or nutrients, dissolved gases.

The three most important factors in this system are I believe, stress to the organism brought on by the changes in pH, metabolism being regulated by temperature, and the thiocyanate ion. I found an increase

in toxicity (ranging from five to ten fold) with a decrease in pH (7->5) at all temperatures. It is possible that since metabolism of the animal is governed by temperature, so might be the interaction with thiocyanate. At all three pH's, an increase in temperature (8 -> 16 °C) increased toxicity. This increase in toxicity ranged from 5.5 to 10 fold. Therefore the combination of the most stressful situation in this case pH 5 and the highest temperature 16° C is found to be the most toxic with an average LC50 value of 0.629 mg/L SCN^-.

SPEYER AND PARKER COMPARISONS

In comparing my data with that found by Speyer and Raymond (1984) I found both similarities and differences in regards to trends. With Daphnia magna an increase in temperature increased toxicity at all three pHs (5, 6, and 7). Speyer and Raymond (1984) working with fish also found this trend but only at pH 6. At lowest pH (6), increasing temperature from 5 to 12 C significantly increased thiocyanate toxicity. At higher pH (8), a simular increase in temperature significantly reduced thiocyanate toxicity, a result we did not find at our highest ph (7).

In Daphnia magna increasing pH (5 to 7) at a given temperature consistently decreased toxicity. Speyer and Raymond (1984) found that increasing pH (6 to 8) at 12 C also significantly decreased toxicity, but at 5 C a similar increase in pH significantly increased toxicity. From their results Speyer and Raymond (1984) concluded that thiocyanate is most toxic at low pH (6) and high temperature 12 C, and conversely the least toxic combination was at high pH and high temperature (12 C). These results only partially agree with our findings. Since the abiotic medium was the same as Speyer and Raymond (1984), the differences were found in the pH range; 6 and 8

vs 5 and 7, and the model species; Daphnia magna vs rainbow trout. Speyer and Raymond (1984) suggest that pH has a greater effect on the toxicity of thiocyanate to rainbow trout than does changing temperatures. They stated that the toxicity of thiocyanate at pH 6 and 12 C was most toxic because of changes in gill membrane permeability and increased metabolic rate.

In tests conducted on rainbow trout by the Environmental Protection Service (EPS) Halifax, Parker (1983) found similar responses to changes in pH. He investigated the toxicity of potassium thiocyanate at three different pHs (5, 7, and 8.5). All tests were conducted at 15 C. A decrease in the pH to 5 from pH 7 decreased the LC 50. Based on his results, Parker (1983) suggests that the toxicity of potassium thiocyanate may be increased with lowering pH but further testing is needed.

These differences or non-conformence in trends with potassium thiocyanate and pH between Speyer and Raymond (1984) and Parker (1983) may be partially due to the different water quality parameters between the two labs. Table 9 displays the differences between the two test waters used. Heming et al. (1985) suggest that thiocyanate toxicity is influenced by multiple factors. These include temperature, water calcium levels, hardness, catecholamine release, photoperiod, stress, and the

TABLE 9 COMPARISON BETWEEN SPEYER AND RAYMOND (1984) AND PARKER (1983) WATER COMPOSITION

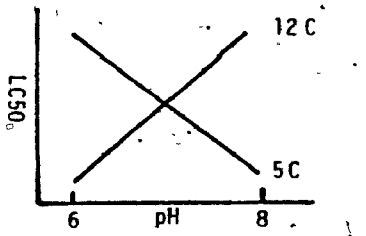
SPEYER (1984)	CHARACTERISTIC	PARKER (1983)
75.00	Hardness mg/L	20.05
250	Conductivity	600-700
68.75	Alkalinity mg/L	10
8.78	Magnesium mg/L	.62-.63
90	Sulfates mg	7.2-7.4
40.25	Sodium mg/L	3.9-4.3
16.5	Calcium mg/L	6.83-7.27
.0025	Copper mg/L	<.01
.0125	Iron mg/L	.11-.12
<.0 025	Zinc mg/L	<.01
11.3	Total Carbon mg/L	6.0
12.25	Chloride mg/L	7.2
2.45	Potassium mg/L	.3-.4

inhibition of thiocyanate uptake by the presence of other univalent anions.

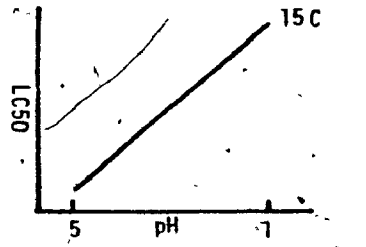
The difference in trends that Speyer and Raymond (1984) found may be due to an above mentioned factor or another factor yet undetermined (Fig. 15). This conflict in results illustrates the importance of using simular experimental protocol when determining the characteristics of thiocyanate toxicity.

FIG 15 Intergrating trends found with those established by Speyer and Raymond (1984) and Parker (1983).

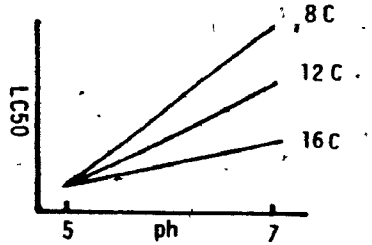
The effect of pH at different temp. on thiocyanate toxicity



Speyer and Raymond (1984)

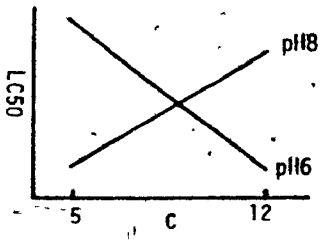


Parker (1983)



Myself

The effect of temp. at different pH on thiocyanate toxicity



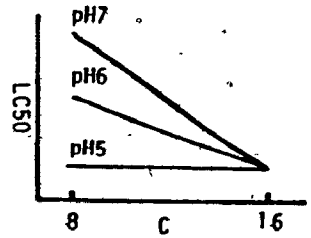


Figure 15.

LITERATURE REVIEW

Having already compared values and trends with Speyer (1982), Parker (1983) and Heming (1985) it is shown that different experimental design and water parameters can alter results, i.e. when documenting the literature, it is obvious that many different protocols were followed. These protocols ranged from lenient to very well defined. It is therefore more advantageous to look at the results in the literature as more of a trend than apply them specifically. The object of this comparison was to see how Daphnia magna 's sensitivity compares with that of other organisms.

The lack of thiocyanate toxicity studies on invertebrates only allows verification that our results were in the same general range as those found by Parkhurst (1979) and Anderson (1946)(Table 10). Parkhurst (1979) found with thiocyanate (species of thiocyanate unknown) the 48 hr LC 50 to be 51.4 mg/L thiocyanate. Anderson (1946) used sodium thiocyanate and found less than 11.3 mg/L immobilized Daphnia magna . Since our test results range from 4.554 to 33.467 mg/L thiocyanate within certain parameters, an LC 50 value of 51.4 mg/L does not seem improbable. This difference in LC50 values could be explained by different anionic composition, hardness, age of animal etc... An immobilization concentration of 11.3

Table 19: Literature comparison.

Author	Species of SCN^-	Species of Test Organism	Duration	Amount of SCN^-	Conclusion
Brun (1936)	KSCN	Gambusia holbrooki mosquito fish	10 days	59 mg/L SCN^-	harmful effects
			2 hrs	2320 mg/L SCN^-	dead
Schaut (1939)	KSCN	unidentified minnows	9-10 days	102 mg/L SCN^-	killed
Herbert (1962)	NaSCN	rainbow trout	---	1800 mg/L SCN^-	killed
Demyanenko (1931)	NH_4SCN	Alburnis alburnis	50 hrs	153 mg/L SCN^-	killed
	NH_4SCN	Bleak	144 hrs	76.5 mg/L SCN^-	not lethal
Shelford (1917)	NH_4SCN	Lepomis numilis Spotted sunfish	1 hr	2205-229.5 mg/L SCN^-	killed
Oshima (1931)	NH_4SCN	young eels	25 hrs	580 mg/L SCN^-	tolerated
		Anguilla japonica	3.7 hrs	5800 mg/L SCN^-	killed
Thumann (1950)	NH_4SCN	rainbow trout	10 hrs	76 mg/L SCN^-	no effect
Walen et al. (1957)	NH_4SCN	mosquito fish	96 hrs	87 mg/L SCN^-	96 hr TLM 87 mg/L /48 hr TLM 420 mg/L /24 hr TLM 920 mg/L
	NH_4SCN	Gambusia affinis	144 hrs	43 mg/L SCN^-	killed
Parkhust (1979)		Daphnia magna	48 hr	51.4	48 LC_{50} 51.4 mg/L SCN^-
Anderson (1946)	NaSCN	Daphnia magna	--	11.3 mg/L SCN^-	immobilized
Huiatt et al. (1982)	NH_4SCN	Canassius auratus mosquito fish	24 hrs	1600 ppm	freshwater 24 hr lethal limit
		Gambusia affinis	24 hrs	910 ppm	static bioassay, freshwater, lab study 24 TLM acute high
Huiah et al. (1982)	KSCN	K. sandvicensis	2 min	10 ppm	salt water, no observable response
			2 mmin	20 ppm	salt water, slight response

mg/L thiocyanate can only be compared very generally in showing the sensitivity of Daphnia magna since we did not investigate that parameter and cannot extrapolate to an LC 50 value.

A comparison between the vertebrate and invertebrates generally suggests that invertebrates are more sensitive than vertebrates. Since the parameters were either undefined or dissimilar a more exact comparison cannot be made.

HYPOTHESIS

Thiocyanate is classified as a pseudohalide, having similar chemical and physical characteristics to the halide group. It is a nucleophile and would be most similar to iodine in regards to its redox potential (Newman 1975). Although similar to halides, thiocyanate also has its own unique properties; isomerism, shape and modification of its chemical reactivity. Potassium thiocyanate salts have a high solubility in aqueous solutions, less so in organic solvents. Like the common alkali metal salts of other strong acids, Potassium thiocyanate is fully dissociated to $K^+ + SCN^-$ in aqueous solutions at all pHs used. This indicates that only the ionized form would exist at the physiological pH. It is this form, SCN-, that is responsible for the biological or toxic effects of potassium thiocyanate (Patai 1977). The decrease in pH from 7 to 5 or increased temperature from 8 to 16 C does not alter the structure of this ion (Westley, personal communication).

The physiological mechanism for thiocyanate toxicity on Daphnia magna is unknown at this point. The mechanism of thiocyanate toxicity in fishes has been investigated by several authors; Heming et al.(1985), Singh et al. (1977), Eales and Shostak (1983), De Renzis and

Barnanan (1977) and Epstein et al. (1975). Through their investigations they have found numerous modes of action of thiocyanate toxicity.

Firstly, cyanide is a precursor of thiocyanate and one of the enzymes responsible for this reaction is rhodenase (thiosulfate cyanide sulfur transferase)(Westley 1981). This enzyme is universally found in both prokaryotes and eukaryotes. This reaction is biologically reversible, not via the reversed reaction, but via an oxidative reaction (Westley 1981). The reversed reaction needs peroxidases to drive it and is usually carried out by methemoglobin and oxyhemoglobin. Heming et al.(1985) found that in rainbow trout exposed to external thiocyanate, some thiocyanate (albeit small but of utmost importance) was converted. This percentage of cyanide, considering the physiological pH of fish, would be un-ionized and present as HCN (Izatt et al.1962). Heming et al.(1985) found the relationship of thiocyanate to endogenously converted cyanide was in a equilibrium ratio of 664:1. Heming et al.(1985) suggests that this might be the trigger for sudden death syndrome (SDS). If this ratio was shifted due to stress, the resultant endogenously generated cyanide could be a contributing factor in SDS.

Secondly, there is a substitution or interference by thiocyanate with Cl^- at the Cl^-/HCO_3^- exchange site of the gill (Fig. 16). Heming et al.(1985),

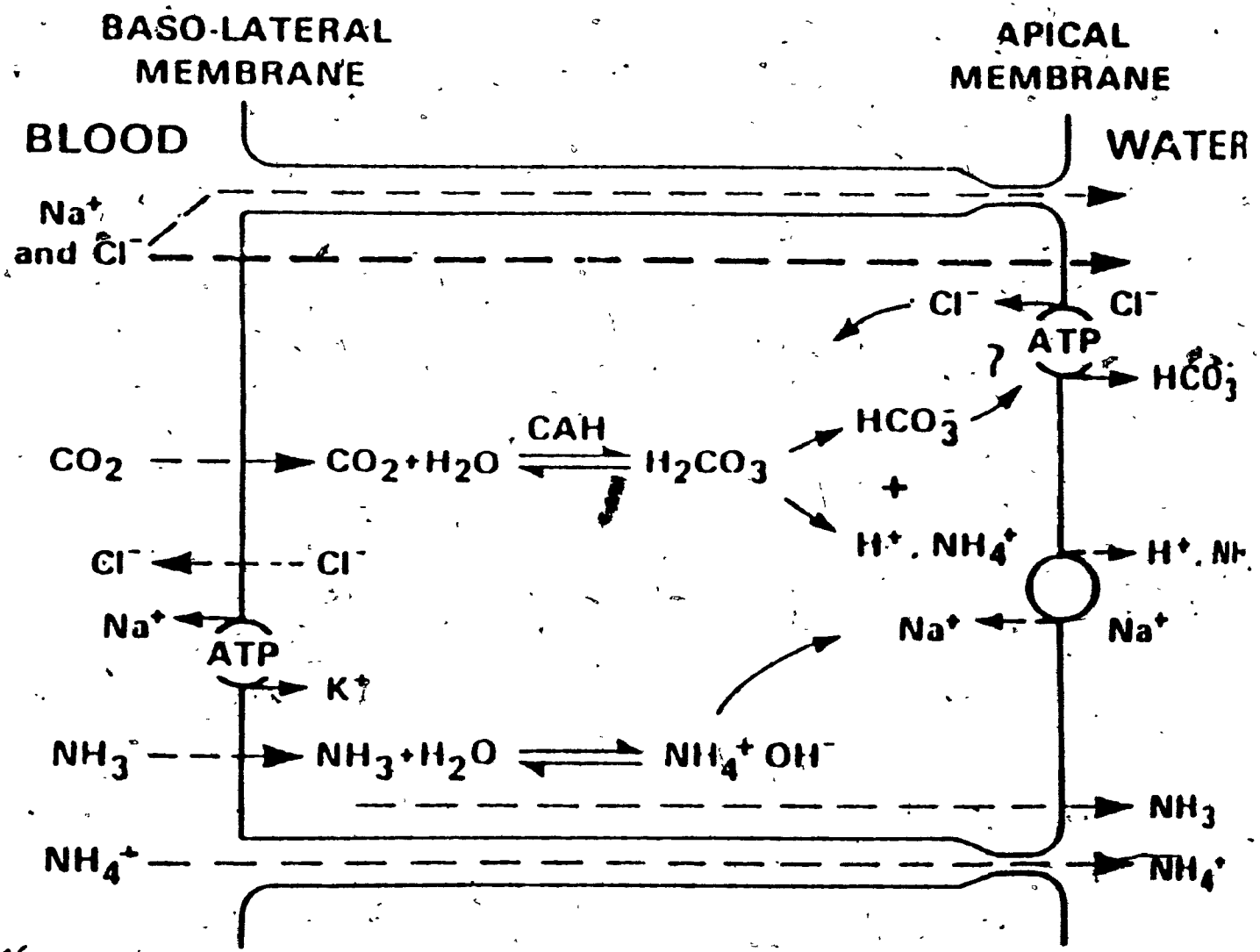

BASO-LATERAL MEMBRANE
APICAL MEMBRANE
BLOOD
WATER
Na+ and Cl-
Cl-
Cl-
ATP
?
HCO3
HCO3-
CAH
CO2
CO2+H2O
H2CO3
+
H+. NH4+
H+. NH
Cl-
Cl-
Na+
Na+
Na+
ATP
K+
NH3
NH3+H2O
NH4+ OH-
NH3
NH4+
NH4+

FIGURE 16.

deRenzis et al.(1977), deRenzis (1975), Epstein et al.(1975) illustrated that thiocyanate was not only able to compete with Cl^- (univalent anion) for transport across the gills, but also accumulated in the plasma against a concentration gradient. Epstein et al.(1975) found this to be reversed in salt water fish i.e. thiocyanate competes with Cl^- to be excreted across the gills, and the plasma concentration of thiocyanate remains lower than the external medium. The mechanism in which thiocyanate is transported bi-directionally across the gills is basically the substitution or alteration of Cl^- at the exchange site of Cl^- /HCO_3^- at the gills. Thiocyanate inhibits the uptake of Cl^- at the gill site yet has no adverse effects on plasma Cl^- and urinary loss, however the end result of thiocyanate exposure is decreased plasma chloride (deRenzis et al 1977). As thiocyanate competes with one univalent anion; Cl^-, it also competes with Br^-, I^-,and F^- for uptake, which can therefore effect toxicity (Heming et al.1985).

Thirdly, thiocyanate has also been shown to compete with halide transport across the cornea, stomach and thyroid. Singh et al.(1977) and Eales and Shipstak (1983) have shown that thiocyanate is an antithyroid compound, in that it is an iodine competitor. The thyroid gland plays an intergal role in the biological organism, interacting with the pituitary,regulating hormones and

metabolism. The consequences due to misfunction of the thyroid result in numerous altercations of signals. For example increased antithyroidism (hyperthyroidism) might induce growth through increased anabolic responses; in contrast hypothyroidism is inhibitory or catabolic (Raymond 1984), illustrating the relationship between the thyroid and metabolism. Different degrees of iodine displacement also leads to the enhancement or reduction in the production of thyroid hormones. Depending on the interference of thiocyanate different levels of T_3 and T_4 will be produced. Generally growth enhancement occurs with low levels of thiocyanate but with higher levels the reverse is true (Raymond 1984). Other effects such as the conversion efficiency of food and growth stimulation are brought on by thiocyanate toxicity. The preceeding effects of thiocyanate illustrate its interaction with the thyroid. The consequences are not spontaneous but rather a result of chronic exposure.

Lastly, thiocyanate has been seen to inhibit enzymes such as carbonic anhydrase (Davenport 1940) succinic dehydrogenase (Solomon et al.1973) and moieties of ATPase (Katz and Epstein 1971, Solomon et al.1973). Carbonic anhydrase regulates the interconversion of CO_2 and HCO_3^- in both gill tissue and blood cells. Therefore the exchange of CO_2 across the gills is regulated by carbonic anhydrase. Reduction of carbonic anhydrase by thiocyante

would result in internal acidosis and higher levels of total CO_2. Neither Heming et al.(1985) nor deRenzis (1975) found any evidence of reduced carbonic anhydrase activivty with normal plasma pH and levels of CO_2.

In reviewing the effects of thiocyanate activity on fish and attempting to extrapolate a hypothesis on the mode of action of thiocyanate in Daphnia magna we must consider the known data, and examine how the findings apply to Daphnia model, if at all. In looking at the four choices, on can eliminate the first choice i.e.the conversion to cyanide, due to the fact that red blood cells i.e. methemoglobin and oxyhemoglobin are not found in Daphnia magna . Although Daphnia magna do have a hemoglobin this is more of a loose arrangement for an attachment with iron. Also the hemoglobin is produced to counteract adverse conditions of low oxygen. Oxygen is transported by a loose association within the hemoceol. It is therefore unlikely that interconversion of thiocyanate to cyanide takes place. Thiocyanate interaction with the thyroid through the inhibition of I^- seems unlikely as a mode of action by thiocyanate on Daphnia magna . Daphnia do not have a physiologically recognized thyroid. Although they may have hormones regulating growth and molting it is unknown if I^- plays a role. The reduction of the enzyme carbonic anhydrase does not seem to figure as a major constituent of thiocyanate toxicity to fish and therefore

would probably not effect Daphnia magna . By elimination it therefore seems that the most likely mode of action would be the substitution or interference with Cl^- at the Cl^-/HCO_3^- exchange site. With fish this exchange site is located at the gills (Fig. 16). In Daphnia magna the Cl^-/HCO_3^- exchange site is located on the epithelium of the thoracic limbs (Stobbart et al.1977). It is possible that it is through the loss of plasma chloride that the toxicity to Daphnia magna is established. It would only be through further research and experiments that this question can be assuredly answered. An experiment that would increase Cl^- in solution in combination with other univalent ions would be of interest. A physiological exploration of the mode of action of thiocyanate within Daphnia magna would help complete the picture.

Temperature and pH add to the toxic effect of thiocyanate by modifying both the condition of the environment and the organism. Temperature is especially important when dealing with ectothermic organisms. This effect is reflected in a Q10 value i.e. the rate of metabolism in aquatic organism more or less doubles with every 10°C rise in temperature (Cairns et al.1975). Daphnia magna are found to be relatively temperature-independant over the wide range of temperature in which Daphnia encounters in nature. This is illustrated by the relative constancy of the temperature coefficient

Q10. The Q10 was calculated as 1.4 over a range of 10-19 °C (Sarviro 1980). Therefore it can be considered that over the range we are studying (8-16 °C) that the increase in metabolism approaches a straight line. This phenomenon of thermal tolerance within the temperature ran e in which the organism is found has been documented by many authors (Macissac et al.1985, Galkovskaya et al.1985, and Ranta and Hakala 1978). Although thermal tolerant, Daphnia still exhibit an increase in metabolism with increased temperature in a ~ar manner (Galkovskaya et al.1985 and schindler 1973). This increase in metabolism corresponds to an increase in respiration and thus reflects faster uptake of a toxicant with increased temperature i.e. effects of thiocyanate would be intensified by a temperature increase. The enzymatic activities and growth of the organism would also be increased. Change in temperature and pH may also result in increased solubilities of some chemical compounds changing the environmental conditions. The effects of pH on Daphnia magna has been investigated by a few researchers; Hovas et al.1984, Stobbart et al.1977, Potts and Fryer 1979, Meinel et al.1985, and Brehm and Meijering 1982. The effects of lower pH on Daphnia magna results in misregulation of the ionic balance. Increasing the H^+ concentration in the external medium has a negative effect on the Na^+ regulation. The Na^+ ion loss rate increases while the rate

of uptake decreases, therefore the Na^+ concentration in the blood drops (Potts and Fryer 1979). This could be explained by competition between Na^+ and H^+ for a carrier binding site, particularly at low Na^+ levels. Furthermore, the Cl^- uptake is also inhibited by a low pH value (Potts and Fryer 1979) resulting in a decrease in the Cl^- content of the plasma. The drop in the Na^+ and the Cl^- plasma concentration is a result of the failure in the Na^+/H^+ and the CL^-/HCO_3^- exchange mechanism. The disruption of the ionic regulation causes acidosis of the hemolymph to occur.

The addition of thiocyanate would enchance the misregulation of the ions as it may compete or disrupt the ionic exchanges. The loss of Cl^- would be increased and death more likely.

The specific ionic composition of the external medium, after manipulation in pH and temperature, was not fully investigated. It would be of interest to monitor ions such as CO_2, HCO_3, $CO_3^=$, univalent ions and Na through the experiment to study their role in thiocyanate toxicity.

SUMMERY

The allowable limits set by the Ontario Ministry of the Environment for thiocyanate concentrations in mine mill effluent are 150 mg/L where receiving waters are less than 200 mg/L hardness. Since the average hardness of Canadian waters is 75 mg/L (Speyer and Raymond 1984), this thiocyanate standard would apply to the majority of water bodies in Canada. Under certain experimental conditions, Daphnia magna have shown to be more sensitive to thiocyanate than rainbow trout. This difference in sensitivity renders them more vulnerable to thiocyanate. The results illustrate the importance of pH and temperature in eter inin thiocyanate toxicity and should be taken into consideration when setting guidelines. The descriptive equation allows government agencies and industries to help establish a guideline for water quality of thiocyanate concentrations with regards to pH and temperature at a constant hardness. The ecotoxicological effects of thiocyanate should be completely investigated combining both lab and field studies before the complete impact of this toxicant can be estimated.

If the SO_2-air method must be implemented, secondary treatment for thiocyanate removal should be strongly considered. The pressure to convert

cyanidation reclamation from the established alkaline chlorination method to the new Inco SO_2- air process is due mainly to higher capital expense. The ideal solution for industries that use this new method is to implement an additional system to remove the elevated thiocyanate levels. The systems available for this procedure range from natural degredation to electrochlorination and include alkaline chlorination, ozonation, electrooxidation method, and biological treatment.

The gold mine industry uses a process called cyanidation for the removal of gold from its ore. This procedure is based on the preferential dissolving action for gold by cyanide. The resulting effluent contains simple cyanides (sodium or calcium), cyanide complexes of copper, iron, nickel, zinc, and thiocyanate. The convential way cyanide was removed was through alkaline chlorination. This process is capable of completely oxidizing cyanide to nitrogen and bicarbonate thus eliminating problems associated with cyanate and thiocyanate release. Justification for this proposed change is that while the capital cost of an alkaline chlorination facility is relatively low, reagent costs can be high; this contributes to low cost efficiency and is the main disadvantage of the process.

The proposed sulfur-dioxide -air process involves the 'selective' oxidation to cyanate of both free

and complexed cyanide species (with the exception of iron cyanide) using a mixture of SO_2 and air at controlled pH in the presence of copper as a catalyst. The advantages of this process are the relatively low reagent costs, the ability to remove iron cyanides, and the ability to achieve low total cyanide concentrations. On the negative side, the process effluent contains elevated levels of cyanates and/or thiocyanates.

Natural degredation or lagooning is effective only if the retention time is adequate and dilution sufficient. Natural degredation is brought about through a combination of many environmental conditions. Photodecomposition aided by sunlight. Carbon dioxide in the atmosphere leads to acidification and thus lowering of pH. Oxygen produces oxidation reactions. Dilution lowers pH. An underlying porous environment lends to seepage plus absorption of solids, and if the retention time is sufficient, biological action.

Alkaline chlorinati~ is the most widely used method of cyanaide reclammation through cyanide destruction, an oxidation process. Because of its history, the engineering expertise, equipment, control techniques, operational know-how, and background experience, have all been established. Alkaline chlorination is the use of chlorine gas, calcium hypochlorite or sodium hypochlorite to achieve the oxidation of cyanide (White 1972).

Oxidation of thiocyanate utilizes vast amounts of chlorine, and although costly, chlorine is available in many different forms

The disadvantages of the alkaline chlorination method are twofold- the previously mentioned high costs and secondly that although through careful control of pH, the formation of cyanogen chloride will not occur, there are other toxic derivatives of chlorine such as chlorinated organic compounds which need an extra step to be eliminated.

Ozonation is the generation of ozone electrically, either from atmospheric oxygen or from pure oxygen. Ozonation will not only destroy thiocyanate but also hydrogen cyanide, cyanide ion, and the complexes of zinc, cadmium and copper. The oxidation reaction between ozone and thiocyanate is demonstrated through the following equation;

$$SCN + O_3 + H_2O \text{ ----------> } CN + H2SO_4$$

(Liptak 1974)

The cyanide ion could then be recycled.

Electrooxidation reactions have not been put into industrial practice as yet. The reaction oxidizes thiocyanate producing cyanate and sulfates.

Electrochlorination is the process involving active chlorination- achieved by the addition of sodium chloride. The active chlorine reacts with

thiocyanate, producing cyanates and sulfates. As with alkaline chlorination, the chloride ion is released, and is available for future reactions.

Biological treatment, although still in the research stage, has promising potential for the removal of thiocyanate. Because of metal accumulation, primary treatment for the removal of metal is necessary. In the biological treatment method, although the necessary nutrients must be added, operation and maintenance costs are comparatively low and thiocyanate removal is near complete with a 92% reduction.

In summation there are practical processes which can be used in conjunction with the Inco Sulfur Dioxide Air method to reduce thiocyanate effluent levels. The choice of which method best serves the needs of 'economy' and 'efficiency' should be left to those who understand fully the implications of both criteria. However it is the mining establishment itself which should accept the responsibility in the removal process, thus enabling the ecosystem as a whole to continue in a healthy manner.

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