Pergamon

Environmental Toxicology and Chemistry, Vol. 13, No. 9, pp. 1423-1432, 1994
Copyright © 1994 SETAC
Printed in the USA
0730-7268/94 $6.00 + 00

0730-7268(94)00092-1

CHRONIC TOXICITY OF WATERBORNE THIOCYANATE TO THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*): A PARTIAL LIFE-CYCLE STUDY

ROMAN P. LANNO and D. GEORGE DIXON*
Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1 Canada

(*Received* 1 *October* 1993; *Accepted* 18 *February* 1994)

Abstract—Juvenile fathead minnows (*Pimephales promelas*) were exposed to thiocyanate (SCN^-) concentrations of 0, 1.1, 7.3, 16.6, or 32.6 mg/L for 124 d while monitoring growth, physiological, reproductive, and histological parameters. The NOEC for decreased egg production, increased time to first spawn, and development of overt goiter was 1.1 mg/L, whereas the LOEC for these parameters was 7.3 mg/L. Histological changes in thyroidal tissue were the most sensitive parameter observed, with an LOEC of 1.1 mg/L. Fish exposed to 16.6 or 32.6 mg SCN^-/L neither completed development of secondary sexual characteristics nor spawned. The LOEC for decreased growth and hematocrit was 32.6 mg/L. SCN^- accumulated in the plasma of fish exposed to 16.6 and 32.6 mg/L, with BCFs of 2.7 and 13.8, respectively. Development and mortality of embryos and larvae to 3 d post-hatch were also monitored in eggs spawned by adults exposed to 0 or 1.1 mg SCN^-/L and subsequently incubated and hatched at 0, 1.1, 7.3, 16.6, or 32.6 mg/L. Eggs spawned by adults exposed to 1.1 mg SCN^-/L exhibited increased percentage of eyeup and hatch, while decreasing time to hatch and mortality. There were no effects of SCN^- concentration during incubation on egg viability. After the 124-d exposure, adults were transferred to SCN^--free water for 30 d. Insufficient numbers of adults were available from groups exposed to 32.6 mg/L to be included in the recovery study. Eggs were spawned by all groups during the recovery period, regardless of the SCN^- preexposure level, although time to first spawn increased with preexposure concentration.

Keywords—Thiocyanate Reproduction Fathead minnow Goiter Chronic toxicity

INTRODUCTION

Thiocyanate (SCN^-) is an inorganic anion with specific antithyroidal properties [1,2]. Of the numerous antithyroidal compounds reported in the endocrinology literature, only SCN^- is likely to occur at biologically active levels in the aquatic environment. This can arise from the treatment of cyanide (CN^-) -rich effluents from gold-ore concentrators, a treatment that uses sulfur dioxide and air, in the presence of a copper sulfate catalyst, to convert CN^- to the less toxic SCN^- [3]. SCN^- levels of 168 to 680 mg/L have been detected in effluent following such treatment [4]. SCN^- may also be formed by aquatic organisms during the metabolic detoxification of CN^- present in industrial effluents, or from the metabolism of dietary CN^- precursors such as cyanogenic glycosides and nitriles [5].

Although the antithyroidal properties of SCN^- have been recognized in mammals for some time [1], lab investigations with aquatic organisms have been restricted largely to studies with doses administered by i.p. injection [6–8]. Waterborne administration of antithyroidal agents has been limited to thiourea [9,10] and bromide [11,12]. SCN^- acts as an inhibitor of both the iodide (I^-) -trapping mechanisms that incorporate plasma I^- into the colloid of thyroid follicles [1] and I^- organification [2]. In teleosts, SCN^- inhibits halide transport across biological membranes, including those at the gill [13,14], and possibly the intestine [15]. SCN^- has also been shown to reduce phosphorus and I^- uptake by the ovaries of fish [7,16].

Given the possibility of exposure of fish populations to SCN^- in areas of mining activity, as well as the potential for antithyroidal effects, we assessed the effects of SCN^- on reproduction in the fathead minnow (*Pimephales promelas*) during a 124-d exposure and a subsequent 30-d recovery period. Reproductive response was determined by following the development of secondary sexual characteristics, spawning behavior, time to first spawn, fertilization rates, and egg production. Thyroid histology, plasma SCN^- concentrations, and growth of the breeding adult fish were also evaluated. In addition, SCN^- effects on the survival and development of eggs, as well as the survival, growth, and sexual maturation of the resulting juveniles, were monitored.

MATERIALS AND METHODS

Supply and maintenance of fish

Juvenile fathead minnows were obtained from the lab breeding culture, University of Waterloo (Ontario). Each brood tank contained two male and four female fish, and received a continuous flow of well water at 23 to 25°C. The photoperiod was maintained at 16:8 h light:dark, with 15-min periods of simulated dusk and dawn. Routine practices for culture maintenance were based on those of the U.S. Environmental Protection Agency (EPA) [17]. Hardness, alkalinity, pH, and DO were monitored routinely and were essentially identical to those reported below for the subsequent

*To whom correspondence may be addressed.

experiment. A detailed characterization of the water supply is available elsewhere [18].

Fish in the lab colony were fed three times daily, 7 d a week. Brood stock were fed frozen adult brine shrimp (*Artemia salina*) (Murex brand; Artemia Canada Ltd., Langley, British Columbia) (manufacturer's analysis: crude protein ≥2.6%; fat ≥0.7%; fiber ≤0.18%; moisture ≤95.7%). Juvenile fish in the colony were fed a combination of frozen *Artemia*, tropical fish flake food (TetraMin®, TetraWerke, Germany) and salmonid starter diet (Martin's Feed Mills, Elmira, Ontario) (manufacturer's analysis: protein ≥52%; fat ≥18%; fiber ≤2.5%). Larval minnows were fed live *Artemia* nauplii three times per day; nauplii were incubated according to manufacturer's directions (Artemia Canada Ltd.) and rinsed with fresh water before feeding. During the actual experiment, adult and juvenile fish were fed frozen *Artemia*. Although the amount was not weighed, similar amounts were offered to each tank during feeding periods. Excess feed and feces were siphoned from the tanks daily. Larval fathead minnows were fed live nauplii and gradually adapted to frozen brine shrimp.

Experimental system

The experimental system comprised fifteen 18-L glass aquaria, each divided into two sections by a glass partition. This number of tanks allowed for five treatment groups (four SCN^- concentrations plus a control) by three replicates. Water flow entered the back portion of the tank, passed over the glass partition, and exited the tank via a standpipe in the front portion of the tank. The rear portion of the tank (6 L) was used for the incubation of eggs and fry, whereas spawning substrates and adult fish were maintained in the front portion (9 L). At a continuous water flow rate of 200 ml/min, 99% replacement times for water in the rear and front portions of the tank were, respectively, 2.5 and 3.5 h [19]. In the front chamber of each aquarium four pieces of 7-mm-o.d. glass tubing were fixed to the sides perpendicularly to the bottom. A flat clay tile was placed on these posts as a spawning substrate.

Dilution water used during the study was the same as that used in the maintenance of the breeding colony and contained no detectable SCN^-. Temperature was measured with a calibrated mercury thermometer; pH with a standard pH meter (Model PHM82, Radiometer A/S, Copenhagen, Denmark); and alkalinity, hardness, and DO according to standard analytical procedures [20]. Mean (SE, n) water quality parameters during the experiment were temperature, 24.6°C (0.16, 98); pH, range, 7.69 to 7.76; DO, 6.41 mg/L (0.4, 11); hardness, 372 mg/L as $CaCO_3$ (10.5, 8); alkalinity, 300 mg/L as $CaCO_3$ (9.6, 8).

The fathead minnow were continuously exposed to nominal SCN^- concentrations of 0, 1, 8, 18, or 36 mg/L. Toxicant was delivered to the experimental system by a proportional diluter [21]. The SCN^- stock solutions were prepared with analytical-grade potassium thiocyanate (BDH Chemicals, Toronto, Ontario) and deionized water. Water samples for the determination of SCN^- concentration were taken from each tank at least weekly. The SCN^- levels were assayed by complexation with ferric nitrate and determination of the optical density at a wavelength of 575 nm [20]; the method was verified by HPLC [22]. Mean (SE, $n = 21$) assayed SCN^- concentrations were 0.06 (0.01), 1.1 (0.07), 7.3 (0.37), 16.6 (0.56), and 32.6 (0.54) mg/L. There were no significant differences in concentrations between replicates within treatments. All results are reported in terms of assayed SCN^- concentrations.

Experimental outline

Ten juvenile fathead minnows, ≈50 to 60 d old, were randomized to each of the 15 exposure aquaria, and SCN^- exposure was initiated. The aquaria were set up in a randomized complete block design so that each toxicant concentration appeared once in each block. Survival and reproductive response were monitored for 124 d. Growth was measured by weighing minnows at the beginning and end of the exposure period. With the first appearance of secondary sexual characteristics in any of the treatment groups (22 d), spawning substrates were introduced into each tank. When the sex of individual fish became apparent, populations in each tank were thinned to give a ratio of four females to two males. In an effort to quantify the progression of reproductive effort at different concentrations of SCN^-, a numerical index of reproductive behavior and performance was developed (Table 1). Observations on behavior and reproductive performance were made every three weeks and assigned a number based on the index.

Egg production was monitored daily. If sufficient numbers of eggs (>300) were produced in a tank during the preceding 16 h, they were gently removed from the spawning substrates [23]. The eggs were examined under magnification (10–40×) to determine fertilization rates [24], and all dead or broken eggs were removed. Twenty eggs were counted randomly into each of 15 egg incubation cups; then the cups were randomized across all treatment blocks, so that the rear portion of each tank contained one cup. This split-plot experimental design allowed us to separate indirect (prespawning exposure of adults) effects on egg and larval viability from direct (postspawning exposure of eggs) effects. The in-

Table 1. Numerical index of reproductive behavior and performance used to quantify reproductive effort of fathead minnows during exposure to SCN^-

Stage	Criteria
0	No changes in secondary sexual characterisitics; no eggs
1	Males with stripes and fat pad barely visible, dorsal fin spot not distinct; nipping and chasing of other fish, including females, but no association with spawning substrate; no eggs
2	Males with prominent fat pads, stripes and dorsal fin spot; occasional herding of females towards substrate; no ovipositor visible on females; no eggs
3	Males with prominent fat pad, stripes and dorsal fin spot; continuous herding of females and cleaning of substrate with fat pad; ovipositor not yet prominent on females; no eggs
4	Males with prominent secondary sexual characteristics and egg guarding behavior; females with prominent ovipositor; eggs present

cubation cups were 100-ml polyethylene beakers, the bottoms of which had been removed and replaced with 0.5-mm nylon screening. A styrofoam sleeve cut from a drinking cup was fitted around each incubation cup for flotation. These incubation cups allowed the exchange of oxygen and wastes with the water in the incubation tank, but prevented larvae from escaping. The eggs were incubated to determine eyeup, time to first hatch, hatching success, and larval survival rates to 3-d post-hatch.

Surplus eggs were placed in the back portions of the exposure tanks or in 2-L beakers in a water bath (25°C) and incubated at the SCN^- concentrations at which they were spawned. Random samples of larvae were examined under a dissecting microscope for gross physical deformities. Survival and development of F_1 larvae in the incubation portions of the tanks were monitored weekly for eight weeks to determine qualitatively the relative survival and time to sexual maturity and spawning.

After 124 d of exposure, five adult spawners from each tank were sampled for hematocrit and histopathology, mortalities permitting. The minnows were sacrificed with an overdose of tricaine methane sulfonate (Syndel Laboratories, Vancouver, British Columbia), weighed, and blood samples collected into heparinized hematocrit tubes after caudal peduncle severance. Hematocrits were determined after centrifuging at 7,100 rpm for 5 min. The hematocrit tubes were scored and broken at a point just above the packed white cell layer. Both ends of the tubes were sealed with hematocrit sealant and the plasma frozen at −20°C pending SCN^- analysis.

After blood samples were taken, an incision was made in the wall of the peritoneal cavity of each fish, and they were fixed whole in 10% buffered formalin. Following fixation, the heads were removed, cut sagitally, and placed in histology cassettes. The tissues were then embedded in paraffin, cleared, and stained with hematoxylin and eosin to observe the occurrence and degree of thyroid hyperplasia. Histological observations were confirmed by D. Speare (Department of Pathology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, P.E.I.).

For the remaining adult fish from each treatment, SCN^- exposure was terminated and they were pooled in duplicate tanks to provide a ratio of three females to two males. The fish were provided with spawning substrates and their reproductive response monitored during a 30-d SCN^--free recovery period.

Plasma SCN^- analysis

Due to the small volume of plasma obtained from the hematocrit tubes, samples were pooled within treatments. Two samples were analyzed for each treatment, except for the 32.6-mg/L exposure, from which only a single sample was obtained. Samples were diluted 10-fold with HPLC-grade water (double distilled over potassium permanganate) and centrifuged through C_{18} solid-phase extraction columns (Supelco Canada Inc., Oakville, Ontario). Precipitation of plasma proteins was not required in conjunction with the C_{18} columns. The eluent was injected directly into the HPLC.

Plasma SCN^- was determined by reversed-phase HPLC (Spectra Physics, model SP8100) with a C_{18} column (length 15 cm; internal diameter 4.6 mm; particle size 5 μm; Supelco Canada Inc.) permanently coated with a cetylpyridinium chloride solution (5×10^{-4} M). Chromatography conditions were similar to Barkley et al. [22], as modified by D.G. Brown et al. (manuscript in preparation).

Statistical analysis

Data were subjected to standard ANOVA procedures to determine significant treatment effects. Data normality and heterogeneity of variance were determined by residuals analysis [25]. If these assumptions were violated, $\log_{10}$ or arcsine angular transformations were applied to the data before statistical analysis. Embryo and larval response parameters were analyzed as a split-plot ANOVA with subsampling, with the adult preexposure SCN^- level as the whole plot and embryo exposure concentration as the split plot. In the event of a significant one-way ANOVA, multiple comparisons were conducted using Tukey's (HSD) test ($\alpha = 0.05$) [26].

RESULTS

Exposure to SCN^- reduced the reproductive response of fathead minnows in a concentration-dependent manner, with reproduction decreasing over the range of concentrations in the study (Fig. 1). In general, the NOEC for impacts on reproduction was 1.1 mg SCN^-/L, whereas the LOEC was 7.3 mg SCN^-/L, giving a chronic value (ChV) of 2.8 mg SCN^-/L (Table 2). The LOEC for growth and survival of adults was 32.6 mg SCN^-/L, whereas the LOEC for overt goiter was 7.3 mg SCN^-/L.

Mortality, growth and hematocrit

Mortality of adult fathead minnows increased ($p = 0.001$) with SCN^- exposure (Table 3). Total mortalities of 30 and 63% were observed at 16.6 and 32.6 mg SCN^-/L, respectively. The first mortality at 32.6 mg/L occurred on day 25, but was delayed until day 56 at 16.6 mg/L. Initial body weight (mean 1.3 g) did not differ between tanks ($p = 0.70$, C.V. = 4.1%). Exposure to SCN^- for 124 d depressed final body weights ($p = 0.001$) and hematocrit ($p = 0.002$) of adult fathead minnows (Table 3). Due to the small sample size ($n = 6$) for fish surviving exposure to 32.6 mg SCN^-/L, the final body weight and hematocrit ANOVAs should be interpreted with caution. The feeding response was diminished in fish exposed to 32.6 mg SCN^-/L; food offered at each feeding was not consumed, whereas it was at the other exposure levels.

Pooled plasma SCN^- samples showed an increase in plasma SCN^- with exposure concentration (Table 3). Waterborne SCN^- was concentrated in the plasma, with BCFs of 2.7 and 13.8 at 16.6 and 32.6 mg SCN^-/L, respectively.

Secondary sexual characteristics and spawning behavior

The LOEC for delayed or completely inhibited development of secondary sexual characteristics and normal spawning behavior was 7.3 mg SCN^-/L (Table 2). Fish exposed to 32.6 mg SCN^-/L neither developed secondary sexual characteristics nor exhibited spawning behavior. Those exposed to 16.6 mg SCN^-/L exhibited incomplete development of

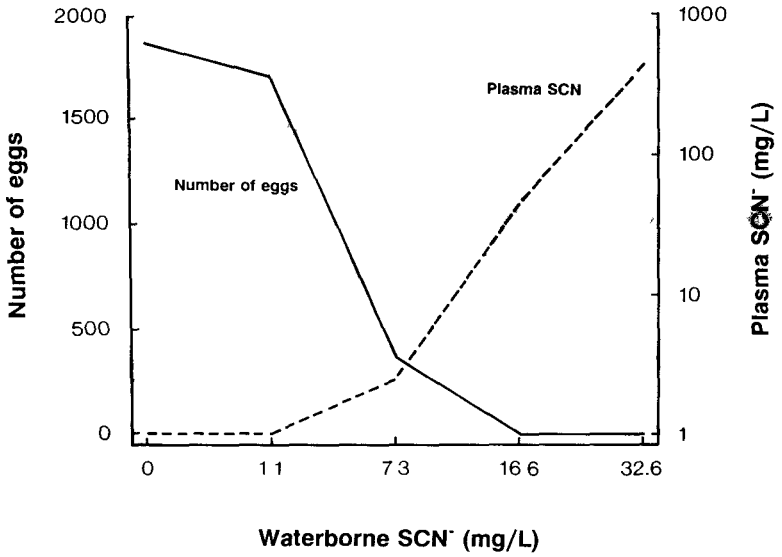

Fig. 1. Generalized reproductive response of fathead minnows exposed to waterborne SCN^- for 124 d. The mean of total egg production per tank over the course of the exposure is used in this figure, although similar responses in relation to plasma SCN^- were found for all reproductive parameters.

secondary sexual characteristics. Mature males developed the dark stripes and dorsal fin spot characteristic of the species, but failed to develop the large fat pad of actively spawning fish. These males were aggressive toward other fish but did not exhibit spawning behavior. Fish exposed to 1.1 and 7.3 mg SCN^-/L developed normal sexual characteristics and followed normal patterns of courtship and spawning, but these processes were delayed in fish exposed to 7.3 mg SCN^-/L. This pattern is reflected in the decreased reproductive index (Table 1) in groups exposed to ≥7.3 mg SCN^-/L ($p = 0.0003$, Table 3).

Reproductive response

Egg production was significantly reduced ($p < 0.0001$) by SCN^- exposure (Table 3). Adult fish exposed to 16.6 or 32.6 mg SCN^-/L failed to spawn successfully. The LOEC for egg production was 7.3 mg SCN^-/L with a NOEC of 1.1 mg SCN^-/L (Table 2). Time to first spawning was delayed by SCN^- exposure ($p = 0.002$), with a LOEC of 7.3 mg/L. Whereas the control and 1.1 mg/L groups spawned within 6 d of the introduction of spawning tiles, groups exposed to 7.3 mg SCN^-/L required an average of 25.7 d. Exposure of adults to SCN^- during spawning had no significant impact on the fertilization rates of eggs spawned by any group ($p = 0.558$, Table 3). No eggs were spawned by fish exposed to 16.6 or 32.6 mg SCN^-/L. The number of larval deformities was not affected by SCN^- exposure level ($p = 0.405$, Table 3), with C- and L-shaped larvae as the predominant types of deformities. Two larvae were observed to have a general edema in the anterior region of the yolk sac.

Response of embryos and larvae

There was no significant effect of the SCN^- concentration at which fertilized eggs were exposed on percentage eyeup ($p = 0.364$), percentage hatch ($p = 0.119$), percentage hatch of eyed eggs ($p = 0.115$), time to completion of hatch ($p = 0.442$), or larval mortalities to 3-d post-hatch ($p = 0.478$) (Table 4). A significant ANOVA interaction between the SCN^- concentration at which the eggs were spawned (0 and 1.1 mg/L) and the postspawning SCN^- incubation concentration was evident for percentage eyeup ($p = 0.009$), percentage hatch ($p = 0.0001$), hatch of eyed eggs ($p = 0.066$), and larval mortalities ($p = 0.040$), but not for time to completion of hatch ($p = 0.954$). The SCN^- level (1.1 mg/L) at which the eggs were spawned increased percentage eyeup ($p = 0.001$), percentage hatch ($p = 0.0001$), and percentage hatch of eyed eggs ($p = 0.001$),

Table 2. Summary of NOEC and LOEC values for growth, reproductive, and physiological parameters for F_0 and F_1 fathead minnows exposed to waterborne thiocyanate (SCN^-)

Parameter	NOEC (mg/L)	LOEC (mg/L)	Chronic value (ChV) (mg/L)
F_0 generation			
Wt. (at 124 d)	16.6	32.6	23.3
Mortality	7.3	16.6	11.0
Hematocrit	16.6	32.6	23.3
Plasma SCN^- (mg/L)	7.3	16.6	11.0
Time to first spawn	1.1	7.3	2.8
Egg production	1.1	7.3	2.8
Spawning	7.3	16.6	11.0
Fertilization	7.3	NA[a]	NA
Overt goiter	1.1	7.3	2.8
Histological thyroid changes	0	1.1	NA
F_1 generation			
Survival at 56 d	7.3	16.6	11.0

[a]Not available, as no eggs were spawned.

Table 3. Growth, physiological, and reproductive responses of adult fathead minnows during 124-d exposure to SCN^-

Parameter	Responses to various SCN^- exposures					SE[a]
	0 mg/L	1.1 mg/L	7.3 mg/L	16.6 mg/L	32.6 mg/L	
Initial wt. (g)	1.42 A	1.32 A	1.31 A	1.34 A	1.28 A	0.129
Final wt. (g)	2.49 A	2.82 A	2.36 A	2.10 AB	1.40 B	0.904
Mortalities (%)	0 A	0 A	3.3 A	30 AB	63 B	13.11
Hematocrit	35.8 A	32 A	29.5 AB	31.9 A	24.0 B	5.671
Plasma SCN^- (mg/L)	ND[b]	ND	0.25[c]	45.5	450	–
BCF	–	–	0.03	2.7	13.8	–
No. eggs produced[d]	1,860 A	1,707 A	369 B	0 C	0 C	346.4
Time to first spawn (d)	5.7 A	2.3 A	25.7 B	–	–	4.726
Fertilization rates (%)	89.4 A	91.1 A	86.1 A	–	–	5.523
Larval deformities (%)	1.76 A	3.76 A	3.14 A	–	–	1.494
Reproductive index	3.87 A	4.0 A	3.23 AB	2.30 BC	1.33 C	0.498

Within rows, treatment means followed by a common letter are not significantly different ($p > 0.05$).
[a]Pooled standard error from ANOVA.
[b]Not detected.
[c]Trace levels detected; assigned value of half the detection limit of 0.5 mg/L for calculation of BCF.
[d]Mean of total egg production per tank over course of exposure.

while decreasing larval mortalities ($p = 0.0001$) and time to completion of hatch ($p = 0.013$).

Reproductive response during recovery period

Adults exposed to SCN^- for 124 d and then transferred to SCN^--free water for 30 d exhibited some recovery of the reproductive response (Table 5). Insufficient numbers of adults were available from the 32-mg SCN^-/L exposure group after the 124-d exposure to conduct a recovery study. Eggs were produced by all groups, but due to the extreme variability in egg production by the group exposed to 16.6 mg SCN^-/L, the ANOVA was not significant ($p = 0.184$). Larval deformities for eggs spawned by all groups during the recovery period were not affected by the SCN^- preexposure level of adults ($p = 0.085$, Table 5). The time to first spawn during the recovery period increased with increasing adult SCN^- preexposure concentration ($p = 0.022$).

Survival and spawning of F_1

F_1 juveniles of controls or adults exposed to 1.1 mg SCN^-/L and reared at the same respective SCN^- concentration survived to sexual maturity and produced viable offspring. Progeny spawned and reared at 7.3 mg SCN^-/L, survived, developed large overt goiters (Fig. 2), and did not spawn. Control eggs incubated, hatched, and reared at 16.6 and 32.6 mg/L showed signs of goiter development at early juvenile stages (three to four weeks), and mortality was complete by eight weeks.

Goiter development and histology

Fathead minnow exposed to 7.3, 16.6, or 32.6 mg SCN^-/L developed visible protrusions from the "throat" area behind the lower jaw (Fig. 2), deformities absent from fish exposed to 1.1 mg SCN^-/L and control fish (Fig. 3). The NOEC and LOEC for overt goiter development were 1.1

Table 4. Eyeup, hatchability, hatch of eyed eggs, larval mortality, and time to hatch of fathead minnow embryos spawned by adult fathead minnows preexposed to 0 or 1.1 mg SCN^-/L and subsequently transferred to 0, 1.1, 7.3, 16.6, or 32.6 mg SCN^-/L for incubation

	Adult SCN^- preexposure (mg/L)	Responses to various larval SCN^- exposures					WP[a]	SP[b]
		0 mg/L	1.1 mg/L	7.3 mg/L	16.6 mg/L	32.6 mg/L		
Eyeup (%)	0	85.9	85.1	82.5	78.9	80.0	1.94	2.76
	1.1	85.6	85.0	84.4	87.8	85.6		
Hatch (%)	0	86.9	82.3	82.5	76.1	76.7	1.31	3.58
	1.1	85.6	83.9	83.9	86.7	83.9		
Eyed eggs to hatch (%)	0	98.3	96.9	97.7	94.3	92.9	1.50	2.04
	1.1	98.7	98.3	98.5	98.9	97.7		
Mortality (3 d post-hatch) (%)	0	1.67	1.67	2.78	2.22	5.0	0.26	2.04
	1.1	2.22	1.67	1.67	1.11	2.22		
Time to hatch (d)	0	7.1	7.3	7.1	7.1	7.2	0.81	0.24
	1.1	6.7	6.8	6.6	6.4	6.6		

[a]Pooled whole-plot SE from ANOVA.
[b]Pooled split-plot SE from ANOVA.

Table 5. Time to first spawn and egg production of adult fathead minnows during 30-d recovery period in SCN^--free water after 124-d exposure

Parameter	Responses to various SCN^- preexposure concns.				
	0.06 mg/L	1.1 mg/L	7.3 mg/L	16.6 mg/L	SE[a]
Time to first spawn (d)	7 A	8.5 A	13 AB	16 B	1.223
Eggs produced (mean/tank)	1,519 A	1,766 A	1,143 A	294 A	557.8
Larval deformities (%)	1.48 A	2.93 A	2.92 A	6.60 A	0.979

Larval deformity rates of successfully hatched offspring are also provided. Within rows, values with a common letter are not significantly different ($p > 0.05$).
[a]Pooled from ANOVA.

and 7.3 mg SCN^-/L, respectively. Subsequent histological examination showed a marked relationship between waterborne SCN^- level and degree of goiter development. Fish exposed to 32.6 mg SCN^-/L exhibited a combination of a diffuse hyperplastic goiter (DHG) and diffuse colloidal goiter (DCG). Few active follicles with little eosinophilic colloid were visible. The areas of DHG were characterized by an apparently low mitotic rate, lack of overt inflammation, and the presence of degenerative and necrotic changes (pyknotic cell nuclei) with a mild macrophage infiltration. Thyroid structure of fish exposed to 16.6 mg SCN^-/L was more organized, with DHG located on the periphery of the tissue and DCG in the central region (Fig. 4). Follicles were filled with eosinophilic colloid with scalloping around the circumference, indicating active metabolism. Thyroid epithelial cell types at the two highest SCN^- exposure levels ranged from high cuboidal to high columnar. Thyroid tissue of fish exposed to 7.3 mg SCN^-/L was characterized by DCG with follicles containing varied amounts of colloid and surrounded by cuboidal epithelium. Less diffuse hyperplasia was evident, with cells more organized into functional glandular units. Large, lobular thyroid follicles with abundant colloid and low cuboidal epithelium were present in fish exposed to 1.1 mg SCN^-/L. Control fish had very few, rounded follicles surrounded by squamous to low cuboidal epithelium, filled with eosinophilic colloid, and with scalloping present around the circumference of the colloid (Fig. 5). The LOEC for histological changes in thyroidal tissue was 1.1 mg SCN^-/L, the lowest tested concentration.

DISCUSSION

The primary effects of chronic SCN^- exposure on fathead minnow reproduction were on the maturation and spawning of late juvenile to adult fish, probably mediated through the antithyroidal activity of SCN^-. Minimal effects on the fertilization, development, and hatchability of embryos, as well as the early survival of larvae, were apparent. For the majority of fish studies with toxic chemicals, the order to sensitivity by life stage (from most to least) is larvae and juveniles > embryos > adults [27-29]. In this study, the ChV for egg production and goiter development in both adult and F_1 minnows (2.8 mg SCN^-/L) was lower than the ChV for either long-term survival (11.0 mg/L) or embryonic and larval mortality (>32.6 mg/L) (Table 2). In other words, SCN^- impacts on sexual maturation and gamete production

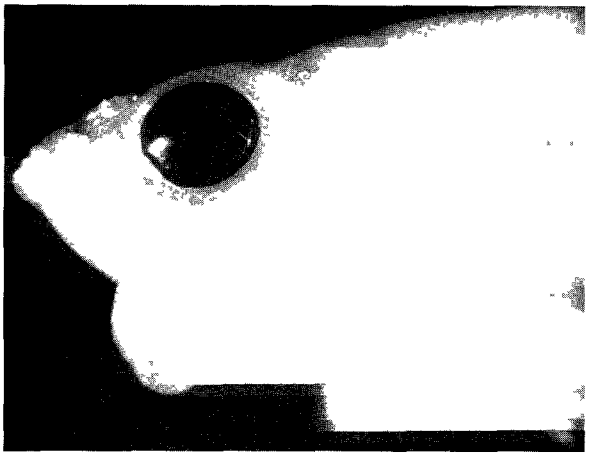

Fig. 2. Overt goiter in a fathead minnow (≈20×) exposed to 7.3 mg SCN^-/L for 124 d. Note the smaller size of the head relative to a control fish (Fig. 3).

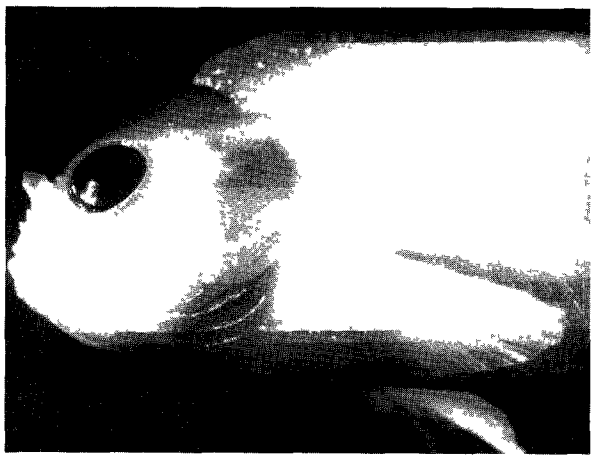

Fig. 3 Control adult male fathead minnow (≈20×) showing the fat pad and coloration typical of mature specimens of the species. Note the absence of overt goiter.

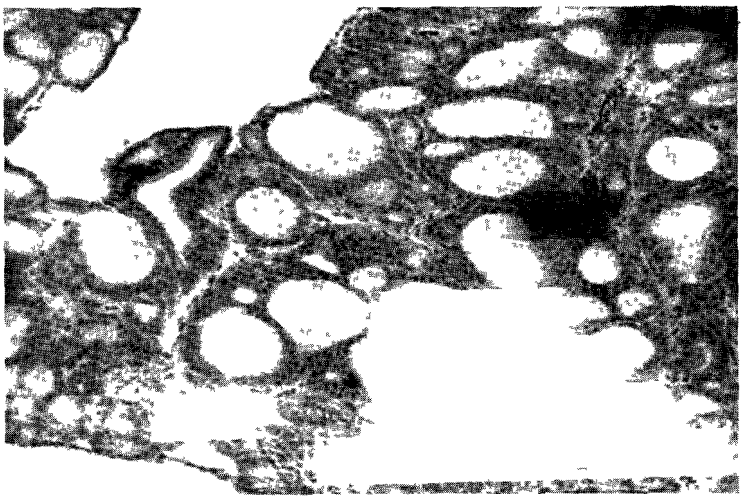

Fig 4 Section (400×) through the thyroidal region of an adult fathead minnow exposed to 16.6 mg SCN^-/L for 124 d. Both diffuse hyperplastic (DHG) and diffuse colloid (DCG) goiter are evident. Relative to the control fish in Figure 5, numerous follicles are surrounded by high-cuboidal to low-columnar epithelium. The follicles contain eosinophilic colloid with clear areas of scalloping around the circumference.

in adult fish occurred at a lower SCN^- concentration than impacts on the survival and development of young.

The most striking morphological effect of SCN^- on both adult and juvenile fathead minnows was the development of overt goitrous nodules in the branchial region. Histological examination confirmed the presence of a combination of diffuse hyperplastic and diffuse colloid goiter that increased in severity with SCN^- concentration and was paralleled by reduced reproductive effort. Previous observations on the antithyroidal effects of SCN^- were derived almost entirely from mammalian research; the presence of an antithyroidal effect of waterborne SCN^- in fish had not been previously verified [8].

Waterborne SCN^- exposure delayed sexual maturation of juvenile fathead minnows and reduced egg production by adults. Because thyroid function has been linked with gonadal activity in teleosts [30,31], chemical interference with normal thyroid function would likely impact on both normal sexual maturation and the spawning process. Antithyroidal agents or thyroidectomy can inhibit the development of secondary sexual characteristics and gonadal maturation in a number of fish species [10–12,32]. SCN^- administered by either waterborne exposure or i.p. injection has been shown to inhibit both halide (Cl^-) movement across gill membranes [13,14] and radioiodide uptake by the thyroid [6,8,33]. Conversely, waterborne halides (Cl^-, Br^-, I^-, F^-) have been shown to inhibit SCN^- uptake from water by rainbow trout (*Oncorhynchus mykiss*) [34]. As reported here, waterborne

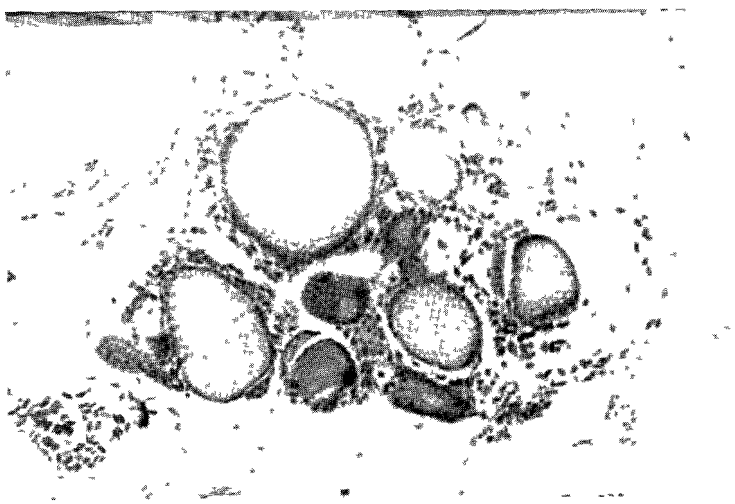

Fig. 5. Section (400×) through the thyroidal region of a control fathead minnow. Only a few thyroid follicles are evident. Those present contain abundant colloid and are surrounded by squamous epithelium

SCN^- exposure leads to increased plasma SCN^- levels, which may result in the competitive inhibition of waterborne and thyroidal I^- uptake. By interfering with I^- uptake and organification, SCN^- may ultimately reduce triiodothyronine (T_3) and thyroxine (T_4) production, which through negative feedback on the pituitary would stimulate the production of thyroid stimulating hormone (TSH). As TSH acts on thyroid follicular cells to increase the rate of I^- incorporation for T_3 and T_4 production, the net result is seen as goiter [35].

Although not thoroughly understood, thyroid-mediated reproductive effects may be due to either indirect or direct gonadal impacts [7]. Indirect antigonadal effects may result from the production of excess TSH at the expense of pituitary gonadotropin (GtH) production. This indirect mechanism for gonadal inhibition was initially based on thyroid and gonad effects on teleosts subjected to thiourea immersion [9]. Singh et al. [7] verified this mechanism by i.p. injection of a number of antithyroids, including SCN^-, in a freshwater catfish (*Heteropneustes fossilis*). The antithyroids increased pituitary TSH production [6] and decreased GtH production. Direct antigonadal effects may result from the reduced uptake of elements, such as phosphorus and I^-, by the gonads. Ovarian accumulation of these elements, essential for the development of eggs and survival of young, has been shown competitively inhibited by plasma SCN^- in both *H. fossilis* and the channel catfish (*Ictalurus punctatus*) [6,7,16].

Most reports on the effects of antithyroids on reproduction have been limited to observations on the development of secondary sexual characteristics and gonadal maturation, with no mention of spawning behavior. The spawning behavior of fathead minnows in this study was qualitatively assessed with a reproductive index (Table 1) and was found to be affected by SCN^- exposure level (Table 2). No courtship behavior [36,37] was observed in groups exposed to 32.6 mg/L. In groups exposed to 16.6 mg/L, males developed secondary sexual characteristics, but exhibited aggression toward all other fish in the tank and courtship behavior did not materialize. In groups where eggs were produced, normal courtship and spawning behavior were observed. Indirect effects of SCN^- on GtH production by the pituitary could possibly interfere with the expression of normal courtship and spawning behaviors.

The LOEC for mortality of adult fathead minnows exposed to waterborne SCN^- for 124 d was 16.6 mg/L. There was no evidence of sudden death syndrome as described by Heming et al. [34]. The LOEC for growth after 124 d was 32.6 mg SCN^-/L. These fish also consumed less feed. It is not clear whether this decrease in growth was due to the specific hypothyroid action of SCN^- or to another more general mode of action. Both thyroidectomy [32,38] and exposure to antithyroidal compounds [39–41] have been shown to reduce growth, and in some cases feed conversion efficiency, in fish.

The LOEC for reduced hematocrit was also 32.6 mg SCN^-/L and may be related directly to the hypothyroid effect of SCN^-. Reduced hematocrit and hemoglobin levels have been reported for both radiothyroidectomized rainbow trout [32] and rainbow trout exposed to 35, 77, or 114 mg SCN^-/L for 16 weeks (R.P. Lanno and D.G. Dixon, manuscript in preparation). The in vivo conversion of SCN^- to CN^- [42] and subsequent depletion of vitamin B_{12} stores [43] by CN^- present an alternative explanation for the reduced hematocrit.

Historically, antithyroids have been administered to fish by either i.p. injection or immersion in a static system at pharmacological, not physiological, doses [8]. Although nominal doses are calculated, quantification of the amount of chemical in the test organism is lacking. The quantification of plasma SCN^- levels in fathead minnows in this study offers a unique situation in which internal SCN^- levels can be related to biological responses. Plasma SCN^- levels were only slightly elevated in fish exposed to 7.3 mg/L. At 16.6 mg/L, SCN^- influx to plasma was substantial (45.5 mg/L), with a BCF of 2.7. Fish exposed to 32.6 mg/L had plasma SCN^- levels of 450 mg/L, with a BCF of 13.8. At this highest exposure level, physiological control over SCN^- uptake and/or excretion appeared to be greatly reduced.

Our results indicate that the SCN^- concentration at which the adults were reared and the eggs were spawned and fertilized (control or 1.1 mg/L) had a statistically significant impact on embryological development, whereas the concentration of subsequent incubation did not (Table 4). Insufficient egg production at 7.3 mg/L did not allow further investigation of this trend with increased levels of waterborne SCN^-. Fertilization rates were not affected by SCN^- when eggs were spawned, suggesting that SCN^- concentrations up to 7.3 mg/L do not impair the fertilization process in the fathead minnow (Table 3). Deformity rates of hatched larvae were not affected by adult preexposure and/or spawning concentration, or by subsequent incubation concentration (Tables 3 and 5). The most common types of deformities were C- and L-shaped larvae, similar to those observed in rainbow trout exposed to SCN^- as fertilized eggs [18] and in white sucker (*Catostomus commersoni*) larvae collected from lakes with elevated levels of copper and zinc [44]. In general, deformity rates at all SCN^- exposures in which eggs were spawned were low and would not be expected to impact on free-living fish populations.

The impact of SCN^- on reproduction appeared to be transient, as illustrated by the production of eggs during the recovery period by adults previously exposed to 7.3 or 16.6 mg SCN^-/L for 124 d. Although exposure to 7.3 mg SCN^-/L significantly decreased egg production during exposure (Table 3), no reduction in egg production was apparent during the recovery period, with all groups of fish spawning (Table 5). Depuration of SCN^- most probably reduced the levels of SCN^- at the site of toxic action below the threshold level required for the inhibition of reproductive activity. If this threshold was assumed to be represented by plasma SCN^- levels (0.25 mg/L) in fish exposed to 7.3 mg SCN^-/L, and given a plasma SCN^- half-life of 2.02 d in rainbow trout (D.G. Brown et al., manuscript in preparation), we estimate that clearance of plasma SCN^- to levels below the threshold would require approximately 14 to 16 d. Our results are supportive of this estimate; during recovery, control fish spawned 8 d after the introduction of substrates, whereas fish previously exposed to 16.6 mg SCN^-/L commenced spawning after 16 d. The transient nature of the re-

productive effect in adult fish exposed to SCN , and the general agreement of the time required to commence spawning and the estimated depuration rate for SCN^- in trout, suggest that the toxic effect of SCN is mediated, at least partially, through some form of competitive inhibition at the site of toxic action No statistical difference in egg production occurred between SCN^- treatment groups during the recovery period, due to the extreme variation in egg production by fish preexposed to 16 6 mg SCN /L (only one replicate actually produced eggs) That only one group of fish preexposed to 16 6 mg SCN /L spawned within the 30 d recovery period suggests some more permanent toxic action of SCN^- present at this exposure level

CONCLUSIONS

The results of this study indicate that the major effects of chronic SCN exposure in fathead minnow are on reproduction and thyroid histology LOEC and NOEC values for all effects are summarized in Table 2 The ChV for egg production, time to first spawn, and goiter development in F_0 and F_1 minnows was 2 8 mg SCN /L The most sensitive indicator of SCN^- exposure was histological changes in thyroidal tissues with a LOEC of 1 1 mg/L Neither long-term juvenile survival (ChV 11 0 mg/L) nor embryonic and larval mortality (ChV > 32 6 mg/L) was as sensitive as reproductive impacts

That increases in plasma SCN^- levels were not evident in fish exposed to 7 3 mg SCN^-/L may be due to uptake of SCN by the thyroid, producing lower levels in the plasma Plasma SCN levels in fish exposed to 16 6 mg/L were 10-fold higher, possibly suggesting saturation of uptake mechanisms in peripheral tissues such as the thyroid and spillover into the plasma The elevated levels of SCN^- in the plasma of fish exposed to SCN^- and the development of goiter suggest that effects on maturation and reproduction are probably mediated through the antithyroidal activity of SCN^-

Acknowledgement—Financial support for this work was provided by the Ontario Ministry of the Environment and Energy (Research Advisory Council Grant 320PL) and the Natural Sciences and Engineering Research Council of Canada (Research Grant A8155) Statistical advice was provided by J O'Hara Hines David Speare provided invaluable interpretation of thyroid histology

REFERENCES

1 **Wolff, J.** 1964 Transport of iodide and other anions in the thyroid gland *Physiol Rev* **44** 45–89
2 **Green, W.L.** 1986 Antithyroid compounds In S H Ingbar and L E Braverman, eds , *The Thyroid A Fundamental and Clinical Text* J B Lippincott, Philadelphia, PA, pp 339–350
3 **Devuyst, E.A., B.R. Conrad** and **V.A Ettel.** 1982 Pilot plant operation of the Inco SO_2/air cyanide removal process *Can Min J* **103** 27–30
4 **Huiatt, J.L., J.E. Kerrigan, F A. Olson** and **G.L. Potter.** 1983 Control and treatment of cyanide mining wastes *Proceedings*, Workshop on Cyanide from Mineral Processing, Salt Lake City, UT, February 2-3, 1982, pp 1–31
5 **Wood, J.L** 1975 Biochemistry In A A Newman, ed , *Chemistry and Biochemistry of Thiocyanic Acid and Its Derivatives* Academic, New York, NY, pp 156–221
6 **Singh, T P.** 1969 Thyroidal radioiodine uptake and thyrotropic potency of the pituitary in a freshwater catfish, *Mystus vittatus* (Bloch), in response to L thyroxin, antithyroid drugs, and heavy doses of ^{131}I *Allg Zellforsch Mikrosk Anat* **94** 172–180
7 **Singh, R., R.B. Raizada** and **T.P Singh.** 1977 Effects of some antithyroid drugs on the pituitary thyroid gonad axis in a freshwater catfish, *Heteropneustes fossilis* (Bloch) *Gen Comp Endocrinol* **31** 451–456
8 **Eales, J G.** and **S. Shostak** 1983 Influence of potassium thiocyanate on thyroid function of rainbow trout, *Salmo gairdneri Gen Comp Endocrinol* **51** 39–43
9 **Grosso, L.L.** 1961 The effect of thiourea, administered by immersion of the maternal organism, on the embryos of *Lebistes reticulatus*, with notes on the adult gonadal changes *Biol Bull Woods Hole* **121** 481–496
10 **Mukherjee, A** 1975 Effects of thiourea treatment on thyroid and ovary of the catfish *Heteropneustes fossilis* (Bloch) *Ind J Exp Biol* **13** 327–332
11 **Canton, J.H., P.W Wester** and **E A M. Mathijssen-Spiekman.** 1983 Study on the toxicity of sodium bromide to different fresh water organisms *Food Chem Toxicol* **21** 369–378
12 **Wester, P W , J.H. Canton** and **J.A M.A. Dormans.** 1988 Pathological effects in freshwater fish *Poecilia reticulata* (guppy) and *Oryzias latipes* (medaka) following methyl bromide and sodium bromide exposure *Aquat Toxicol* **12** 323–344
13 **De Renzis, G.** 1975 The branchial chloride pump in the goldfish *Carassius auratus* Relationship between Cl /HCO_3^- and Cl /Cl^- exchanges and the effect of thiocyanate *J Exp Biol* **63** 587–602
14 **Maetz, J.** 1976 Transport of ions and water across the epithelium of fish gills *Proceedings*, Symposium on Lung Liquids, London, England 1975 CIBA Foundation Series 38 Elsevier, Amsterdam, The Netherlands, pp 133–159
15 **Katz, U., K.R. Lau, M M.P. Ramos** and **J.C Ellory.** 1982 Thiocyanate transport across fish intestine (*Pleuronectes platessa*) *J Membrane Biol* **66** 9–14
16 **Lindsay, R.H., C. Romine, F. Zacharewicz, H.K. Dupree** and **K.E. Sneed.** 1966 Accumulation of ^{131}I by channel catfish (*Ictalurus punctatus*) ovaries in vivo and in vitro *Gen Comp Endocrinol* **6** 231–238
17 **Denny, J.S** 1987 Guidelines for the culture of fathead minnows *Pimephales promelas* for use in toxicity tests EPA 600/3 87 001 U S Environmental Protection Agency, Duluth, MN
18 **Kevan, S.D.** and **D.G. Dixon.** 1991 The acute toxicity of pulse-dosed thiocyanate (as KSCN and NaSCN) to rainbow trout (*Oncorhynchus mykiss*) eggs before and after water hardening *Aquat Toxicol* **19** 113–122
19 **Sprague, J.B.** 1973 The ABCs of pollutant bioassay with fish In J Cairns, Jr and K L Dickson, eds , *Biological Methods for the Assessment of Water Quality* STP 528 American Society for Testing and Materials, Philadelphia, PA, pp 6–30
20 **American Public Health Association, American Water Works Association** and **Water Pollution Control Federation.** 1980 *Standard Methods for the Examination of Water and Wastewater*, 15th ed American Public Health Association, Washington, DC
21 **Mount, D I.** and **W.A. Brungs.** 1967 A simplified dosing apparatus for fish toxicology studies *Water Res* **21** 21–29
22 **Barkley, D J., T.E. Dahms** and **K.N. Villeneuve.** 1987 Permanently coated ion exchangers for liquid chromatographic determination of anionic species in samples from environmental control processes *J Chromatogr* **395** 631–640
23 **Gast, M.H.** and **W.A. Brungs.** 1973 A procedure for separating eggs of the fathead minnow *Prog Fish -Cult* **35** 54
24 **Manner, H.W.** and **C.M. Dewese.** 1974 Early embryology of the fathead minnow *Pimephales promelas* Rafinesque *Anat Rec* **180** 99–109
25 **Montgomery, D.C.** 1984 *Design and Analysis of Experiments* John Wiley & Sons, New York, NY
26 **Sokal, R.** and **F. Rohlf** 1981 *Biometry*, 2nd ed W H Freeman, San Francisco, CA
27 **Eaton, J G., J.M McKim** and **G.W. Holcombe.** 1978 Metal toxicity to embryos and larvae of seven fresh water fish species 1 Cadmium *Bull Environ Contam Toxicol* **19** 95–103

28. **McKim, J.M., J.G. Eaton** and **G.W. Holcombe.** 1978. Metal toxicity to embryos and larvae of eight species of freshwater fish. II. Copper. *Bull. Environ. Contam. Toxicol.* **19**:608–616.
29. **Ward, G.S.** and **P.R. Parrish.** 1980. Evaluation of early life-stage toxicity tests with embryos and juveniles of sheepshead minnows (*Cyprinodon variegatus*). In J.G. Eaton, P.R. Parrish and A.C. Hendricks, eds., *Aquatic Toxicology (Third Conference)*. STP 707. American Society for Testing and Materials, Philadelphia, PA, pp. 243–247.
30. **Eales, J.G.** 1979. Thyroid functions in cyclostomes and fishes. In E.J.W. Barrington, ed., *Hormones and Evolution*, Vol. 1. Academic, New York, NY, pp. 341–436.
31. **Cyr, D.G.** and **J.G. Eales.** 1988. Influence of thyroidal status on ovarian function in rainbow trout, *Salmo gairdneri. J. Exp. Zool.* **248**:81–87.
32. **LaRoche, G., A.N. Woodall, C.L. Johnson** and **J.E. Halver.** 1966. Thyroid function in the rainbow trout (*Salmo gairdneri* R.). 2. Effects of thyroidectomy on the development of young fish. *Gen. Comp. Endocrinol.* **6**:249–266.
33. **Hunn, J.B.** and **P.O. Fromm.** 1964. Uptake, turnover and excretion of ^{131}I by rainbow trout (*Salmo gairdneri*). *Biol. Bull. Woods Hole* **126**:282–290.
34. **Heming, T., R.V. Thurston, E.L. Meyn** and **R.K. Zajdel.** 1985. Acute toxicity of thiocyanate to trout. *Trans. Am. Fish. Soc.* **114**:895–905.
35. **Leduc, G.** 1984. Cyanides in water: Toxicological significance. In L.J. Weber, ed., *Aquatic Toxicology*, Vol. 2. Raven, New York, NY, pp. 153–224.
36. **Pyron, M.** and **T.L. Beitinger.** 1989. Effect of selenium on reproductive behaviour and fry of fathead minnows. *Bull. Environ. Contam. Toxicol.* **42**:609–613.
37. **Cole, K.S.** and **R.J.F. Smith.** 1987. Male courting behaviour in the fathead minnow, *Pimephales promelas. Environ. Biol. Fishes* **18**:235–239.
38. **Gross, W.L., P.O. Fromm** and **E.W. Roelofs.** 1963. Relationship between thyroid and growth in green sunfish *Lepomis cyanellus* (Rafinesque). *Trans. Am. Fish. Soc.* **92**:401–408.
39. **Eales, J.G.** and **R.J. Omeljaniuk.** 1981. Effect of pyrazole on thyroid function in rainbow trout, *Salmo gairdneri. Gen. Comp. Endocrinol.* **45**:279–282.
40. **Pandey, S.** and **J.F. Leatherland.** 1970. Comparison of the effects of methallibure and thiourea on the testis, thyroid, and adenohypophysis of the adult and juvenile guppy, *Poecilia reticulata* Peters. *Can. J. Zool.* **48**:445–450.
41. **Leatherland, J.F.** and **J.W. Hilton.** 1988. Thyroidal compensation in rainbow trout (*Salmo gairdneri*) fed canola meal. *Fish Physiol. Biochem.* **5**:199–207.
42. **Goldstein, F.** and **F. Rieders.** 1953. Conversion of thiocyanate to cyanide by an erythrocytic enzyme. *Am. J. Physiol.* **173**: 287–290.
43. **Smith, A.D.M., S. Duckett** and **A.H. Waters.** 1963. Neuropathological changes in chronic cyanide intoxication. *Nature* **200**: 179–181.
44. **Munkittrick, K.R.** and **D.G. Dixon.** 1989. Effects of natural exposure to copper and zinc on egg size and larval copper tolerance in white sucker (*Catostomus commersoni*). *Ecotoxicol. Environ. Saf.* **18**:15–26.