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# The Effect of Sublethal Cyanide Exposure on Plasma Vitellogenin Levels in Rainbow Trout (*Salmo gairdneri*) During Early Vitellogenesis

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**Abstract.** Female rainbow trout *Salmo gairdneri* were exposed to 0.01 mg $L^{-1}$ hydrogen cyanide (HCN) for 12 days at 12.5 ± 0.5°C at the onset of vitellogenesis (May–June). Plasma vitellogenin levels were measured, utilizing a homologous radioimmunoassay specifically developed for this species. Exposure to HCN reduced vitellogenin levels in the plasma to levels recorded in the control non-vitellogenic group. Similarly, the gonadosomatic index declined steadily throughout the experimental period in vitellogenic females exposed to cyanide while no difference was observed in hepatosomatic indices relative to controls. The high sensitivity of the radioimmunoassay for vitellogenin utilized in this study has permitted a rapid assessment of the effects of low levels of this toxicant on yolk production. The findings indicate that exposure of naturally reproducing female rainbow trout to this low concentration of HCN during early vitellogenesis would eliminate an important source of yolk precursor synthesized in the liver and sequestered by the ovary.

Cyanide was chosen as the toxicant for this study, because of its widespread occurrence in terrestrial and aquatic ecosystems attributable to both natural and man-made sources. Cyanides are considered among the major pollutants of receiving waters (Leduc *et al.* 1982). Its presence at sublethal levels in the aquatic environment affects fish physiology, particularly reproduction by reducing the number of eggs spawned and the viability of eggs (Koenst *et al.* 1977; Lind *et al.* 1977; Kimball *et al.* 1978; Cheng and Ruby 1981). Furthermore, Lesniak and Ruby (1982), working with sexually maturing female rainbow trout during mid-summer (July–August), have indicated that cyanide at both 0.01 and 0.02 mg $L^{-1}$ HCN can have a critical effect on reducing the number of viable eggs by delaying the process of secondary yolk deposition in the ovary.

Vitellogenin, a glycolipophosphoprotein present in the plasma of non-mammalian oviparon female vertebrates during the process of yolk formation, (Follet *et al.* 1968; Wallace and Dumont 1968; Bergink and Wallace 1974; Campbell and Idler 1976) is synthesized in the liver (Plack and Fraser 1970; Sundararaj and Nath 1981) under the stimulation of estrogen (Emmersen and Petersen 1976; Hara and Hirai 1978; Campbell and Idler 1980). It is released into the circulation and subsequently sequestered by the ovary (Campbell andd Jalabert 1979; Ng and Idler 1978a, 1978b). Plasma vitellogenin levels in fish may be used as one of the criteria to assess the state of oocyte development particularly during vitellogenesis. So *et al.* (1985) demonstrated a linear relationship between the concentration of plasma vitellogenin and the development of the gonad expressed as gonadosomatic index (GSI) and the increase corresponds with the appearance of yolk granules in the oocytes of fish (Plack *et al.* 1971; Aida *et al.* 1973). Recently, So *et al* (1985) developed an homologous radioimmunoassay which permitted a highly sensitive detection of plasma vitellogenin in salmonids.

The present study was undertaken to examine the sublethal effects of cyanide exposure on plasma vitellogenin in rainbow trout at the onset of vitellogenesis in May–June. The concentration of cyanide selected was 0.01 mg $L^{-1}$ HCN. It was based on a previous study by Lesniak and Ruby (1982), which indicated that no significant effect on yolk forma-

tion in rainbow trout occurred during early summer below 0.02 mg $L^{-1}$ HCN.

## Materials and Methods

Rainbow trout, *Salmo gairdneri,* were purchased from La Pisciculture Mont Sutton, Sutton, Quebec. Fish weights ranged between 150–300 g ± S.D. 10.4. Prior to the experiment, fish were held in the laboratory for two months at 12.5 ± 1°C and were fed trout chow (Martin's Feed Mills, Elmira, Ontario) at a level of approximately 2% of their body weight/day. Experiments were performed between late May and early June when vitellogenin levels in the plasma begin to rise.

The experimental apparatus consisted of fiberglass tanks equipped with flow meters which regulated the flow of water at 4 L/min and provided a 99% replacement time every 4.5 hr as calculated from Sprague (1973). The experimental apparatus was supplied with dechlorinated City of Montreal water. The mean alkalinity was 83.0 mg $L^{-1}$ $CaCO_3$, total hardness 122.0 mg $L^{-1}$, $CO_2$ content 0.5 mg $L^{-1}$, and pH 7.2 throughout the experimental period. Mariotte bottles (Leduc 1966) were used to deliver the cyanide stock solution into the dilution water, and cyanide levels in the experimental tanks were maintained at 0.01 mg $L^{-1}$ HCN throughout the experimental period. Cyanide levels were monitored daily using the method of Lambert *et al.* (1975). Dissolved oxygen remained above 85% saturation thoughout the experimental period. Fifteen female fish were acclimated in each of the experimental tanks for one month prior to commencement of the experiment. During the experimental period, they were fed at 1% of their body weight but otherwise maintained in conditions identical to those prior to the experiment. Five fish were sampled on days 0, 6, and 12. Blood samples were collected from the caudal blood vessels, centrifuged and frozen in liquid nitrogen and stored at −60°C. Total body weight, liver and ovarian weights were recorded for subsequent calculation of the GSI and hepatosomatic index (HSI). Gonads were examined histologically to confirm the sex. Vitellogenin was measured in individual serum samples using a homologous radioimmunoassay (RIA) system identical to that described for Atlantic salmon (*Salmo salar*) (So *et al.* 1985). The assay was performed on plasma samples using $I^{131}$ radio-iodinated and "cold" purified plasma vitellogenin from rainbow trout as the tracer and standard, respectively. Barbital buffer containing 0.5% bovine serum albumin and 0.01% thimersal was used as the diluent for the assay. Control and cyanide exposed samples along with vitellogenin standards were prepared in serial dilutions and incubated with 200 μL of antibody (at a dilution of 1:250,000) and 200 μL of $I^{131}$ radiolabelled vitellogenin (about 10,000 cpm) at 4°C for three days. Normal rabbit serum (100 μL in 1:80 dilution) and goat anti-rabbit γ-globulin antibody (100 μL in 1:20 dilution) were added 24 hr prior to counting.

The standard curve ranged in concentration from 0.2–208 ng and was reliable for the range from 1 to 100 ng. The log/logit regression lines of the dose-response curve (n = 10) had $R^2$ = 0.98 with a slope of −1.61, an intercept of 1.03, and $B/B_0$ (50%) = 4.37 ng.

Data from vitellogenic and non-vitellogenic control females were tested with a one way anova (Zar 1984, p 163) followed by multiple comparisons by Student-Newman Keuls multiple range test (Zar, 1984, p. 190) to determine any significant differences between plasma vitellogenin levels on days 0, 6, and 12. Control and cyanide-treated vitellogenic females were tested on days 6 and 12, using the student 'T-test' for unequal sample sizes (Sokal and Rohlf 1969, p 220).

**Table 1.** Gonadosomatic and hepatosomatic indices and plasma vitellogenin levels in non-vitellogenic and vitellogenic female rainbow trout Salmo gairdneri (May–June, 1984)[a]

| | Day | | |
|---|---|---|---|
| | 0 | 6 | 12 |
| **Vitellogenic Females** | | | |
| GSI (%) | 0.36 ± 0.10 | 0.41 ± 0.08 | 0.69 ± 0.21 |
| Vg (mg/mL) | 0.32 ± 0.10 | 0.33 ± 0.09 | 0.55 ± 0.17 |
| HSI (%) | 1.15 ± 0.01 | 1.73 ± 0.16 | 1.23 ± 0.12 |
| **Non-Vitellogenic Females** | | | |
| GSI (%) | 0.21 ± 0.02 | 0.21 ± 0.04 | 0.21 ± 0.02 |
| Vg (mg/mL) | 0.012 ± 0.001[b] | 0.015 ± 0.002[b] | 0.013 ± 0.002[b] |
| HSI (%) | 1.20 ± 0.05 | 1.41 ± 0.06 | 1.51 ± 0.06 |

[a] Values represent means ± SEM

[b] Values significantly different from vitellogenic females

## Results

Values for vitellogenin, GSI and HSI in vitellogenic females are presented in Table 1. The results indicate that among vitellogenic females there was a gradual increase in both GSI and plasm vitellogenin levels throughout the experimental period. While these increases were not statistically significant between day 0 and 12, they nevertheless confirm that fish were entering the vitellogenic phase. In non-vitellogenic females, the vitellogenin levels and GSI remained unchanged throughout the experimental period. Plasma vitellogenin levels rose from 0.32 ± 0.10 mg/ml to 0.55 ± 0.17 mg/ml in vitellogenic females. This represented an average increase of 20 μg/ml/day in control fish during the 12 day experiment. In non-vitellogenic females, plasma vitellogenin levels were significantly lower ($p < 0.05$) than in vitellogenic females throughout the experiment (Table 1).

Fish exposed to 0.01 mg $L^{-1}$ HCN demonstrated an initial increase in plasma vitellogenin to 0.40 ± 0.27 mg/ml at day 6 of the experiment, followed by a rapid decline to 0.005 ± 0.002 mg/ml, a level similar to that in non-vitellogenic fish by day 12 (Figure 1). There was a statistically significant difference ($p < 0.05$) between the cyanide exposed and control fish by day 12 of the experiment.

The GSI in females treated with 0.01 mg $L^{-1}$ HCN declined steadily throughout the experimental period. The mean level in these fish was below control levels for non-vitellogenic fish following the twelve day exposure and showed a significant difference ($p < 0.05$) from the vitellogenic control at the end of the experiment (Figure 2).

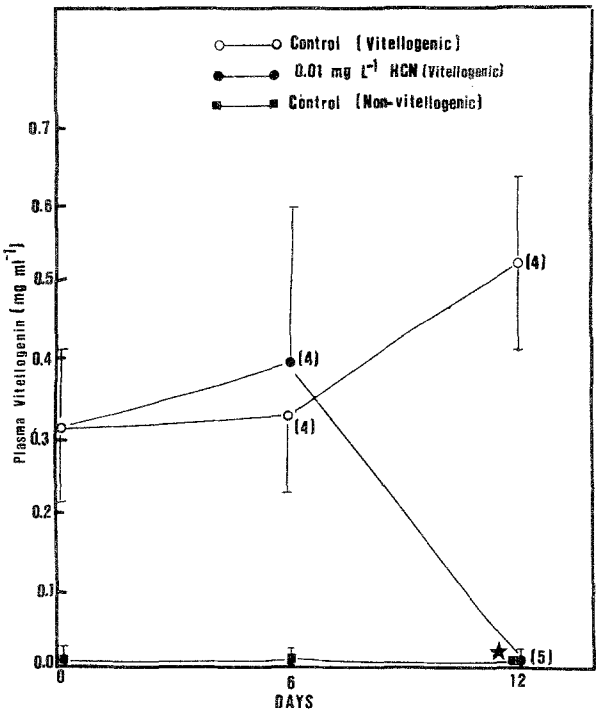


**Fig. 1.** Plasma vitellogenin levels in female rainbow trout *Salmo gairdneri* following exposure to 0.01 mg $L^{-1}$ HCN for 12 days during early vitellogenesis. Data represents $\bar{X} \pm$ SEM; *significantly different from control at $p < 0.05$

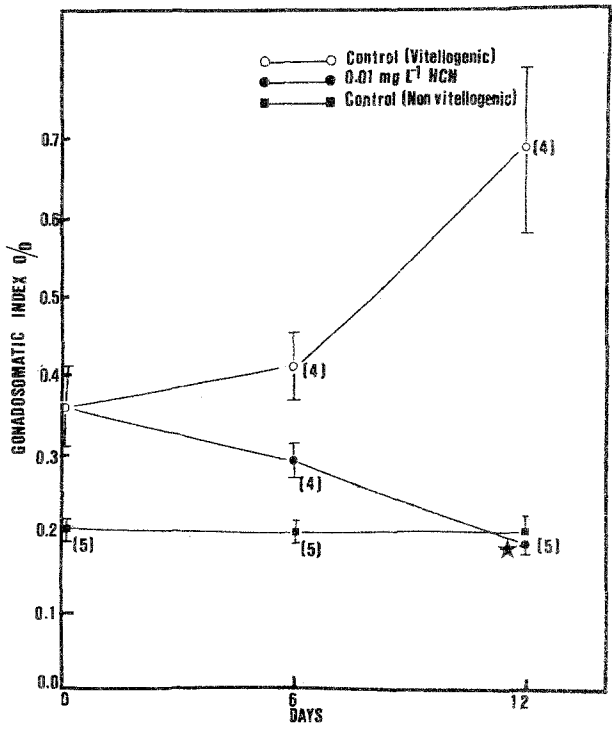


**Fig. 2.** Gonadosomatic Index in female rainbow trout *Salmo gairdneri* following exposure to 0.01 mg $L^{-1}$ HCN for 12 days during early vitellogenesis. Data represents $\bar{X} \pm$ SEM; *significantly different from control at $p < 0.05$

The HSI varied insignificantly in vitellogenic and non-vitellogenic females throughout the experiment. While there was a trend toward lower mean values (1.09 ± 0.05 and 1.04 ± 0.09) in cyanide-exposed fish on day 6 and 12, respectively, there was no statistically significant difference between the cyanide treated fish and both controls.

## Discussion

Exposure of female rainbow trout at the onset of vitellogenesis to 0.01 mg $L^{-1}$ HCN rapidly reduced plasma vitellogenin to levels below that found in non-vitellogenic fish. This implies that production of exogenous yolk precursors by the liver, which acts as an important source of yolk for the ovary, is highly sensitive to low levels of cyanide in the early stages of vitellogenesis. A previous study (Lesniak and Ruby 1982), in which rainbow trout were exposed to this level of cyanide for 20 days during early vitellogenesis (May–June), showed no significant effect upon yolk deposition in the ovary. However, at a higher concentration (0.02 mg/$L^{-1}$ HCN) under the same conditions, yolk deposition was delayed among 93% of the oocytes when compared with controls. The present study, utilizing a highly sensitive homologous radioimmunoassay, has for the first time clearly demonstrated that the primary yolk source for ovarian development outside the ovary is eliminated, following a short twelve day exposure to 0.01 mg $L^{-1}$ HCN.

The synthesis of vitellogenin is directly regulated by estrogen (Tata and Smith 1979) which, in turn, is synthesized in the ovary (Kagawa 1981). Several studies in fish have confirmed the role of estrogen in this process (Plack *et al.* 1971; Emmersen and Petersen 1976; Campbell and Idler 1980; Idler and Campbell 1980). Elliot *et al.* (1979) reported that the estrogen synthetic capacity of the ovary increases gradually in rainbow trout from May during early vitellogenesis to a maximum in October during exogenous vitellogenesis. In the present study, the effect of cyanide on vitellogenesis may be related to a decreased availability of estrogen to the liver at this critical phase when plasma vitellogenin levels are beginning to increase. However, sublethal cyanide may inhibit any of the events which result from the action of estrogen on liver cells (Tata and Smith 1979).

The mechanism whereby sublethal cyanide could lower plasma vitellogenin levels is unclear, although there is some evidence to suggest that observed effects may be related to thiocyanate tox-

icity. Low levels of cyanide are detoxified by conversion to thiocyanate (Leduc 1984). The enzyme rhodenase is involved in this conversion and its presence has been reported in fish liver (Sido and Koj 1972). Raymond (1979) has shown that in rainbow trout at the concentration of cyanide utilized in the present study, a steady bioaccumulation of thiocyanate occurred in the plasma up to 10 to 15 days. Following a 20 day exposure, cyanide-exposed fish contained 3.5 to 5 times the level of thiocyanate as the controls. High levels of thiocyanate also occur in mammals including man (Bourdoux *et al.* 1978) following continuous consumption of low levels of cyanide from food. Elevated thiocyanate inhibits iodide ($I^-$) transport and subsequently lowers thyroxine biosynthesis (Williams 1981). Through a feedback mechanism, TSH production is stimulated. There is some evidence in fish that increased TSH activity accompanies increased levels of thiocyanate. Singh *et al.* (1977) reported injections of thiocyanate in fresh water catfish, *Heteropneustes fossilis* for eight weeks reduced iodide while the pituitary gland showed increased TSH activity. Simultaneously, there was reduced gonadotropic potency and reduced $^{32}p$ uptake at the ovarian level. While gonadotropin functioning is not clearly understood in fish, the dependency of ovarian estrogen on the pituitary has been established (Crim and Idler 1978), utilizing salmon pituitary extract in trout, and by Fostier *et al.* (1979) with carp. It has been suggested (Ng and Idler 1983) that low levels of a maturational carbohydrate-rich gonadotropin initiates vitellogenesis through stimulation of ovarian estrogen.

Serial dilution of rainbow trout plasma from individual vitellogenic females in both control and cyanide treated fish exhibited parallelism to the standard curve for vitellogenin, indicating the high sensitivity of the RIA as a direct measure of circulating vitellogenin in the plasma. Levels of plasma vitellogenin recorded among vitellogenic females in this study which ranged from 0.2–0.8 mg/ml at the onset of endogenous vitellogenesis correlate well with previous studies. So *et al.* (1985) reported that plasma vitellogenin levels in 3-year old female landlocked Atlantic salmon reached its lowest level in March (0.12 ± 0.02 mg/ml) prior to the onset of endogenous vitellogenesis. Van Bohemen and Lambert (1981) reported that in rainbow trout plasma vitellogenin measured by densitometric scanning, following polyacrylamide gel electrophoresis, rose from 0.1 mg/ml in May during endogenous vitellogenesis to 0.4 mg/ml at the beginning of exogenous vitellogenesis in July.

The GSI in control and cyanide-exposed fish demonstrated similar trends to those observed for plasma vitellogenin. GSI values increased in the controls with increasing levels of plasma vitellogenin while the reverse response was observed in the cyanide-exposed fish. GSI values decreased with decreasing levels of plasma vitellogenin. Previous investigators (Idler *et al.* 1981) have shown that a significant correlation exists between the stage of gonadal development and plasma vitellogenin concentration among Atlantic salmon (*Salmo salar*) captured at sea. A similar relationship is present between gonadal development and the gonadosomatic index.

The hepatosomatic indices did not reflect changes observed in plasma vitellogenin. This is probably related to the low levels of liver vitellogenin relative to the total weight of the liver. Histological damage was not observed in liver tissue of females exposed to 0.01 mg $L^{-1}$ HCN in the present study. Dixon and Leduc (1981) demonstrated wide-spread cyanide-induced degenerative necrosis of liver cells at the same concentration; however, the mean weight of rainbow trout utilized in that study was 11 g while in the present study the mean weights ranged between 150–300 g. The larger size of trout in the present study may permit a more rapid detoxification following sublethal cyanide exposure, and subsequently explain the different results observed in the two studies.

In conclusion, the high sensitivity of the radioimmunoassay for vitellogenin utilized in this study has permitted detection of the effect of a low level of cyanide on plasma vitellogenin early in the reproductive cycle. The loss of vitellogenin in the plasma would remove a major source of yolk which is essential for the normal development of eggs during vitellogenesis.

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