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Environmental Contamination and Toxicology

Ovarian Effects of a Sublethal Concentration of Mercuric Chloride in the River Frog, *Rana heckscheri* (Anura: Ranidae)

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The introduction of toxic materials into the environment as a result of industrial processes has caused serious problems to a variety of organisms inhabiting diverse habitats. Previous studies have shown that numerous metals such as cadmium, copper, lead, arsenic and mercury can act as systemic toxicants in man and other species (Goyer 1986). Although many studies have focused on the effects of pesticides and metals on survivorship, growth, metabolism , neural processes and reproduction in a number of different taxa, little information is available on the effects of sublethal concentrations of metals on the reproductive physiology of amphibians in general (Punzo 1983) , and specifically on gonadal tissues (Kanamadi and Saidpur 1991). Mining activities and industrial discharges can release large concentrations of heavy metals such as mercury into aquatic ecosystems (Dustman et al. 1970 ; Goyer 1986). Since most amphibians have obligate aquatic larval stages, they are extremely susceptible to pollutants discharged into the environment (Byrne et al. 1975 ; Punzo et al. 1979 ; Power et al. 1989). Furthermore, amphibians significantly accumulate heavy metals (Hall and Mulhern 1984). What little information is available on the effects of heavy metals on amphibian reproduction is namely limited to embryonic development and early larval stages (Power et al. 1989). Kanamadi and Saidapur (1991) reported on the effects of mercuric chloride on the ovary of the frog, Rana cyanophlyctis which provides the only available data concerning the effects of a heavy metal on adult frog gonadal tissues. Because of the recent concern over worldwide declines in amphibian populations (Berger 1989 ; Baringa 1990) and the susceptibility of amphibians to environmental toxicants (Punzo 1983) , the present study was conducted to further evaluate the effect of a sublethal concentration of mercuric chloride on the ovary of the river frog, Rana heckscheri Wright , an important component of the aquatic fauna of the southeastern United States.

MATERIALS AND METHODS

Rana heckscheri is commonly found associated with the shores of ponds,

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bayous and river swamps (Ashton and Ashton 1988). All animals were collected from the Hillsborough River in Tampa, Hillsborough County, Florida, during 1990, as previously described by Punzo (1991). Adult females weighing 39.6 ± 1.1 g (mean ± SD) and 9.3 ± 0.9 cm in length were used in the present study and held under laboratory conditions for two weeks prior to testing. Animals were kept by south-facing windows and exposed to normal seasonal photoperiod regime. The protocol used in these experiments was similar to that described by Kanamadi and Saidapur (1991). Mercuric chloride was obtained from Sigma Chemical Co. (St. Louis, Missouri). Dechlorinated tap water (DTW) was used as exposure / control water for all experiments. Exposure water was examined at 24-hr intervals using appropriate chemical tests (Hach Chemical Co., St. Louis, MO) and atomic absorption analyses in order to determine water quality, mercuric chloride concentrations and pH. Water quality parameters of exposure water were as follows (± SD of the measurements : pH 7.24 (± 0.18) ; conductivity, 723 (± 31.7) umhos; total hardness, 352.4 (± 16.4) mg / L as $CaCO_3$; total alkalinity, 279.3 (± 22.7) mg / L as $CaCO_3$; nitrate , 0.77 (± 0.11) mg / L ; nitrite, 0.009 (± 0.003) mg / L ; ammonia, 0.34 (± 0.04) mg / L ; calcium, 85.7 (± 5.8) mg / L ; magnesium, 30.1 (± 2.5) mg / L ; and copper, 0.003 (± 0.0001) mg / L . Mercuric chloride concentrations were determined by comparison with aqueous standards using a Perkin - Elmer 5000 atomic absorption spectrophotometer with a fuel-lean air-acetylene flame as described by Bowen (1966).

Toxicity to mercuric chloride was assessed as a 96-hr LC50 (Litchfield and Wilcoxin 1949). Frogs were placed individually in 20 - L aquaria containing various concentrations of mercuric chloride in 2 L of DTW . One-fifth of the LC50 value (0.88 mg / L) was then used as a sublethal concentration to determine the effects of this toxicant on gonadal tissue since mercuric chloride levels of this magnitude have been previously reported from aquatic habitats from selected sites in south-central Florida near landfills where batteries and other electrical components have been discarded (Irwin and Bonds, 1987). Twenty animals were randomly assigned to either a control group (dechlorinated tap water) or an experimental group exposed to 0.88 mg / L of mercuric chloride. Frogs were continuously exposed to test conditions throughout the experiments. Fresh water and mercuric chloride solutions were used daily. Frogs were provided with guppies ad libitum as a food source on alternative days. An initial series of experiments was conducted on animals in the post-breeding phase of their reproductive cycle, during November and December (Ashton and Ashton 1988). These frogs were sacrificed on day 30 and body, ovary and oviduct masses were weighed . Ovaries were fixed in Bouin's fluid (Carolina Biological Supply, Burlington, North Carolina) for analyses of ovarian follicle characteristics. A second series of experiments was conducted on frogs during the normal breeding period (June - July). Similar measurements were taken on these animals on days 30 and 60 .

Oocytes were classified according to Rugh (1962) and Kanamadi and Saidapur (1991) as follows : (1) first growth phase (FGP) ; (2) second growth phase characterized by medium-sized oocytes (MSGP) with small amounts of yolk and no clear, visible distinction between vegetal and animal poles ; (3) large-sized, second growth phase (LSGP) oocytes with vegetal and animal poles clearly differentiated. When viewed under a dissecting microscope, previtellogenic atretic follicles (AF) fixed in Bouin's were transparent and easily distinguished from yolk-containing material which appeared much more opaque and contained widely dispersed, dark granules. The number of various oocyte stages was determined from a sample of ovarian tissue from each frog as described by Saidapur (1989), as well as by changes in the percent mass of oviducts (Table 1). Data were expressed as means $\pm$ SD . Data were analyzed using a Scheffe F - test (Sokal and Rohlf 1981), with $P < 0.05$ regarded as significant.

RESULTS AND DISCUSSION

There was no mortality as a result of mercuric chloride exposure in control and experimntal animals. The LC50 (96 hr) value for mercuric chloride was 4.4 mg / L (95% fiducial limits : 3.22 - 5.37). Animals exposed to 0.88 mg / L of toxicant lost 6.9 $\pm$ 0.4 g of body weight after 30 days , and 11.7 $\pm$ 0.6 g after 60 d . Exposure to mercuric chloride resulted in a significant decrease in ovarian mass under all test conditions (Table 1). This agrees with the results reported from India for R. cyanophlyctis (Kanamadi and Saidapur 1991).

For postbreeding individuals, there was a significant decrease (51 %) in the number of FGP oocytes (Table 1) as well as for the LSGP oocytes (60%) after 30 d . For frogs during the breeding phase, exposure to mercuric chloride for 30 d only affected LSGP oocytes (52 % reduction). Increasing the period of exposure to 60 d resulted in a more severe response in that both LSGP oocytes and previtellogenic atretic follicles (AF) were affected. The AF number increased significantly from a mean of 1.9 to 3.7. This suggests that mercuric chloride can cause a degeneration of the ovarian follicles in adult amphibians. Changes in total oocyte number were similar to the changes observed for the FGP oocytes (Table 1). Oviduct mass decreased significantly after 30 d exposure for frogs in the postbreeding phase but not for those in the breeding period. However, after 60 d there was a significant reduction in oviduct mass for this group, indicating that these oviduct changes are associated with concomitant changes in the number of LSGP oocytes. These results suggest that if the concentration of mercuric chloride used in this study were present in the environment, ova production and maturation in this species would be significantly impaired. Previous studies on the hormonal regulation of follicular development in ranid frogs (see reviews by Bentley 1977 ; Saidapur 1989) have indicated that the recruitment and development of SGP oocytes requires pituitary

Table 1. Effect of exposure to mercuric chloride (0.88 mg / L) on the ovary and oviduct of Rana heckscheri .

Group	Mean Number of oocytes / frog x 10^3					Mass (g / 100 g)	
	FGP	MSGP	LSGP	AF	Total	Ovary	Oviduct
Postbreeding (Nov - Dec)							
30 - Day autopsy							
Control	27.1 (2.1)	1.34 (0.13)	1.97 (0.11)	3.77 (0.12)	34.2 (2.2)	7.1 (0.6)	4.2 (0.5)
Mercuric chloride	13.3 (1.6) $p < 0.05$	1.12 (0.03) NS	0.78 (0.02) $p < 0.05$	3.69 (0.16) NS	18.9 (1.1) $p < 0.05$	4.3 (0.1) $p < 0.05$	3.1 (0.5) $p < 0.05$
Breeding (June - July)							
30 - Day autopsy							
Control	19.2 (2.4)	1.47 (0.6)	4.96 (0.18)	3.11 (0.7)	28.7 (3.9)	11.1 (1.1)	5.3 (0.8)
Mercuric chloride	18.4 (2.7)	1.58 (0.4)	2.43 (0.29)	3.24 (0.5)	25.7 (3.1)	6.9 (0.8)	4.8 (0.7)
	NS	NS	$p < 0.05$	NS	NS	$p < 0.05$	NS
60 - Day autopsy							
Control	19.5 (2.6)	2.2 (0.3)	4.11 (0.4)	1.92 (0.3)	27.7 (3.2)	11.9 (1.8)	5.1 (0.3)
Mercuric Chloride	19.8 (1.9)	2.1 (0.2)	1.96 (0.3)	3.71 (0.4)	27.5 (2.7)	7.1 (0.5)	3.4 (0.4)
	NS	NS	$p < 0.05$	$p < 0.05$	NS	$p < 0.05$	$p < 0.05$

Values expressed as means. Numbers in parentheses represent ± SD . N = 20 surviving frogs for each group . P values calculated using a Scheffe F - test (Sokal and Rohlf, 1981) .

gonadotropins (luteinizing hormone, LH and follicle-stimulating hormone, FSH) as well as somatotrophin (STH) and estradiol. Conversely, the recruitment of FGP oocytes appears to be independent of hormones (Saidapur 1989). The results reported in this study show that mercuric chloride impaired the formation of FGP oocytes as well as the growth and maintenance of SGP oocytes. This suggests that this toxicant may act on the pituitary gland , ovaries , or even the hypothalamus. Mercuric chloride exposure has been shown to impair the estrous cycle as well as the growth of ovarian follicles in mammals by reducing the secretion of pituitary gonadotropins and ovarian steroids (Lamperti and Niewenhuis 1976 ; Clarkson et al. 1983). The precise mechanisms by which mercury acts on amphibian reproduction are not presently known (Power et al. 1989). Mercury has been shown to impair the development of primordial germ cells, cleavage processes, embryonic development and hatching success in amphibians (Byrne et al. 1975 ; Power et al. 1989). Additional information is needed concerning the effects of mercury on gonadal tissues as well as other physiological systems in many more species of amphibians in order to more fully understand the impact of heavy metal toxicants on their overall fitness which may be contributing to the worldwide decline in amphibian populations.

In summary, mercuric chloride can present a very real environmental hazard for the reproductive physiology and overall viability of amphibians, especially in localized areas where items containing mercury have been discarded thereby contaminating groundwaters and adjacent aquatic habitats through runoff. Mercury has the ability to impair the process of fertilization, ova maturation, postembryonic development, normal neurochemical events associated with central nervous system integration as well as neuroendocrine regulation.

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