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Embryotoxic Action of Methyl Mercury on Coho Salmon Embryos

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Environmental levels of heavy metals like mercury have increased significantly over the last 100 years in response to anthropogenic activities (Leonard et al. 1983). More recently the increase in acid precipitation may make the situation worse in that it appears to increase the production of methyl mercury (MeHg) in the surface waters and benthic regions (Winfrey and Rudd, 1988). Concentrations of mercury in surface waters are normally relatively low in the ng/L range, yet levels of mercury in fish tissue in the mg/L range are not uncommon (Eisler, 1987). There have been several well-publicized accounts of acute mercury poisoning from consuming highly contaminated fish (Kudo and Miyahara, 1991). Less is known about the health risks associated with consumption of fish with low levels of mercury contamination (Piotrowski and Inskip, 1981). Human embryos are especially sensitive to MeHg's teratogenic effects. For example, psychomotor retardation is a common outcome of fetal MeHg exposure. Such neural effects may be the result of abnormal migration of neurons during development (Chio, 1991).

A number of different species have been utilized to investigate the effects of mercury on teleost development (Devlin and Mottet, 1991: Sharp and Neff, 1982). Coho salmon, like all teleostean embryos possess mitotic cells that provide a useful model for the study of MeHg teratogenesis. Due to their large size, coho salmon embryos are easily observed and maintained in the laboratory. In addition large numbers of eggs are available from one female thus minimizing effects of variability among individuals. The objective of the present study is to characterize the toxic levels of MeHg and study their uptake. In addition some of the teratogenic effects of MeHg on coho salmon embryos will be described.

MATERIALS AND METHODS

Coho salmon, *Oncorhynchus kisutch* were from a population that return to the University of Washington holding pond to spawn. Eggs and sperm were stripped, then fertilized in the laboratory and placed in egg cups in raceways containing 10 C carbon-filtered Seattle City water. Sperm and eggs were from one male and one female were used in any given experiment to minimize variability. Eggs were allowed to water harden then treated with a fungicide (wescodyne). 50 fertilized eggs were placed in 100-mm square plastic petri dishes containing 25 mL carbon-filtered city water with 0, 6, 13, 29, 62 and 139 ug/L MeHg from a 1 *M* stock solution of methyl mercury hydroxide (Alpha Chemicals). Three replicates of control and MeHg exposed embryos were then placed in a 10 C +0.5 C incubator. Solutions were changed daily and mortalities were recorded and dead embryos were removed.

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Embryos were removed at hatching, dechorionated, fixed in neutral buffered formalin, embedded in paraffin, sectioned at 7 microns and stained with Hematoxylin and eosine for light microscopy. Blastula stage embryos were fixed in Half-strength Karnovsky's with 0.2 *M* Sorenson's buffer (pH 7.2) containing 1% sucrose and postfixed 1% osmium tetroxide solution. Embryos were then embedded in epoxy-resin. Thin sections were cut on a porter-blum MT-2 ultramicrotome, mounted on grids and stained with lead citrate and uranyl acetate. Thin sections were examined with an AEI 801 and a JEOL 100-B transmission electron microscope.

Mercury concentrations in the embryos and stock solutions were determined by cold vapor atomic absorption analysis with an IL551 AA/AE spectrophotometer as follows. The embryos or stock solutions were digested in concentrated nitric-sulfuric acid, then potassium permanganate, potassium persulfate and hydroxylammonium chloride reagents were sequentially added. The sample was then reduced with stannous chloride in hydrochloric acid. Readings were then taken of the total mercury content of the sample. The lethal levels (LC50's) and their confidence limits were calculated with an interactive computer program used by the U.S. Environmental Protection Agency (U.S. EPA, 1988).

Effects of MeHg on the cell cycle were determined using flow cytometric analysis. 28-day-old embryos were dechorionated in cold buffer, the yolk sac was removed, and the complete embryo was placed in 0.27 ml DAPI (4',6-diamidino-2-phenylindol), a fluorescent DNA stain, and drawn into a 1.0 mL syringe. Cells were mechanically dissociated by passing them back and forth through a 26.5 gauge needle 5 times. Samples of individual embryos were then placed in a tube with 10% DMSO and frozen until they were read. Embryonic cells were then injected into a Phywe ICP-22 flow cytometer and the DNA content of the injected cells was recorded (Rabinovitch, 1983). The percentage of cells in each of the phases of the cell cycle was determined from the flow cytometer data. Comparisons were then made between the control and MeHg exposed cells.

RESULTS AND DISCUSSION

In this study the embryonic development of the coho salmon at 10 C took from 40 to 48 days. They have large eggs (8.0 mm + 0.5 mm, 0.19 g + 0.01 g) that are available in large numbers from a single female. A series of three 48-d prehatching LC50s of MeHg on coho salmon were run. The 48-d LC50 estimates generated ranged from 54 to 71 ug/L in three different females (see Table 1).

Table 1. Results of three independent experiments and the estimates of pre-hatching (48-d) LC50 values of MeHg for coho salmon embryos. The tests utilized 50 embryos in the control and each of the treatment groups. Temperature was maintained at 10 C + 0.5 C throughout the tests. Each test utilized gametes from one male and one female.

Test Number	LC50 (ug/L)	Std. Error (ug/L)	95% C.I. (ug/L)
Coho Salmon I	54.1	2.8	48.9-60.3
Coho Salmon II	64.7	3.4	58.4-72.3
Coho Salmon III	70.8	5.5	61.2-84.0

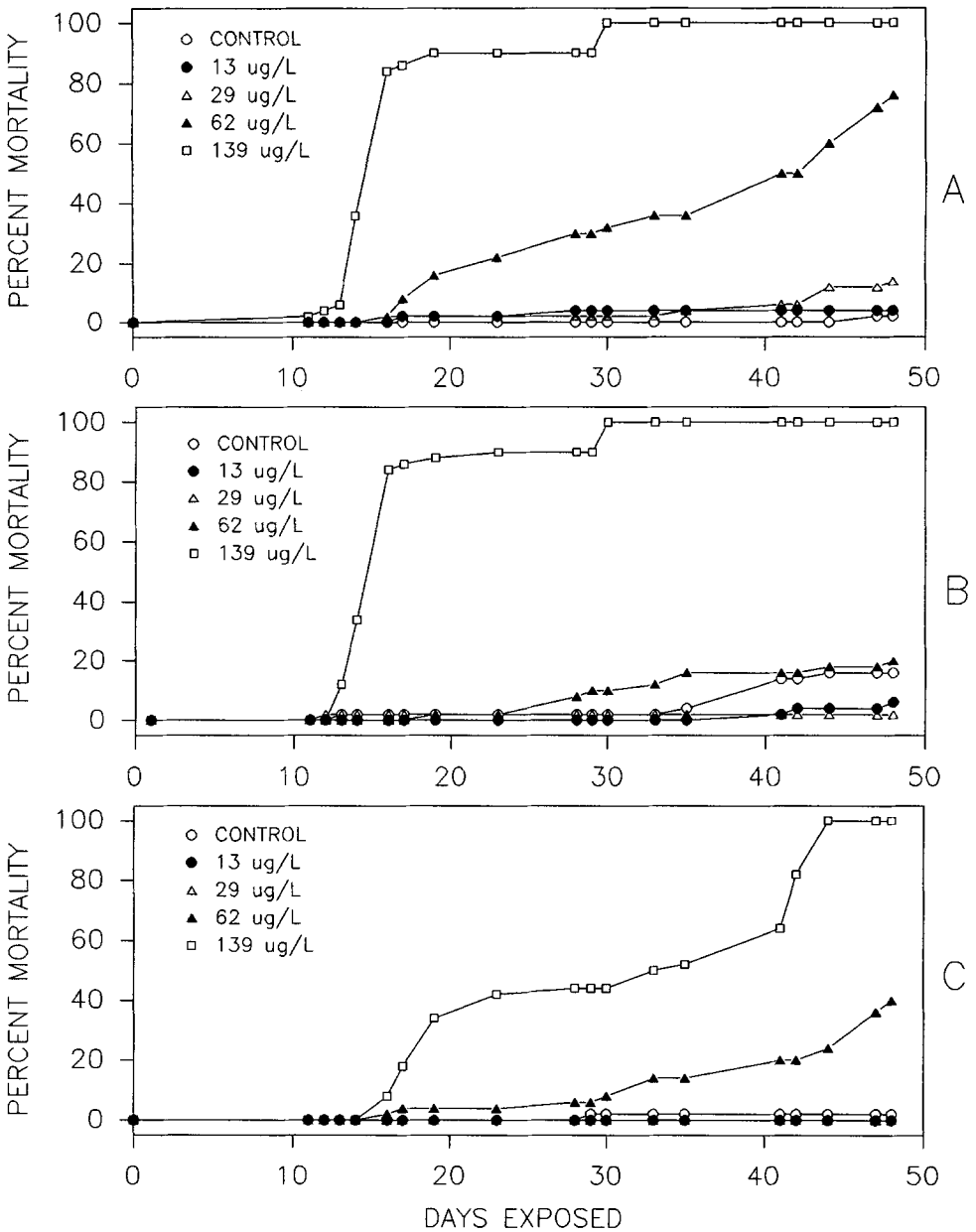

Figure 1. This figure illustrates the mortality curves for the three 48-d LC50 tests. Little mortality was seen in the early stages of development until about day 12. Graph A and B show a straight line in the highest concentration which represents complete mortality at about day 30. Graph C does not achieve complete mortality until about day 48. LC50 estimate for this graph was 65 ug/L.

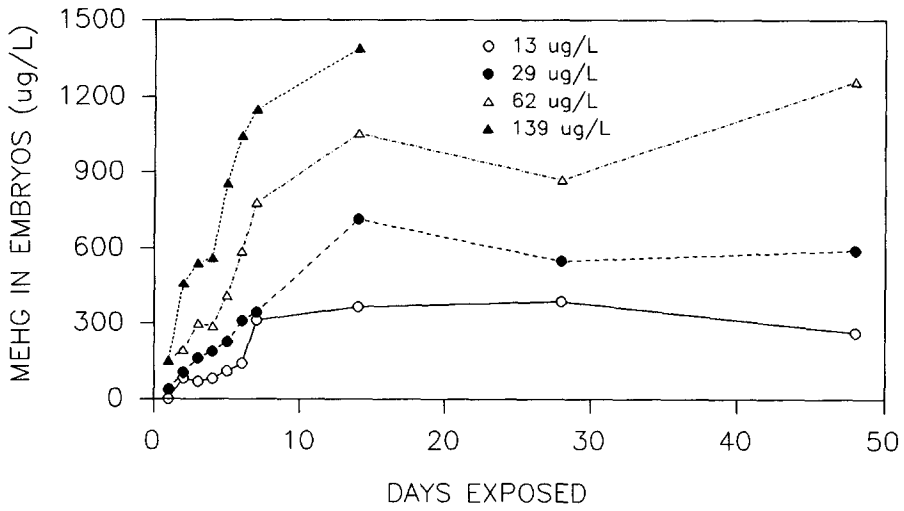

Figure 2. This figure illustrates the accumulation of MeHg in coho salmon. Embryos were continuously exposed to solutions containing 13, 29, 62, and 139 ug/L MeHg. The line representing 139 ug/L exposure stops at 12 d due to complete mortality of the embryos at this concentrations. Note that the concentrations of mercury in the embryos is up to 20X greater than the exposure solution concentration.

The mortality curves indicate that the early stages of development (blastula, gastrula) are relatively insensitive to MeHg toxicity and little mortality was found. A fairly constant rate of mortality was seen at the higher MeHg concentrations from about day 12 onward (see Figure 1). These LC50 estimates are in the range reported for other fish embryos: 67 ug/L in the killifish, *Fundulus heteroclitus* (Sharp and Neff, 1982) and 55 ug/L for the Japanese medaka, *Oryzias latipes* (Sakaizumi, 1980).

The lethal exposure concentrations of MeHg to which the coho embryos are exposed does not reflect the mercury levels within the embryo. We have shown that coho salmon embryos rapidly take up mercury from their exposure solution. This process occurs within hours following initial exposure and continues throughout the embryonic period as long as samples were taken.

Mercury concentrations were found in the embryo that were up to 20 times greater than the mercury level present in the exposure solution (see Figure 2). Other workers have found even greater rates of uptake of inorganic forms of mercury by teleostean embryos (Heisinger and Green 1975). The effect of mercury on embryonic cells was examined using flow cytometry. 28-d old control and embryos exposed to 62 ug/L MeHg continuously following fertilization were dissociated into cells and prepared for flow cytometric analysis.

In a series of experiments the number of cells in each of the cell cycle phases was compared for both the control and MeHg exposed cells. It appears as if the MeHg exposed embryonic cells are piling up in the G1 phase of the cell cycle and passing more quickly through the S phase.

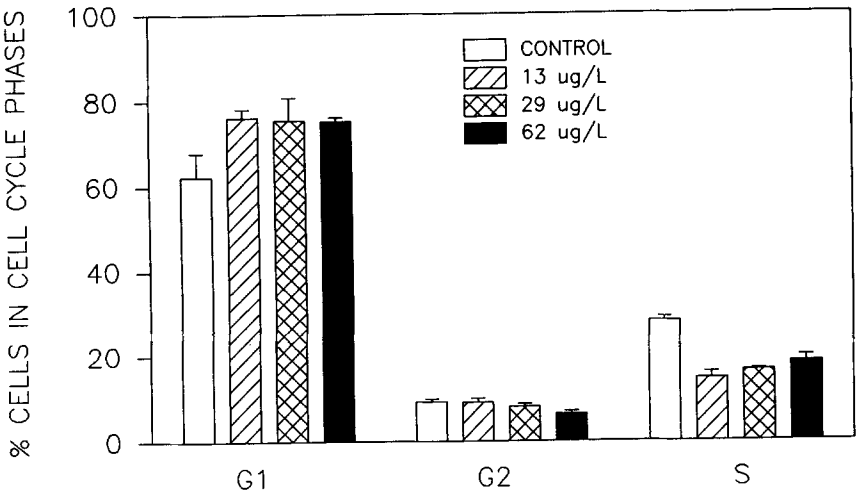

Figure 3. This graph summarizes the results of a series of experiments in which the S, G1, and G2 phases of the cell cycle for 5 treated and 5 control rainbow trout embryos are compared for both MeHg treated and control embryonic cells using flow cytometric analysis. Whole 28-d old embryos were mechanically dissociated and cells were stained with fluorescent stain. It appears as if the MeHg exposed embryonic cells are piling up in the G1 phase of the cell cycle and passing more quickly through the S phase.

Light microscopic analysis consisted of analyzing serial sections of control and MeHg exposed (29 ug/L) 40-d old embryos. A number of anomalies were noted in the MeHg embryos including; highly vacuolated enlarged cells of the notochord and edema of the skeletal muscle. No other organs were grossly affected. This was somewhat unexpected. Other authors have found necrosis of the liver (Bano and Hasan, 1990) and abnormal eye development (Dial, 1978) in other species of mercury exposed fish. A number of macroscopic gross anomalies were also noted including; an enlarged pericardial coelom, stunted growth, and abnormal flexures of the embryonic axis. These anomalies have also been noted by the authors in rainbow trout embryos (Devlin and Mottet, 1991).

Blastula stage embryos were examined ultrastructurally. No consistent trends were found to differentiate the control from the MeHg exposed (62 ug/L) embryos. This was also somewhat unexpected. Brovyagian et al.(1989) found swelling of cilia, dilation of the RER and nuclear membrane along with increased numbers of lysosomes in cells of the bleak fish in response to mercury exposure. A possible mechanism of action of mercury involves the depletion of ATP due to the inhibition of oxidative phosphorylation which may involve depolarization of the mitochondrial membranes (Nieminen et al, 1990). Hence we anticipated some mitochondrial effects. The mitochondria of our MeHg exposed blastula stage embryos appeared normal. The authors think that the early blastula stage embryos were too undifferentiated to demonstrate any clear trends in subcellular pathology. It should be noted that higher MeHg exposure levels (>100 ug/L) major necrosis of the blastomeres occurred along with the breakdown of the cellular membranes.

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