Arch. Environ. Contam. Toxicol. 12, 415—419 (1983)

Archives of Environmental Contamination and Toxicology

Joint Toxicity of Mercury and Selenium in Salmonid Eggs

J. F. Klaverkamp, W. A. Macdonald, W. R. Lillie, and A. Lutz

Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba, Canada R3T 2N6

Abstract. Toxic interactions between mercury and selenium in fertilized eggs of rainbow trout (*Salmo gairdneri*) and lake trout (*Salvelinus namaycush*) were investigated and compared to the pronounced synergistic effect previously reported in carp (*Cyprinus carpio*) eggs. Mercury produced concentration-dependent decreases in median survival times and caused decreases in median hatch times. Selenium, at concentrations up to 10 mg Se/L, had no effect on hatching times, and did not produce mortality, and had no effect on mercury toxicity. At concentrations of 100 mg Se/L and higher, an apparent protective effect of selenium on mercury toxicity was observed in lake trout eggs. Requirements for additional research are discussed with emphasis on predictions of region-wide damage to freshwater fish populations due to atmospheric deposition of these chemical elements.

Mercury and selenium are discharged to the atmosphere in massive amounts by industrial activities, such as fossil fuel combustion and metal smelting (Klein *et al.* 1975; Wheatley 1979). Global anthropogenic emissions of mercury arising from these activities (Harriss and Hoehenemser 1978; Airey 1982) have been estimated to be 1×10^7 kg per year (National Academy of Sciences 1978). Selenium emissions from coal combustion in the United States alone have been estimated to be 5×10^5 kg per year (Vokal-Borek 1979). Release of these elements to the atmosphere and subsequent deposition to the aquatic environment is resulting in gradual increases in contamination of freshwater ecosystems (Lantzy and Mackenzie 1979; National Academy of Sciences 1981).

While biological interactions between mercury and selenium generally result in decreased joint toxicity (Klaverkamp *et al.* 1983), a rare example of toxicity enhancement (synergism) has been reported in fertilized carp (*Cyprinus carpio*) eggs (Huckabee and Griffith 1974). These investigators suggested that this synergistic effect and increased deposition from the atmosphere could result in damaged fish populations on a region-wide basis. To confirm the possibility of this result, joint toxicity studies of these elements on fertilized eggs of other fish species are required. The purpose of this study is to evaluate toxic interactions of mercury and selenium on fertilized eggs of rainbow trout (*Salmo gairdneri*) and lake trout (*Salvelinus namaycush*).

Materials and Methods

Three experiments were conducted to determine toxic interactions of mercuric chloride (analytical grade, Fisher Chemical) and sodium selenite (A. K. Petro Chemical Ltd., Downsview, Ontario, analyzed at 102.7% Se and 98.0% Na) on fertilized eggs from rainbow trout in experiments 1 and 2 and from lake trout in experiment 3. Exposures occurred under static conditions in polyethylene containers described in schematic manner in Figure 1. Up to 40 exposure containers were supported in a temperature controlled water bath. Individual eggs in 1 cm × 1 cm chambers were supported by nylon mesh screening in an acrylic grid system of about eighty chambers (Figure 1—right panel) per container. Water aeration and circulation through the screen were continuously provided by an air lift located at the center of the chamber grid. Experimental designs and conditions for the three experiments are described in Table 1. Criteria for death were formation of white protein coagulate in chorionated embryos and immobility with cessation of heart rate in fry.

Water samples were analyzed for selenium content by atomic absorption spectrophotometry using techniques described in Klaverkamp *et al.* (1983). For mercury analyses, samples were collected in 250 ml Pyrex bottles which had been heated in a muffle furnace at 450°C overnight. Samples were preserved with addition of 3 ml conc HNO_3 and 1 ml 5% potassium dichromate. Portions were digested with permanganate and potassium persulfate (Kopp *et al.* 1972) and analyzed by flameless atomic absorption spectrometry (Armstrong and Uthe 1971). Total ammonia concentrations in water samples from control (un-treated)

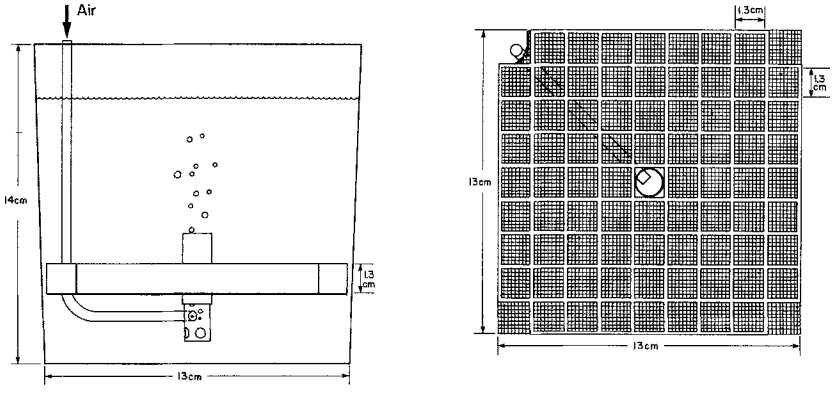

Fig. 1. A schematic representation of exposure container used in fish egg experiments. Left panel presents side view and right panel presents top view

Table 1. Experimental designs and conditions for investigations on toxic interactions of selenium and mercury on fertilized trout eggs

	Experiment 1	2	3
Design	6^2 factorial; nominal Hg and Se concentrations of 0, 0.1, 0.3, 1.0, 3.0 and 10 mg element/L	4^2 factorial; nominal Hg and Se concentrations of 0, 0.1, 0.3 and 1.0 mg element/L	4^2 factorial set with nominal Hg and Se concentrations of 0, 0.01, 0.1, 1.0 and 10 mg element/L and a 2^2 factorial set with 0 and 20 mg Hg/L and with 10, 20, 50, 100 and 200 mg Se/L
Fish species	Rainbow trout	Rainbow trout	Lake trout
Stage of egg development	Late-eyed	Late-eyed	Early-eyed
No. of eggs container	25	25	20
No. of containers/ concentration	1 (4 extra controls)	2	1
Temperature (°C)	13 −16	8.5 −10.0	9.9−10.2
pH	6.10− 6.55 (n = 39)	7.79− 7.89 (n = 39)	—
Water source	L239, E.L.A.	U.V. dechlorinated Winnipeg city tap water	U.V. dechlorinated Winnipeg city tap water
Frequency of water changes	Every 48 hr	Every 48 hr	Not changed
Duration of exposure	120.5 hr	184 hr	576 hr
Observation intervals	Ranged from 2 to 24-hr intervals	Every 8 hr	Every 12 hr

containers at 360 and 576 hr of exposure were obtained by the methods described by Stainton *et al.* (1974). Median survival times (MST) and median hatch times (MHT) were estimated by graphical interpolation on probit log plots of cumulative percent mortality agianst exposure time, except as noted in Results and Discussion.

Results and Discussion

Analytical chemistry data for water-borne mercury and selenium in the trout egg experiments are presented in Table 2. Mercury concentrations were extremely variable and generally much lower than expected. Selenium concentrations were generally less variable and nearer to those expected. In experiment 3, total ammonia concentrations were 410 μg N/L at 360 hr and 1250 μg N/L at 576 hr of exposure. These concentrations would provide un-ionized ammonia concentrations of 6 and 18 μg N/L, respectively, at 10°C and pH 7.9 (Trussel 1972). These are acceptable levels according to Stephan (1975).

Table 3 presents values for MHT (left panel) and MST (right panel) for late-eyed stage eggs of rainbow trout exposed to mercury and selenium (Experiment 1, Table 1) in water from Lake 239 in the Ex-

Table 2. Chemical analyses of water-borne mercury and selenium in experiments on toxic interactions in fertilized trout eggs

Experiment 1[a]			Experiment 2[a] Mercury Concentrations (mg/L)			Experiment 3[b]		
Expected	Actual	% Expected	Expected	Actual	% Expected	Expected	Actual	% Expected
			0	0.0038	—			
0.1	0.013	13	0	0.0003	—	0.01	0.0052	52
0.1	0.020	20	0.1	0.0094	9	0.1	0.014	14
0.3	0.028	9	0.1	0.050	50	1.0	0.059	6
1.0	0.39	39	0.3	0.120	43	10.0	5.64	56
3.0	1.94	65	0.3	0.360	40	10.0	5.98	60
3.0	1.36	45	0.3	0.710	120	20.0	14.6	73
10.0	4.15	42	1.0	0.710	71			
10.0	4.02	40	1.0	0.460	46			
			1.0	0.850	85			
			Selenium Concentrations (mg/L)					
Expected	Actual	% Expected	Expected	Actual	% Expected	Expected	Actual	% Expected
0.1	0.1	100	0	<0.005	—	0.01	0.005	—
0.3	0.33	110	0	<0.005	—	0.1	0.11	110
0.3	0.33	110	0.1	0.086	86	1.0	0.96	96
0.3	0.27	90	0.1	0.011	110	10.0	10.0	100
1.0	1.0	100	0.3	0.30	100	20.0	20.0	100
1.0	0.55	55	0.3	0.34	113	50.0	53.0	106
10.0	8.1	81	1.0	1.05	105	100.0	110.0	110
10.0	7.6	76	1.0	1.05	105	200.0	200.0	100

[a] Water samples were taken at the end of 48 hr exposure periods
[b] Water samples were taken at the end of 24 hr exposure periods

Table 3. Median hatch times and median survival times of fertilized rainbow trout eggs in late-eyed stage of development exposed to combinations of mercury and selenium. Experimental design and conditions are described under Experiment 1 in Table 1

Median hatch time (hr)	Mercury concentration (mg Hg/L)						*Median survival time* (hr)	Mercury concentration (mg Hg/L)					
Selenium Concentration (mg Se/L)	0	0.1	0.3	1.0	3.0	10.0		0	0.1	0.3	1.0	3.0	10.0
0	77	71	45	51	N.H.[a]	N.H.	0	N.M.[a]	N.M.	115	107	68	12
0.1	82	76	40	32	N.H.	N.H.	0.1	N.M.	N.M.	115	95	111	14
0.3	76	72	45	45	76	N.H.	0.3	N.M.	N.M.	96	107	84	13
1.0	76	76	44	61	N.H.	N.H.	1.0	N.M.	N.M.	96	98	78	17
3.0	73	75	55	56	100	N.H.	3.0	N.M.	N.M.	128	110	96	19
10.0	71	72	67	61	N.H.	N.H.	10.0	N.M.	N.M.	175*	133*	107	26

[a] N.H. = no hatch N.M. = no mortality * by extrapolation beyond experiment duration

perimental Lakes Area (Klaverkamp *et al.* 1983.). The control (no added Hg or Se) was replicated four additional times. The percent coefficient of variation in MHT of eggs in these 5 controls was less than 8%, indicating good reproducibility. At mercury concentrations of 0.3 mg Hg/L and 1.0 mg Hg/L (Table 3—left panel), there were decreases in MHT to approximately 58% and 66%, respectively of control values. Most of the eggs did not hatch in 3 mg Hg/L and 10 mg Hg/L. Mercury at concentrations ranging from 0.3 mg Hg/L to 10 mg Hg/L, produced a concentration dependent decrease in MST (Table 3—right panel). At the lower mercury concentrations of 0.3 mg Hg/L and 1.0 mg Hg/L, mortality occurred after the eggs hatched. Selenium, at concentrations up to 10 mg Se/L, did not affect the mercury-induced inability to hatch, the decreases in MHT and the decreases in MST. Selenium alone had no effect on hatching time, and did not produce mortality.

Table 4. Median hatch times and median survival times of fertilized rainbow trout eggs in late-eyed stage of development exposed to combinations of mercury and selenium. Values are the means of duplicate exposures. Experimental design and conditions are described under Experiment 2 in Table 1

Selenium Concentration (mg Se/L)	Median hatch time (hr) Mercury concentration (mg Hg/L)				Median survival time (hr) Mercury concentration (mg Hg/L)				
	0	0.1	0.3	1.0		0	0.1	0.3	1.0
0	175	150	102	>178	0	N.M.[a]	N.M.	97	102
0.1	175	151	101	>178	0.1	N.M.	N.M.	111	121
0.3	173	144	92	>178	0.3	N.M.	N.M.	111	119
1.0	171	152	115	>102	1.0	N.M.	N.M.	115	109

[a] N.M. = no mortality

In a second experiment (Experiment 2; Table 1), a different batch of rainbow trout eggs was exposed to the lower concentration range of mercury and selenium combinations used in Experiment 1. This was conducted in duplicate, using dechlorinated Winnipeg city tap water (approximate hardness as Ca and Mg = 90 mg $CaCO_3$/L), with other experimental conditions similar to the first experiment (Table 1). Table 4 presents MHT and MST calculations obtained from Experiment 2. Mercury alone produced a decrease in MHT at lower concentrations and mortality at higher concentrations. Selenium did not produce mortality and did not affect the mercury-induced decrease in MHT or mortality. While both of the mercury-induced effects were observed at approximately one-half an order of magnitude lower mercury concentrations than in experiment 1, the trends in these observations generally confirm those of Experiment 1.

A third experiment was conducted with fertilized lake trout eggs (Experiment 3; Table 1). High concentrations (1,10 and 20 mg Hg/L) of mercury produced mortality, but lower concentrations (0.01 and 0.1 mg Hg/L) did not decrease MHT as they did with rainbow trout eggs. Selenium at concentrations up to 50 mg Se/L had no effects on mercury-induced mortality, but at concentrations of 100 and 200 mg Se/L, selenite delayed mercury-induced (20 mg Hg/L) mortality by factors of 2.1 and 2.5 , respectively. Selenium at 200 mg Se/L decreased MHT.

The marked synergistic effect between mercury and selenium described for carp eggs (Huckabee and Griffith 1974) was not observed in this study on eggs from rainbow trout and lake trout. Mercury toxicity to these eggs was evident as decreased median hatch and median survival times at approximately the same concentrations as in carp eggs, however, selenium did not influence mercury toxicity with the exceptions of concentrations of 100 mg Se/L or higher in lake trout. At these concentrations, an apparent protective effect of selenium on mercury toxicity was observed. There were major differences in experimental procedures between the investigation on carp eggs and this study. For example, investigations on carp eggs were conducted over the entire embryological period of development, at temperatures which were higher by 10 to 17°C, and on eggs which were fertilized in a saline solution. It is interesting to note that preliminary studies in our laboratory suggest that selenium but not mercury, accumulation in lake trout eggs depends upon the ionic strength of the solution surrounding the eggs. Biological factors, such as species differences and permeability differences in primary envelope or vitelline membrane, may also be responsible for the different observations between carp and these salmonids. Additional research on processes of mercury and selenium transport and toxicology is required to understand these differences. Knowledge of these processes is necessary for making predictions on region-wide damage to fish populations from atmospheric deposition of mercury and selenium of freshwater ecosystems.

Acknowledgments. We express sincere appreciation and gratitude to Robert Corbett, Helve Hobe, and Judy Klaverkamp for technical assistance and Laurie Taite for preparing the graphic illustrations.

References

Airey, D.: Contributions from coal and industrial materials to mercury in air, rainwater, and snow. Sci. Total Environ. **25**, 19 (1982).

Armstrong, F. A. J., and J. F. Uthe: Semi-automated determi-

nation of mercury in animal tissue. Atom. Absorpt. Newsletter **10,** 101 (1971).

Harriss, R. C., and C. Hohenemser: Mercury: Measuring and managing the risk. Environment **20,** 25 (1978).

Huckabee, J. W., and N. A. Griffith: Toxicity of mercury and selenium to the eggs of carp (*Cyprinus carpio*). Trans. Amer. Fish. Soc. **103,** 822 (1974).

Klaverkamp, J. F., D. A. Hodgins, and A. Lutz: Selenite toxicity and mercury-selenium interactions in juvenile fish. Arch. Environ. Contam. Toxicol. **12,** 405 (1983).

Klein, D. H., A. W. Andren, and N. E. Bolton: Trace element discharges from coal combustion for power production. Water, Air, and Soil Pollut. **5,** 71 (1975).

Kopp, J. F., M. C. Longbottom, and L. B. Lobring: "Cold Vapor" method for determining mercury. J. Amer. Water Wks. Assn. January, p. 20 (1972).

Lantzy, R. J., and F. T. Mackenzie: Atmospheric trace metals: Global cycles and assessment of man's impact. Geochim. Cosmochim. Acta **43,**511 (1979).

National Academy of Sciences: An assessment of mercury in the environment. Panel on mercury (F. M. D'Itri, chairman). Nat. Acad. Sci: Washington DC (1978).

———Atmosphere-biosphere interactions: Toward a better understanding of the ecological consequences of fossil fuel combustion. (D. W. Schindler, chairman). National Academy Press: Washington DC (1981).

Stainton, M. P., M. J. Capel, and F. A. J. Armstrong: The chemical analyses of freshwater. Fish. Res. Bd. Can. Spec. Publ. 25. Freshwater Institute, Winnipeg, Manitoba, Canada. (1974).

Stephan, C. E.: Methods for acute toxicity tests with fish macroinvertebrates and amphibians. Ecological Research Series. EPA-660/3-75-009. Corvallis, OR. (1975).

Trussel, R. P.: The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperatures. J. Fish. Res. Bd. Can. **29,** 1505 (1972).

Vokal-Borek, H.: Selenium. USIP Report 79-16. University of Stockholm, Institute of Physics. Vanadisragen 9, S-113 46 Stockholm, Sweden. (1979).

Wheatley, B.: Methylmercury in Canada. Health and Welfare Canada, Medical Services Branch. Ottawa, Canada (1979).

Received for publication June 12, 1982 and in revised form January 21, 1983.