Toxicity of Metal Mixtures to Chick Embryos

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Considering the number and diverse sources of metallic contaminants entering the environment, assessments of biological impact necessarily must include considerations of the combined toxicological effects of metal mixtures. For example, mercury and selenium are but two of numerous metals which occur in fossil fuels, and which are released simultaneously to the environment during fuel utilization. Selenium has been reported at concentrations of 2.20-2.86 ppm in coal and up to 9.35 ppm in the fly ash originating from coal-fired power plants (TALMI AND ANDREN 1974). Mercury concentrations in U.S. coal, though highly variable, have been reported up to 33 ppm, and certain crude oils contain as much as 21 ppm (WALLACE et al. 1971, SANDHOLM et al. 1973). HUCKABEE AND GRIFFITH (1974) recently have demonstrated marked synergism in the effects of mercury/selenium mixtures on embryonic development in the carp. Mercury and selenium are known to concentrate in eggs of avian species to levels which reduce or preclude hatchability (FRANKE et al. 1936, TEJNING 1967, FIMREITE 1971), and the chick embryo is sensitive to a wide range of metallic toxicants (RIDGWAY AND KARNOFSKY 1952, BIRGE AND JUST 1974). The purpose of this study is to examine the toxic effects of mercury/selenium and certain other metal mixtures on the chick embryo, to determine whether antagonistic, additive or synergistic interactions occur.

MATERIALS AND METHODS

White Plymouth Rock chicken eggs were treated by yolk injection with cadmium chloride, mercuric chloride, zinc chloride and sodium selenate. Individual metals and metal mixtures were administered in 0.1 ml aliquots of deionized, distilled water at concentrations calculated to dilute egg yolk to test levels of 1 ppb (1 ng/gm) to 10 ppm (10 μg/gm). Average yolk weight was 18.41 ±0.10 gm, based on measurements of 200 eggs. Test aliquots were injected prior to incubation using the needle track procedure of BIRGE AND JUST (1974). Eggs were then maintained under incubation through hatching, at a temperature of 38° C and a relative humidity of 60-65%. Using a sample size of 200, percent survival was determined as hatchability of experimental eggs/controls. Control eggs were injected with 0.1 ml blanks of distilled water. Hatchability of control eggs

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 3 © 1976 by Springer-Verlag New York Inc.

averaged 83%. Concentrations of test solutions, based on metal content of the above salts, were confirmed analytically using a model 503 Perkin-Elmer AAS unit equipped with a graphite furnace. Mercury was analyzed by the cold vapor method, and flameless procedures were used for the other metals (PERKIN-ELMER 1973). Metal mixtures used included mercury/cadmium, mercury/selenium, mercury/zinc, cadmium/selenium, and cadmium/zinc. Initially, all mixtures were prepared using metals in equal proportions. A limited number of tests were conducted using additional mixing ratios as shown in Table 3.

RESULTS AND DISCUSSION

Results obtained with 1:1 metal mixtures are given in Tables 1 and 2. Hatchability frequencies for individual metals were averaged to give theoretical additive values for the combinations of metals used. These are given parenthetically for comparative purposes. Except for mercury/selenium, all other metal mixtures gave actual values that were within 5% of those for additive toxic effects. This agreement holds for the full range of test concentrations, including the TL_{50} values, and is especially close for zinc/mercury and selenium/cadmium. Actual hatchability frequencies for test concentrations of mercury/selenium are 10-13% lower than the predicted additive values, indicating a moderate degree of synergism.

Using a concentration of 0.05 ppm, which falls midway between the individual TL_{50} values for mercury and selenium, these metals were combined at mixing ratios ranging from 3:1 to 1:3 (Table 3). Compared to the calculated additive values, synergistic responses of similar magnitude occurred for all mixing ratios. Also, changing the mixing ratios did not alter the additive effects of mercury/cadmium mixtures (Table 3).

The results indicate that the strong mercury/selenium synergism which affects embryonic development in the carp does not apply for the chick embryo. In addition, we may conclude that most two-way combinations of cadmium, mercury, selenium and zinc exert purely additive effects on chick hatchability, at least when the toxicants are administered just prior to incubation and survival is tabulated immediately posthatching. Also, these metal mixtures give no discernible antagonistic interactions which affect survival of chick embryos. However, antagonism between zinc and cadmium has been reported in certain studies on mammalian teratogenesis (O'DELL 1968, FERM et al. 1969). The additive and sometimes moderately synergistic effects of metal mixtures on chick hatchability should be considered in evaluating the impact of metallic contaminants upon avian reproduction.

TABLE 1

TOXICITY OF METAL MIXTURES (1:1) TO CHICK EMBRYOS

CONCENTRATION[1] (ppm)	PERCENT SURVIVAL[2,3]					
	Cd	Cd/Hg	Hg	Hg/Se	Se	Se/Cd
0.001	66	73 (72)	78	60 (71)	64	63 (65)
0.01	55	61 (64)	73	45 (58)	43	51 (49)
0.05	48	59 (56)	64	39 (51)	38	41 (43)
0.10	41	49 (51)	61	36 (46)	30	33 (36)
0.50	25	36 (41)	56	30 (40)	24	24 (25)
1.00	8	25 (30)	51	26	0	2 (4)
5.00	0	0	37	0	0	0
10.00	0	0	8	0	0	0

[1]Concentrations based on metal content of cadmium chloride, mercuric chloride, and sodium selenate. Mixtures were prepared using metals in equal proportions.

[2]Theoretical values for additive effects given in parentheses.

[3]Survival was defined as completion of the hatching process.

TABLE 2

TOXICITY OF ZN/CD & ZN/HG MIXTURES TO CHICK EMBRYOS

CONCENTRATION[2] (ppm)	PERCENT SURVIVAL[1] Cd	Cd/Zn	Zn	Zn/Hg	Hg
0.001	66	78 (75)	83	80 (81)	78
0.01	55	61 (65)	75	73 (74)	73
0.05	48	60 (59)	69	66 (67)	64
0.10	41	55 (53)	64	61 (63)	61
0.50	25	38 (42)	58	54 (57)	56
1.00	8	24 (29)	49	52 (50)	51
5.00	0	0	35	35 (36)	37
10.00	0	0	9	8 (9)	8

[1]Theoretical values for additive effects given in parentheses.

[2]Concentrations based on metal content of cadmium chloride, zinc chloride, and mercuric chloride. Mixtures were prepared using metals in equal proportions.

TABLE 3

TOXICITY OF HG/CD & HG/SE MIXTURES TO CHICK EMBRYOS

CONCENTRATION[2] (ppm)	PERCENT SURVIVAL[1]				
	Hg	Hg/Cd 3:1	Hg/Cd 1:1	Hg/Cd 1:3	Cd
0.10	61	55(56)	49(51)	47(46)	41
	Hg	Hg/Se 3:1	Hg/Se 1:1	Hg/Se 1:3	Se
0.05	64	47(58)	39(51)	32(45)	38

[1]Theoretical values for additive effects given in parentheses.

[2]Test concentrations and mixing ratios based on metal content of mercuric chloride, cadmium chloride and sodium selenate.

ACKNOWLEDGEMENTS

Research support was obtained from the U.S. Department of the Interior (agreement no. 14-31-001-4088) and the National Science Foundation (grant no. G.I.-43623).

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