Bull. Environ. Contam. Toxicol. (1999) 63:590-597

Bulletin of Environmental Contamination and Toxicology

Selenium and Other Trace Metals in Pelicans Dying at the Salton Sea

G. Bruehler, A. de Peyster

Graduate School of Public Health, San Diego State University, San Diego, CA 92182-4162, USA

Received: 17 March 1999/Accepted: 30 July I999

The Salton Sea is the largest inland body of water west of the Rocky Mountains and is an important wildlife habitat. Originally created in 1905 when the Colorado River overflowed its man-made irrigation canal and flooded the dry alkaline basin of the Imperial valley, the Salton Sea is now replenished largely by runoff from surrounding mountains and agricultural areas. The only outflow is by evaporation, which concentrates salts and environmental contaminants in the Salton Sea. Selenium and other trace inorganics, DDT and other pesticides have been found at concentrations that may threaten fish and other wildlife populations (Setmire et al. 1990; Setmire et al. 1993). Phosphatic fertilizers commonly used in this region can contain cadmium, nickel, zinc, iron, calcium, vanadium, strontium and uranium (Koranda et al. 1979). Higher concentrations of nickel, chromium, and zinc than are typically found in soils of the Western United States have been detected in sediments of the Whitewater River, another source of water replenishment for the Salton Sea (Setmire et al. 1990).

Major disease outbreaks in fish and birds at the Salton Sea have focused attention on the degraded status of that ecosystem. In 1996, a severe botulism (Type C) outbreak killed over 15,000 pelicans and other fish-eating birds. This was the largest pelican die-off ever reported in the U.S., killing 15% of the population of western white pelicans *(Pelecanus erythrorhyncos)* and as many as 1,400 endangered brown pelicans *(Pelecanus occidentalis).* Although their fish diet was implicated as the probable source of the disease agent, type C botulism had not been associated previously with exposure to fish or caused such high mortality in fish-eating birds. Questions were raised about whether immune suppression might have contributed to the pelican die-offs in this case (U.S. Fish and Wildlife Service, 1997). Elevated tissue levels of selenium and other metals may suppress avian immune system responses to diseases such as avian botulism (Bobker 1993). Chronic contaminant exposure can result in increased metabolic rates thus requiring more energy for maintenance and leaving less available for disease suppression (Peterle 1991). Selenium and other trace metals were the focus of our study. Specific objectives were to determine whether metal levels were different in brown and white pelicans from the Salton Sea compared to concentrations in healthy pelicans from another site not known to have high levels of contaminants, and to relate tissue concentrations to levels known to have immunotoxic and other adverse health effects.

Correspondence to: G. Bruehler, Disney's Animal Kingdom Conservation Station, Post Office Box 10,000, Lake Buena Vista, FL 32830, USA

MATERIALS AND METHODS

With the assistance of U.S. Fish and Wildlife Service personnel, we collected liver and kidney samples from brown pelicans (n=9) and white pelicans (n=10) during the 1996 die-off at the Salton Sea National Wildlife Refuge. These tissues were from terminally ill birds to be euthanized and incinerated to prevent further spread of the disease. Additional liver and kidney samples were collected soon after from healthy brown pelicans (n=4) at Sea World of California, San Diego. These birds had been euthanized by Sea World veterinarians because of broken wings but were otherwise considered free of disease. All samples were collected using acid washed instruments and containers and were kept frozen at -20°C until analysis.

Tissue samples were dried and then digested with 70% HNO_3, using 45-mL microwave digestion bombs (Parr Microwave Acid Digestion Bomb, Model 4782; Parr Instruments Co., Moline, IL 61265). Selenium, cadmium, chromium, and lead concentrations were determined by graphite furnace atomic absorption spectrometry (AA) using a Perkin Elmer SIMAA 6000 transverse heated graphite atomizer equipped with Zeeman background correction (Perkin Elmer Corporation, Norwalk, CT). Furnace programs were optimized following recommendations from the manufacturer. All of these analyses were performed in duplicate. Copper, zinc and iron concentrations were determined using conventional flame AA (Perkin-Elmer 2380) equipped with an impact bead and deuterium arc background correction. Copper, zinc, and iron concentrations reported here were averages of five readings. Analytical accuracy was evaluated using certified reference standards. Recovery of bovine liver standard reference material (SRM 1577b, National Institute of Standards and Technology) was 116% for selenium, 81% for cadmium, 112% for zinc, 98.5% for copper, and 116% for iron. No lead was recovered from the reference material. Chromium was measured but expected values were not given for this material. Tissue spike recoveries were also determined for metals analyzed by graphite furnace AA. Average spike recoveries were 103% for selenium, 104% for cadmium 104%, 95% for lead, and 100% for chromium. Residues reported are actual measurements not corrected for recovery. Results are presented on a dry-weight basis with moisture content noted to allow conversion to wet weight.

Data were analyzed by SPSS (Version 6.1, 1994, Chicago, IL). Nonparametric tests were used because of the relatively small group sizes. Significant differences between groups were determined using Mann-Whitney U tests with Bonferroni corrections for multiple comparisons. Two-tailed p-values ≤ 0.05 were considered significant.

RESULTS AND DISCUSSION

Table 1 shows detection frequencies and geometric means for each metal by group. Mean concentrations of selenium in liver tissue were significantly higher in both brown ($p=0.009$) and white pelicans ($p=0.005$) from the Salton Sea compared with

Table 1. Metals Detected in Pelican Tissues from the Salton Sea and Sea World (ug/g dry weight)[1]

		Salton Sea Brown Pelicans			Salton Sea White Pelicans			Sea World Brown Pelicans (controls)		
Metal	Tissue	Detection Frequency	Mean[2]	Range	Detection Frequency	Mean	Range	Detection Frequency	Mean	Range
Cd	liver	9/9	0.58	0.27-1.62	7/10	0.05*	<.00075-1.1	4/4	0.59	0.4-1.5
	kidney	9/9	2.3	0.96-7.22	10/10	0.9	0.3-4.8	4/4	3.6	2.7-4.8
Cr	liver	1/9	-	<0.0025-0.478	1/10	-	<0.0025-0.392	2/4	-	<0.0025-0.323
Cu	liver	9/9	18.6	8.5-34.4	10/10	16.5	6.1-42.6	4/4	15.1	9.1-38.8
	kidney	9/9	9.2	6.7-11.3	10/10	7.2	5.3-10.4	4/4	7.2	6.2-9.5
Fe	liver	9/9	2,513*	942-4,927	10/10	2,804*	1,270-6,778	4/4	7,342	5,570-13,189
Pb	liver	0/9	-	<0.0125	1/10	-	<.0125-0.2	0/4	-	<0.0125
Se	liver	9/9	16.9*	13.0-34.3	10/10	19.3*	13.9-48.7	4/4	9.3	4.4-13.1
	kidney	9/9	18.8	13.8-27.2	10/10	15.1	10.0-24.6	4/4	13.9	6.5-28.7
Zn	liver	9/9	148*	71-294	10/10	176*	135-320	4/4	238	139-422
	kidney	9/9	86	30-156	10/10	61	47-80	4/4	86	67-110

[1] Average moisture content was 72.8% for liver and 75.7% for kidney.
[2] Geometric mean. No mean was calculated if more than half of the samples were below the detection limit.
* Significantly different from Sea World pelican control tissue concentration ($p<0.05$).

pelicans from Sea World of California (SWC). Geometric mean concentrations in white and brown pelicans from the Salton Sea were approximately twice that of pelicans from SWC. Kidney selenium concentrations were also higher in both brown and white pelicans from the Salton Sea but these differences were not statistically significant.

Dietary selenium is necessary for maintaining normal immune function, but excessive exposure to selenium as selenomethionine (2.2 mg/L selenium) in drinking water suppresses the immune system of mallards (Fairbrother and Fowles 1990). Selenomethionine-treated birds displayed significantly impaired delayed-type hypersensitivity responses to tuberculin *(Mycobacterium bovis).* Significantly increased serum alanine aminotransferase activity in these birds also suggested toxicity to the liver, which produces immunoglobulins. Selenium concentrations in liver tissue from the selenomethionine-treated group in that study were approximately 16 ug/g dry weight. Eleven of the 19 birds sampled from the Salton Sea in our study had liver selenium concentrations at or above this value, which suggests that immune function could have been altered in these birds, and that could have contributed to their deaths.

Geometric mean liver cadmium concentration in Salton Sea brown pelicans (0.58 ug/g) was nearly identical to that of Sea World pelicans (0.59 ug/g). White pelicans from the Salton Sea averaged 0.05 ug cadmium/g liver and had significantly lower liver cadmium concentrations than both groups of brown pelicans sampled. Kidney cadmium concentrations were higher than liver concentrations in all groups studied, kidney concentrations typically being four- to eight-fold the liver concentrations (0.25-7.22 ug/g). Cadmium has the potential to accumulate at the Salton Sea, which is replenished by agricultural runoff containing phosphatic fertilizers often containing cadmium. Fumess (1996) suggests that about 40 ug/g wet weight (132 ug/g dry weight) in the liver or 100 ug/g wet weight (400 ug/g dry weight) in the kidney should be considered tentative threshold tissue concentrations above which cadmium poisoning in birds might be expected. Although no studies we found allow direct comparison of measured tissue concentrations with cadmium levels that specifically alter immune function in birds, tissue levels in all pelicans in our study were several orders of magnitude below threshold tissue concentrations producing other toxic effects in birds.

Lead was detected in the liver of only one white pelican from the Salton Sea with a tissue concentration of 0.2 ug/g. Lead has been found to accumulate in waterfowl wintering in the Imperial valley near the Salton Sea (Koranda et al. 1979); however, waterfowl are usually exposed to lead in the form of spent lead shot from hunting. This type of exposure would typically not occur in pelicans because they feed on fish in the water column and do not contact mud and sediment as waterfowl do while feeding. Lead is one of the most well documented metals in terms of known immunotoxic effects (Burns et al. 1996), but our results indicate that lead was not

found in these affected pelicans from the Salton Sea and probably did not contribute to the die-off

Chromium was detected in four pelicans in this study but with no greater frequency in Salton Sea pelicans. One brown and one white pelican from the Salton Sea had liver concentrations of 0.478 ug/g and 0.392 ug/g, respectively. Two Sea World pelicans had concentrations of 0.001 ug/g and 0.323 ug/g. Chromium levels detected in this study are also within the typical range found in other seabirds (Thompson 1990).

Zinc and copper are micronutrients and their uptake depends on the biological demands of the organism. Both zinc and copper concentrations reported in this study are within the range reported for other seabirds (Thompson 1990). Additionally, they are within the range reported for cormorants and waterfowl at the Salton Sea in 1986-1987 (Setmire et al. 1990), neither of which were reported to be experiencing ill effects. On the other hand, liver zinc was statistically lower in the affected Salton Sea pelicans compared with healthy pelicans from SWC ($p<0.05$). Copper levels were similar.

Like zinc and copper, iron is also an essential element, yet it may also accumulate to toxic levels. Little information could be found on iron levels in seabirds. Iron is considered unlikely to accumulate from the environment and is thought to be less of a potential threat for seabirds than other metals (Thompson, 1990). Mean liver iron concentrations were significantly lower in both brown ($p=0.003$) and white pelicans ($p=0.014$) from the Salton Sea than in Sea World pelicans. Geometric mean liver iron concentrations in pelicans from the Salton Sea were approximately one-third that of Sea World pelicans. These lower liver iron stores could also possibly be related to liver damage caused by the botulinum toxin. Although the mechanism is not clear, dark swollen livers and kidneys have been reported in necropsy findings of chickens with type C botulism (Roberts and Collings 1973). Liver cirrhosis and acute hepatitis also cause release of iron from the liver. Lower liver iron concentrations in the Salton Sea pelicans could also possibly be related to absorption problems or increased utilization associated with the botulism intoxication or other bacterial infection. The concentrations reported here for the pelicans from the Salton Sea are, however, within the range reported for cormorants and other waterbirds from the Salton Sea in 1986-1987 (Setmire et al. 1990).

This study provides new information on trace metals in moribund pelicans sampled at the Salton Sea during the 1996 die-off. Findings indicate that selenium was higher in brown and white pelicans at the Salton Sea. Tissue selenium concentrations were comparable to levels found to be immunotoxic in at least one other bird species. While this indicates that selenium exposure could be a factor in susceptibility to pathogenic organisms, it is not clear that this immune suppression would influence the susceptibility to avian botulism toxin. Unfortunately, functional measurements of immune status were unable to be conducted due to the animals

previously being euthanized. Additionally, susceptibility to selenium toxicity varies among species, and we found no information on selenium levels considered to be immunotoxic in pelicans. Immunotoxicity studies of pelicans are needed to provide proof that selenium impacted the response of white and brown pelican species to botulinum toxin. Meanwhile, tissue levels present and known effects of selenium overexposure make it reasonable to speculate that selenium overexposure may have contributed to the impaired health and susceptibility to botulinum toxin in the birds that succumbed during the 1996 die-off

Selenium was the only analyte we studied that was found at excessively high levels in the affected pelicans by all criteria applied, but other possible contributors to the die-off should be considered. For example, the Salton Sea birds sampled had lower liver zinc and iron. Zinc deficiency has been associated clinically with both increased susceptibility to disease and hemolytic anemia (Goyer 1996). Whether the relatively lower zinc and iron body stores reflected in the liver contributed to greater susceptibility to botulism at that time, or were a result of it, is unknown. In addition, implicating only selenium must be done with caution, since metals can act synergistically, additively or antagonistically, and the form and valence state can make a difference. Several metals known to be immunotoxic (for example arsenic and mercury) were not measured in this study because accurate speciation for meaningful assessment of toxicity requires more specialized equipment and expertise than was available to us at that time. Sources of disease agents must also be considered, focusing on unusually high tissue levels of analytes of concern found in larger prey fish of affected birds at the time of the die-off. Pesticides, PCBs, and other anthropogenic pollutants have also been shown to alter immunity in avian species (Fairbrother and Fowles 1990; Fairbrother et al. 1994; Luebke et al. 1997; Bishop et al. 1998; Zelikoff 1998; Wickliffe and Bickham 1998). Possible contributions of these compounds or naturally occurring toxins (e.g., algal toxins) bioaccumulating in fish also should not be ruled out entirely without a more exhaustive analysis of this incident.

The endangered status of the brown pelican makes it difficult and often inappropriate to obtain samples for studies like this. Only a few pelicans could be sampled from each site. Consistency and control is often difficult to achieve with samples of opportunity. Gender and age, both of which can influence trace element levels (Thompson 1990) were not determined at the time of sampling.

Future laboratory studies could include screening of analytes of concern in this region in avian-specific immunotoxicity assays (Baecher-Steppan et al. 1989; Zelikoff 1998). Measurement of antibody-forming cell response, cell proliferation, and macrophage function are becoming standardized and well validated (Burns et al. 1996). Some immunotoxicology assays are also amenable to application to field-collected samples where exposure to immunomodulating agents is suspected (Zelikoff 1998). Functional measurements of immune status require living cells and tissues (e.g., blood samples or spleen, thymus or other lymphatic tissue, if sacrifice

of the animal is necessary or otherwise warranted). Laboratories must be prepared to deal with samples of opportunity collected on short notice if functional measurements of immune status are to be made.

Studies of the Salton Sea should continue to address the possibility of contamination of the larger prey fish of the brown and white pelican that suffered great losses during the 1996 die-off in order to anticipate or prevent similar epidemics. Such studies would also provide information on how contaminants may adversely affect other wildlife at the Salton Sea.

Acknowledgments. We wish to thank Carol Roberts of the U.S. Fish and Wildlife Service for the opportunity to collect tissue samples at the Salton Sea. This study would not have been possible without her help. We also thank Walter Hayhow for technical assistance with chemical analysis and Sea World of California for allowing the collection of pelican tissues used as healthy reference tissues.

REFERENCES

Baecher-Steppan L, Nakaue HS, Matsumoto M, Gainer JH, Kerkvliet NI (1989) The broiler chicken as a model for immunotoxicity assessment. 1: Standardization of in vitro immunological assays. Fund Appl Toxicol 12:773-786.

Bishop CA, Boermans HJ, Ng P, Campbell GD, Struger J (1998) Health of tree swallows *(Tachycineta bicolor)* nesting in pesticide-sprayed apple orchards in Ontario, Canada. I. Immunological parameters. J Toxicol Environ Health 55:531-559

Bobker G (1993) Death in the Ponds: Selenium-induced waterbird deaths and deformities at agricultural evaporation ponds. The Bay Institute of San Francisco, Sausalito

Bums LA, Meade BJ, Munson AE (1996) Toxic responses of the immune system. In: In: Klaassen CD (ed) Casarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, New York

Fairbrother A, Fowles J (1990) Subchronic effects of sodium selenite and selenomethionine on immune functions of the mallard. Arch Environ Contam Toxicol 19:836-844

Fairbrother A, Fix M, O'Hara T, Ribic CA (1994) Impairment of growth and immune function of avocet chicks from sites with elevated selenium, arsenic and boron. J Wildlife Dis 30:222-233.

Furness RW (1996) Cadmium in birds. In: Beyer WN, Heinz GH, Redmon-Norwood AW (eds) Environmental contaminants in wildlife: Interpreting tissue concentrations. SETAC Press, Pensacola

Goyer RA (1996) Toxic effects of metals. In: Klaassen CD (ed) Casarett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, New York

Koranda JD, Stuart M, Thompson S, Cornado C (1979) Biogeochemical studies of wintering waterfowl in the Imperial and Sacramento Valleys: Lawrence

Livermore Laboratory Report on Department of Energy Contract W-7405-Eng-48, UCID-18288.

Luebke RW, Hodson PV, Faisal M, Ross PS, Grasman KA, Zelikoff J (1997) Aquatic pollution-induced immunotoxicity in wildlife species. Fund Appl Toxicol 37:1-15

Peterle TJ (1991) Wildlife Toxicology. Van Nostrand Reinhold, New York, NY. 322 p.

Roberts TA, Collings AM (1973) An outbreak of type C botulism in broiler chicken. Avian Dis 17:650-658

Setmire JG, Wolfe JC, Stroud RK (1990) Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Salton Sea Area, 1986-87. USGS, Sacramento

Setmire JG, Schroeder RA, Densmore JN, Goodbred SL, Audet DJ, Radke WR (1993) Detailed study of water quality, bottom sediment, and biota associated with irrigation drainage in the Salton Sea area, 1988-90. USGS, Sacramento

Thompson DR (1990) Metals in marine vertebrates. In: Furness RW, Rainbow PS (eds.) Heavy Metals in the Marine Environment. CRC Press, Boca Raton.

U.S. Fish and Wildlife Service (1997). Saving the Salton Sea: A Research Needs Assessment. U.S. Department of the Interior, Fish and Wildlife Service. October, 1997.

Wickliffe JK, Bickham JW (1998) Flow cytometric analysis of hematocytes from brown pelicans (Pelicanus occidentalis) exposed to planar halogenated hydrocarbon and heavy metals. Bull Environ Contam Toxicol 61:239-246

Zelikoff JT (1998) Biomarkers of immunotoxicity in fish and other non-mammalian sentinel species: Predictive value for mammals? Toxicol 129:63-71.