

EFFECTS OF MERCURY IN FLEDGLING AND ADULT CORMORANTS AND EGRETS ALONG THE CARSON RIVER, NEVADA

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ABSTRACT

Effects of mercury in fledgling and adult cormorants and egrets along the Carson River, Nevada. Hoffman, D.J.¹, Henny, C.J.², Hill, E.F.² and Grove, R.A.² ¹USGS-Patuxent Wildlife Research Center, Laurel, MD and ²USGS-Forest and Rangeland Ecosystem Science Center, Corvallis, OR. High concentrations of mercury (Hg) from historical mining activities have accumulated in the food chain of fish-eating birds nesting along the mid to lower Carson River. Activities of ten plasma and tissue enzymes and other biochemistries were measured for double-crested cormorant, *Phalacrocorax auritus* (DCC) and snowy egret, *Egretta thula* (SE) fledglings and adults from a high mercury site (LCRS) and a low mercury reference site. Geometric mean blood Hg concentrations at the LCRS site were 5.4 and 17.1 ppm (ww) for fledgling and adult DCC, and 2.7 and 5.9 for SE. Compared to adult birds, fledglings had 2 to 3 times as many biochemical variables that were significantly linked to Hg. Mercury-related oxidative stress in DCC included 3-fold increases in hepatic thiobarbituric acid reactive substances (TBARS) indicative of lipid peroxidation. In young DCC, Hg decreased hepatic reduced glutathione (GSH) concentration and GSH-S-transferase activity, but increased the ratio of oxidized glutathione (GSSG) to GSH and selenium-dependent GSH peroxidase activity. In adult DCC, hepatic G-6-PDH activity decreased with increasing Hg. Young DCC from the LCRS exhibited 2-fold elevations in activities of five plasma enzymes (GSSG-reductase, ALT, AST, ChE, and LDH-L) linked to mercury-induced hepatotoxicity in birds. Oxidative stress was also apparent in DCC brain and kidney. In fledgling SE with lower concentrations of mercury than DCC, oxidative stress was less, and evidence of compensatory mechanisms were apparent. These included increased activities of hepatic G-6-PDH and GSH-S-transferase activities in LCRS birds. Responses to mercury in fish-eating birds appear to be dependent upon dietary exposure, species, and age. (This study was funded in part by the U. S. EPA)

INTRODUCTION

During the Comstock mining era of the mid to late 1800s, liquid mercury was used to process gold and silver ore mined from Virginia City, Nevada and nearby areas. Along with the waste rock, known as tailings, millions of pounds of mercury were released into the Carson River watershed and washed downstream into the lower reaches of the Carson River, Lahontan Reservoir, and wetlands in the Lahontan Valley at the terminus of the system. Lahontan Reservoir is the most important sport and commercial fishery on the Carson River system; the Lahontan Valley wetlands provide important habitat to a wide variety of birds, including many migratory species. The Carson River Mercury Site was listed as a Federal Superfund Site on the U.S. Environmental Protection Agency's (EPA) National Priorities List in August 1990.

Whole body burdens of total mercury (THg) in walleye (*Stizostedion vitreum*) ranged from 4.2 to 6.6 ug/g (wet weight, ww) and THg concentrations in double-crested cormorant (*Phalacrocorax auritus*) feathers were from 39 to 187 ug/g (dry weight, dw). EPA's assessed identified fish-eating birds as the wildlife most likely to be harmed by the mercury contamination at the site.

This investigation, conducted in 1998, was designed to help determine whether mercury from historic mining activities is adversely affecting fish-eating birds and their reproduction in the Carson River system. This portion of the study evaluates possible sublethal effects of mercury on plasma and organ biochemistries in double-crested cormorants (DCC) and snowy egret (SE) fledglings and adults.

METHODS

Field surveys and sampling have focused on the Lower Carson River System (LCRS), including a high mercury area in Churchill Co., NV on Lahontan Reservoir and Carson Lake. A low mercury reference site, Humboldt River, was situated about 475 km east in Elko Co., NV east of Elko near Ryndon and immediately south of Interstate 80. Activities of ten plasma and tissue enzymes and other biochemistries were measured for double-crested cormorant, *Phalacrocorax auritus* (DCC) and snowy egret, *Egretta thula* (SE) fledglings and adults from the LCRS site and the low mercury reference site.

Biochemical measurements chosen were either indicators of mercury toxicity in birds or ones that would reflect organ damage and related physiological disturbances. Basic methods and assay conditions are described by Hoffman and Heinz (1998; Environ. Toxicol. Chem. 17:161-166). Measurements included: Plasma - glutathione peroxidase (GSH-oxidase), glutathione reductase (GSSG - reductase), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), cholinesterase (ChE), uric acid (UA), creatinine (CRN), glucose (GLU), total plasma protein (TPP), albumin (ALB), cholesterol (CHL), triglycerides (TRG), calcium and inorganic phosphorus; Liver - GSH-oxidase, GSSG-reductase, glutathione-S-transferase (GSH-S-transferase), glucose-6-phosphate dehydrogenase (G-6-PDH), reduced glutathione (GSH), oxidized glutathione (GSSG), total sulfhydryl concentration (TSH), and thiobarbituric acid reactive substances (TBARS); Kidney - GGT, UA, GLU, and enzymes related to glutathione metabolism, oxidative stress and TBARS as for liver assays; Brain - adenosine triphosphate (ATP), cholinesterase (AChE) and all liver variables.

Data between the high mercury and the low mercury site was compared by t-test (p<0.05). Regression analysis was used to define relationship between mercury residues in different tissues and biochemistries.

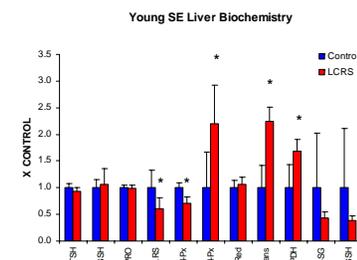
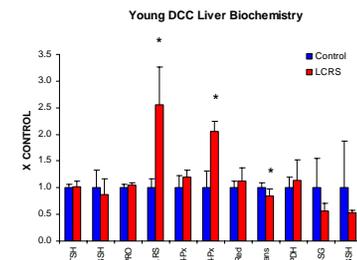
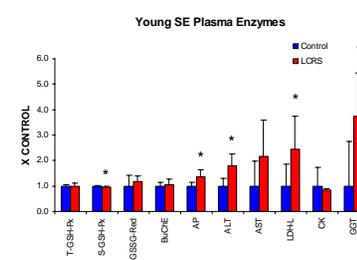
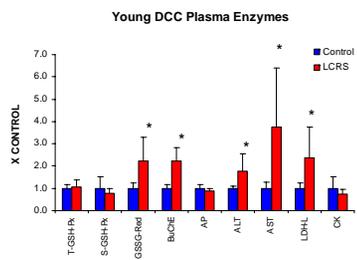


Table 1. Changes in Plasma and Organ Biochemistries in Double-Crested Cormorants at a High Mercury Site (LCRS) Relative to a Low Mercury Site.¹

Adult	Young
Plasma	Plasma
(+) BuCHE	(+) GSSG-reductase, ALT, AST, BuCHE, LDH-L
(-) T-GSH-Px	(-) Calcium, inorganic phosphorus
Brain	Brain
(+) ATP	(+) TBARS
(-) T-GSH-Px, GSSG-reductase	(-) GSH, GSSG-reductase
Liver	Liver
(+) TBARS	(+) TBARS, S-GSH-Px, GSH-S-transferase
Kidney	Kidney
(+) TBARS, GGT	(+) T-GSH-Px, TSH
(-) GSH	(-) GSSG

¹ Increased (+) or decreased (-) relative to reference site (P < 0.05). Geometric mean Hg for blood of adult and young at LCRS were 17.1 and 5.4 ppm (ww) and for liver were 134.8 and 10.9 ppm (ww).

Table 2. Changes in Plasma and Organ Biochemistries in a Snowy Egret at a High Mercury Site (LCRS) Relative to a Low Mercury Site.¹

Adult	Young
Plasma	Plasma
(+) ALP, ALT, GGT, LDH-L, uric acid, glucose, inorganic phosphorus	(-) S-GSH-Px
(-) CK, inorganic phosphorus	Brain
Brain	(+) G-6-PDH, T-GSH-Px, GSSG/GSH, TBARS
(-) GSH, protein	(-) T-GSH-Px, TBARS
Liver	Liver
(+) S-GSH-Px	(+) S-GSH-Px, G-6-PDH, GSH-S-transferase
(-) T-GSH-Px	(-) T-GSH-Px, TBARS
Kidney	Kidney
(+) S-GSH-Px	(+) GSH-S-transferase
(-) G-6-PDH	(-) S-GSH-Px, T-GSH-Px, GSH, TBARS

¹ Increased (+) or decreased (-) relative to reference site (P < 0.05). Geometric mean Hg for blood of adult and young at LCRS were 5.9 and 2.7 ppm (ww) and for liver were 43.7 and 2.7 ppm (ww).



Table 3. Relationship between hepatic mercury and biochemical variables associated with oxidative stress in double-crested cormorants, 1998.

Species/Age	Form of hepatic mercury	Hepatic variable measured	Pearson r	P-value
Cormorant				
Adult				
	MeHg _{org}	TBARS	0.758	0.029
	MeHg _{org}	G-6-PDH	-0.720	0.044
	MeHg	TBARS	0.743	0.035
	loHg	TBARS	0.794	0.019
	THg	TBARS	0.758	0.004
Young				
	MeHg	S-GSH-Px	0.971	0.0003
	MeHg	GSSG-GSH	0.869	0.011
	MeHg	GSH	-0.759	0.048
	loHg _{org}	S-GSH-Px	0.979	0.0001
	loHg _{org}	GSSG-GSH	0.828	0.021
	THg _{org}	S-GSH-Px	0.935	0.0001
	THg _{org}	TBARS	0.832	0.0008
	THg _{org}	GSH-S-transferase	-0.632	0.020
	THg _{org}	GSSG	-0.592	0.04

Table 4. Relationship between hepatic mercury and biochemical variables associated with oxidative stress in snowy egret, 1998.

Species/Age	Form of hepatic mercury	Hepatic variable measured	Pearson r	P-value
Snowy Egret				
Adult				
	THg _{org}	T-GSH-Px	-0.677	0.022
Young				
	loHg	GSH	-0.951	0.013
	THg _{org}	GSH-S-transferase	0.932	0.0001
	THg _{org}	GSSG-GSH	-0.755	0.001
	THg _{org}	G-6-PDH	0.774	0.002
	THg _{org}	T-GSH-Px	-0.719	0.006
	THg _{org}	S-GSH-Px	0.668	0.013

SUMMARY and CONCLUSIONS

- Geometric mean blood Hg concentrations for fledgling and adult DCCs at the high mercury site (LCRS) were 5.4 and 17.1 ppm (ww) versus 0.8 and 5.4 ppm at the low mercury site. Blood concentrations for SEs were 2.7 and 5.9 ppm at the LCRS versus 0.3 and 2.7 ppm at the low mercury site. Liver concentrations for DCCs at the LCRS were 10.9 and 134.8 versus 1.8 and 17.9 at the low site. Liver concentrations for SEs were 2.7 and 43.7 ppm at the LCRS versus 0.4 and 7.9 ppm at the low site. Livers with higher total mercury concentrations had lower percentages of toxic methylmercury (Henny et al, 2002; *Ecotoxicology* 11, 213-231).
- Young DCCs from the LCRS exhibited 2-fold elevations in activities of five plasma enzymes (GSSG-reductase, ALT, AST, ChE, and LDH-L) linked to mercury-induced hepatotoxicity in birds. Young SEs exhibited elevations in four plasma enzymes. These effects were generally not apparent in adults.
- Compared to adult birds, fledglings had 2 to 3 times as many hepatic biochemical variables that were significantly linked to Hg by regression analysis. Mercury-related oxidative stress in DCCs included 3-fold increases in hepatic thiobarbituric acid reactive substances (TBARS) indicative of lipid peroxidation. In young DCC, Hg decreased hepatic reduced glutathione (GSH) concentration and GSH-S-transferase activity, but increased the ratio of oxidized glutathione (GSSG) to GSH and selenium-dependent GSH peroxidase activity.
- In fledgling SEs with lower concentrations of mercury than DCCs, hepatic oxidative stress was less, and evidence of compensatory mechanisms were apparent by regression analysis. These included increased activities of hepatic G-6-PDH and GSH-S-transferase activities accompanied by decreased ratio of GSSG to GSH.
- Responses to mercury in fish-eating birds appear to be dependent upon dietary exposure, species, and age. Adult DCCs and SEs were able to tolerate relatively high levels of mercury apparently through demethylation processes that occur above certain threshold concentrations (Henny et al, 2002; *Ecotoxicology* 11, 213-231).