

Tissue Distribution of Trace Elements and DDE in Brown Pelicans

Harry M. Ohlendorf,¹ Daniel W. Anderson,² Diane E. Boelstorff,² and Bernard M. Mulhern³

¹U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Pacific Coast Field Station, c/o Wildlife and Fisheries Biology, University of California, Davis, CA 95616, ²Wildlife and Fisheries Biology, University of California, Davis, CA 95616, and ³U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD 20708

Trace elements and organochlorine pollutants commonly occur in avian tissues. However, concentrations vary among species in the same geographic area, and some compounds can be distributed differently in body tissues among different species (e.g., Finley and Stendell 1978; Turner et al. 1978; White and Finley 1978; Finley et al. 1979; Furness and Hutton 1979, Osborn et al. 1979, Olsen et al. 1980, Hutton 1981, Ohlendorf et al. 1981, Ohlendorf and Miller 1984; see also review by Ohlendorf et al. 1978). In addition, some heavy metal concentrations vary with the bird's age (e.g., Furness and Hutton 1979; Hutton 1981). Seasonal patterns in metal concentrations can also occur (Osborn 1979), further complicating measuring levels in avian species.

The brown pelican (*Pelecanus occidentalis*) is a long-lived, coastal piscivorous bird. During the 1960s and 1970s, brown pelicans in the Los Angeles area of California were exposed to high levels of DDT contamination. Some fish there still have high DDE concentrations (Stout and Beezhold 1981, Gossett et al. 1983). Cadmium concentrations in plankton off Baja California are also known to be elevated (Martin and Broeknow 1975), although the cadmium may originate from natural sources. However, some heavy metals in petrels (Procellariidae and Hydrobatidae) from California coastal waters near San Francisco are believed to originate from industrial sources (Anderlini et al. 1972).

In Southern California the resident brown pelican population is considered Endangered (Gress and Anderson 1983), although it has been recovering in recent years (Anderson and Gress 1983). Larger and less contaminated populations of this species occur in the Gulf of California (Keith et al. 1971), and there is much interchange between the two populations during the non-breeding season (Anderson and Anderson 1976).

Our purpose was to obtain a sample of brown pelicans from these populations to determine: (1) which brown pelican tissues contain the highest concentrations of organochlorine pollutants and several trace elements, (2) how much variation might exist in a general sample, and (3) what interrelationships might exist among the tissues and compounds studied.

MATERIALS AND METHODS

Nine brown pelicans were shot in the vicinity of Bahia de los Angeles, Gulf of California, on 30 November and 1 December 1980. Steel shot ammunition was used to preclude lead contamination of tissues. We also obtained one pelican found dead at Anacapa Island, CA on 28 July 1980. All birds were frozen soon after collection. Later they were thawed for dissection and tissues were then refrozen pending analysis.

Soft tissues (liver, kidneys, pancreas, and gonads) were removed and homogenized in a blender. Two separate 5-g portions were taken; one for mercury analysis and one for lead, copper, cadmium, zinc, and chromium analyses. A 1- or 2-g portion of each tissue was analyzed for arsenic and/or selenium. Ten to 12 tertial feathers were dissolved in nitric acid, diluted, then portioned and treated similarly to soft tissue. Two-g portions of bone (humerus and ribs analyzed separately) were dry ashed, diluted to a final volume of 30 ml, and analyzed for Cd, Cr, Pb, and Zn.

Samples for Hg determination were prepared and analyzed as described by Hoffman and Moore (1979), and those for As or Se determination as described by Haseltine et al. (1981). All other samples were prepared and dry ashed according to methods described by Custer and Mulhern (1983); they were analyzed by atomic absorption spectrophotometry with a Perkin-Elmer* model 5000 equipped with deuterium arc and tungsten halide background correction, an AS-50 autosampler, and a PRS-10 printer. Metals were determined by measuring atomic absorption of each element in an air-acetylene flame, and background correction was used wherever feasible. The lower limit of reportable residues was 0.1 ppm (wet weight) except for chromium and arsenic (0.05 ppm), and mercury (0.02 ppm). Metal recoveries from fortified bone meal, eagle feathers, or chicken livers ranged from 81-110%. Residues were not corrected for percent recovery. Results are presented on a dry-weight basis to avoid errors associated with varying moisture content levels in soft tissues, as suggested by Adrian and Stevens (1979). Moisture percentage is presented to facilitate conversion to wet-weight basis.

We analyzed breast and wing muscle for organochlorines to compare with the carcass homogenate, which is frequently analyzed in field-collected birds (see Ohlendorf and Miller 1984). Tissues for organochlorine analysis were homogenized, mixed with anhydrous sodium sulfate, and extracted with hexane in a soxhlet apparatus for 7 hours (Cromartie et al. 1975). Lipids were removed by Florisil Sep-Pak cartridge (Clark et al. 1983) and PCBs separated from pesticides by silica gel (Davison grade 923, 100-120 mesh) chromatography (Kaiser et al. 1980).

*Mention of a specific brand name does not constitute government endorsement.

Samples were analyzed by electron-capture gas chromatography using a 1.5/1.95% SP-2250/2401 column. Pesticide and PCB recoveries from fortified chicken eggs ranged from 88-108%, except for trans-nonachlor (33%). The lower limit of reportable residues was 0.1 ppm (wet-weight) for pesticides and 0.5 ppm for PCBs.

Statistical tests were performed using the MINITAB computing system (Ryan et al. 1976). Correlations are either Pearson's Product-Moment or Spearman's Rank correlations, depending on whether values were normally distributed. One-way analysis of variance and matched pair t-tests (with Bonferroni general inequality adjustment, Neter and Wasserman 1974) were used to determine significant differences. When necessary, data were transformed to increase normality.

RESULTS AND DISCUSSION

For each tissue, selenium and 7 metals usually occurred above detection limits in all (or nearly all) of the birds or in only 1 or 2 of them (i.e., they seldom fell between the extremes) (Table 1). Lead occurred more frequently in hard tissues (feather and bone) than in soft tissues (χ^2 , $P < 0.05$), whereas Cd occurred more frequently in soft tissues than in hard tissues (χ^2 , $P < 0.05$).

Livers contained the highest levels of Cu and Se (Table 1). Kidneys contained the highest concentrations of Cd and Cr, and bones had the highest levels of Pb and Zn. Mercury occurred at similar levels in liver, kidneys, and feathers, although concentrations in feathers tended to be somewhat higher. However, variation among individuals was too great to enable us to test for significant differences using the Bonferroni matched-pair t-test. Arsenic occurred in the liver and kidney of one pelican and in the liver (only) or kidney (only) of two others.

We found higher concentrations of Cd, Cr, and Se than reported by Blus et al. (1977) for brown pelicans from the U.S. Atlantic Coast (comparisons are based on approximate conversions of their reported values to dry-weight basis). In contrast, we found lower levels of As and Hg than reported for the Atlantic Coast birds.

Although Cd, presumably of natural origin, occurs at elevated levels in plankton off Baja California (Martin and Broenkow 1975) we found lower concentrations of Cd, Hg, and Se in pelican tissues than those reported for several other Atlantic seabird species (Bull et al. 1977; Furness and Hutton 1979; Hutton 1981). Yet, concentrations of Cd, as well as some other metals, were higher in petrels from California and Antarctica (Anderlini et al. 1972) than in Pacific pelicans. However, for certain others (e.g., Cu and Zn, which are considered essential trace elements) the levels were generally similar in pelican and petrel tissues.

Significant correlations occurred between certain metals within tissues (Table 2); however, because of our small samples, the

Table 1. Concentrations of trace elements (ppm, dry weight) and moisture content in brown pelican tissues.

	Liver 10 ^a	Kidneys 10	Feathers 8	Humerus 10	Ribs 10	Pancreas 8	Gonads 9
Moisture (%) ^b	68.0 ± 2.5	74.6 ± 1.4	18.0 ± 4.6	32.2 ± 9.7	35.7 ± 16.9	66.2 ± 4.8	72.4 ± 4.6
Trace elements ^c							
As	2 0.32- 0.72	2 0.21- 0.92	NA ^d	NA	NA	NA	NA
Cd	10 5.80 B ± 3.14	9 18.2 A ± 6.80	3 0.15- 0.35	1 0.20	2 0.13- 0.18	8 2.63 B ± 1.62	9 4.09 B ± 1.23
Cr	10 0.493 C ± 0.251	10 3.71 ABC ± 3.68	NA	10 1.26 A ± 0.269	10 0.840 B ± 0.173	NA	NA
Cu	10 23.5 A ± 8.94	10 10.8 B ± 3.06	8 3.99 C ± 1.43	NA	NA	NA	NA
Hg	10 0.751 ± 0.384	10 0.678 ± 0.312	8 0.970 ± 0.324	NA	NA	NA	NA

Table 1. Continued.

	Liver 10 ^a	Kidneys 10	Feathers 8	Humerus 10	Ribs 10	Pancreas 8	Gonads 9
Pb	2 0.74- 0.89	2 0.58- 1.6	7 0.902 B + 0.846	10 7.44 A + 2.52	9 6.84 A + 4.28	NA	NA
Se	10 21.1 A + 6.85	10 13.9 B + 3.76	8 2.36 C + 0.742	NA	NA	NA	NA
Zn	10 98.1 AB + 27.3	10 77.2 B + 12.2	8 65.9 B + 15.9	10 108.1 A + 13.0	10 104.5 A + 9.4	8 96.3 AB + 35.9	NA

^aNumber of samples analyzed.

^bArithmetic mean + standard deviation.

^cWhen present in more than half of the samples values are number with quantifiable residue, and arithmetic mean + standard deviation. When in less than half they are the number with residue and the range of concentrations detected (these values were not used in statistical comparisons). Upper-case letters next to means indicate differences among tissues for each element; means that do not share the same letter are significantly different ($P < 0.05$). For Hg, variation among tissues was too high to test for differences using Bonferroni matched-pair t-test (Neter and Wasserman 1974).

^dNA = Not analyzed.

Table 2. Significant correlations between trace elements within tissues and correlations between tissues for trace elements (ppm dry-weight) in brown pelicans^a.

Tissue	Trace element pairs: ^b			Tissue pairs: ^b			
	N ^c	Elements	R ^{2d}	Elements	N ^c	Tissues	R ^{2d}
Liver	10	Zn Cu	60.8*	Cd	10	Liver Kidney	54.1*
		Zn Cr	64.8**				
		Cd Cr	93.3***				
		Cu Cr	69.7**				
Kidney	10	Cd Cu	62.8*	Hg	10	Liver Kidney	92.4***
		Cd Cr	80.5**				
		Hg Cu	65.9**				
Humerus	10	Zn Pb	33.3*	Pb	8	Humerus Feather	92.8***
		Pb Cr	64.0*				
		Zn Cr	77.3***				
Ribs	10	Zn Pb	31.2*	Se	8	Liver Feather	54.6*
		Zn Cr	75.8***				
Pancreas	8	Zn Cd	86.5***	Zn	7	Feather Pancreas	45.5*

^aElements found in less than half of the samples were omitted; or tissues in which less than half of the samples contained a particular element were omitted.

^bPairs of variables not listed were not significantly correlated ($p > 0.10$).

^cNumber of samples analyzed.

^dSignificance levels of correlation coefficients (of best fit regressions), after Bonferroni general inequality adjustment: * = $p < 0.10$; ** = $p < 0.05$; *** = $p < 0.01$; R² value is adjusted for d.f.

regressions we observed do not seem noteworthy here. Concentrations of Cr and Zn were most frequently correlated with other metals, and sometimes with each other. Concentrations of Se were not significantly correlated ($P > 0.10$) with those of any metal, but each of the metals (except As, which was not tested) was correlated with at least one other metal. Relationships between Hg and Se in marine birds are not clear (Koeman et al. 1975; Beijer and Jernelov 1978; Fimreite 1979), but concentrations of these elements in marine mammals are usually highly correlated (Koeman et al. 1973, 1975; Kari and Kauranen 1978; Smith and Armstrong 1978). Lead and Zn were negatively correlated in ribs whereas the correlation was positive in humerus.

Correlations between tissues for trace elements also occurred (Table 2). As expected, concentrations in other tissues were most frequently related to those in the liver. Correlations between tissues for trace element levels have been shown in some other avian species (e.g., Finley and Stendell 1978, White and Finley 1978, Furness and Hutton 1979, Hutton 1981). However, in some instances there are few correlations (Osborn et al. 1979), which suggests that relationships cannot be generalized from one species to another.

The only organochlorine compound found at quantifiable levels was *p,p'*-DDE. It occurred at low concentrations in all samples except for two wing muscles. On a wet-weight or lipid-weight basis DDE concentrations were highest in carcass homogenate (Table 3). Because of differences in lipid content among the three types of tissue, concentrations on a lipid-weight basis were more similar than on a wet-weight basis. Yet, relative lipid levels among tissues were significantly correlated, as expected (Table 4). Highest residue correlations were between carcass homogenate and breast muscle on lipid-weight basis and wing and breast muscle on wet-weight basis (both $P < 0.001$; Table 4). Correlations of DDE concentrations among tissues for other species have been reported (see Ohlendorf and Miller 1984).

Table 3. Concentrations of DDE in brown pelican tissues.

Tissue	N ^a	% Lipid (\bar{x} +SD)	Parts per million ^b	
			Wet-weight	Lipid-weight
Carcass homogenate	10/10	13.8+4.5	0.93(0.61-1.43)	7.2(5.0-10.2)
Breast muscle	10/10	11.1+3.0	0.65(0.42-0.99)	6.0(4.2- 8.7)
Wing muscle	8/10	3.6+1.2	0.19(0.10-0.34)	5.6(3.3- 9.3)

^aN = number of tissues in which DDE was detected/number of tissues analyzed.

^bMean and (95% confidence interval).

Our results lead us to conclude that for pelicans, the following tissues contain the highest concentrations of, or are most

practical to analyze for, the elements listed: liver - Cu, Hg, and Se; kidney - Cd and Cr; humerus - Pb and Zn. We did not find any tissue particularly useful for As analysis but liver is probably best. Further analysis of liver (for Cd, Cr, and Zn), kidneys (Cu and Hg) and humerus (Cr) may help further define the apparent interrelations between metals within tissues and between tissues for various elements. Breast muscle seems to be the best alternative to analyzing carcass homogenate for organochlorines.

Table 4. Correlations of DDE (as parts per million) or lipids (as %) in brown pelican tissues (N = 10).

<u>ANALYSIS</u> Tissue	<u>Correlation^a</u>	
	Breast	Wing
<u>LIPID-WEIGHT ppm:</u>		<u>LIPID-WEIGHT ppm:</u>
Carcass homogenate	0.927***	0.796**
Breast muscle	-	0.845**
<u>WET-WEIGHT ppm:</u>		<u>WET-WEIGHT ppm:</u>
Carcass homogenate	0.681*	0.683*
Breast muscle	-	0.954***
<u>PERCENT LIPIDS:</u>		<u>PERCENT LIPIDS:</u>
Carcass homogenate	0.635*	0.636*

^aLevels of significance: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; residue comparisons use Spearman's Rank correlations and percent lipids use normal correlations on log-normalized values.

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REFERENCES

- Adrian WJ, Stevens ML (1979) Wet versus dry weights for heavy metal toxicity determinations in duck liver. *J Wildl Dis* 15:125-126
- Anderlini VC, Connors PG, Risebrough RW, Martin JH (1972) Concentrations of heavy metals in some Antarctic and North American sea birds. In: Parker BC (ed) *Colloquium on Conservation Problems in Antarctica*. Allen Press, Lawrence, Kansas, pp 49-61
- Anderson DW, Anderson IT (1976) Distribution and status of brown pelicans in the California current. *Am Birds* 30:3-12
- Anderson DW, Gress F (1983) Status of a northern population of California brown pelicans. *Condor* 85:79-88
- Beijer K, Jernelov A (1978) Ecological aspects of Mercury-Selenium interactions in the marine environment. *Environ Health Perspect* 25:43-45

- Blus LJ, Neely BS Jr, Lamont TG, Mulhern B (1977) Residues of organochlorines and heavy metals in tissues and eggs of brown pelicans. *Pestic Monit J* 11:40-53
- Bull KR, Murton RK, Osborn D, Ward P, Cheng L (1977) High levels of cadmium in Atlantic seabirds and sea-skaters. *Nature* 269:507-509
- Clark DR Jr, Clawson RL, Stafford CJ (1983) Gray bats killed by dieldrin at two additional Missouri caves: aquatic macro-invertebrates found dead. *Bull Environ Contam Toxicol* 30:214-218
- Cromartie E, Reichel WL, Locke LN, Belisle AA, Kaiser TE, Lamont TG, Mulhern BM, Prouty RM, Swineford DM (1975) Residues of organochlorine pesticides and polychlorinated biphenyls and autopsy data for bald eagles. *Pestic Monit J* 9:11-14
- Custer TW, Mulhern BL (1983) Heavy metal residues in prefledgling black-crowned night-herons from three Atlantic coast colonies. *Bull Environ Contam Toxicol* 30:178-185
- Finreite N (1979) Accumulation and effects of mercury on birds. In: Nriagu JO (ed) *The Biogeochemistry of Mercury in the Environment*. Elsevier/North Holland Biomedical Press, Amsterdam-New York-Oxford, pp 601-627
- Finley MT, Stendell RC (1978) Survival and reproductive success of black ducks fed methyl mercury. *Environ Pollut* 16:51-64
- Finley MT, Stickel WH, Christensen RE (1979) Mercury residues in tissues of dead and surviving birds fed methylmercury. *Bull Environ Contam Toxicol* 21:105-110
- Furness R, Hutton M (1979) Pollutant levels in the great skua *Catharacta skua*. *Environ Pollut* 19:261-268
- Gossett RW, Puffer HW, Arthur RH Jr, Young DR (1983) DDT, PCB and benzo(a) pyrene levels in white croakers (*Genyonemus lineatus*) from Southern California. *Mar Pollut Bull* 14:60-65
- Gress F, Anderson DW (1983) The California Brown Pelican Recovery Plan. U.S. Fish and Wildlife Service, Portland, Oregon
- Haseltine, SD, Heinz GH, Reichel WL, Moore JF (1981) Organochlorine and metal residues in eggs of waterfowl nesting on islands in Lake Michigan off Door County, Wisconsin, 1977-78. *Pestic Monit J* 15:90-97
- Hoffman DJ, Moore JF (1979) Teratogenic effects of external egg applications of methyl mercury in the mallard, *Anas platyrhynchos* *Teratology* 20:453-462.
- Hutton M (1981) Accumulation of heavy metals and selenium in three seabird species from the United Kingdom. *Environ Pollut Ser A* 26:129-145
- Kaiser TE, Reichel WL, Locke LN, Cromartie E, Krynitsky AJ, Lamont TG, Mulhern BM, Prouty RM, Stafford CJ, Swineford DM (1980) Organochlorine pesticides, PCB, and PRB residues and necropsy data for bald eagles from 29 states - 1975-77. *Pestic Monit J* 13:145-149
- Kari T, Kauranen P (1978) Mercury and selenium contents of seals from fresh and brackish waters in Finland. *Bull Environ Contam Toxicol* 19:273-280
- Keith JO, Woods LA Jr, Hunt EG (1971) Reproductive failure in brown pelicans on the Pacific coast. *Trans N Am Wildl Nat Resour Conf* 35:56-64

- Koeman JH, Peeters WHM, Koudstaal-Hol CHM, Tjioe PS, de Goeij JJM (1973) Mercury-selenium correlations in marine mammals. *Nature* 245:385-386.
- Koeman JH, van de Ven WSM, de Goeij JJM, Tjioe PS, van Haften JL (1975) Mercury and selenium in marine mammals and birds. *Sci Total Environ* 3:279-287
- Martin JH, Broenkow WW (1975) Cadmium in plankton: elevated concentrations off Baja California. *Science* 190:884-885
- Neter J, Wasserman W (1974) *Applied Linear Statistical Models*. Richard D Irwin, Inc, Homewood, Illinois
- Ohlendorf HM, Miller MR (1984) Organochlorine contaminants in California waterfowl. *J Wildl Manage* (in press)
- Ohlendorf HM, Risebrough RW, Vermeer K (1978) Exposure of marine birds to environmental pollutants. *US Fish Wildl Serv Wildl Res Rep No. 9*
- Ohlendorf HM, Swineford DM, Locke LN (1981) Organochlorine residues and mortality of herons. *Pestic Monit J* 14:125-135
- Olsen P, Settle H, Swift R (1980) Organochlorine residues in wings of ducks in South-Eastern Australia. *Aust Wildl Res* 7:139-147
- Osborn D (1979) The significance of metal residues in wild animals. In: *Management and Control of Heavy Metals in the Environment, an International Conference*. CEP Consultants, Edinburgh, pp 187-190
- Osborn, D, Harris MP, Nicholson JK (1979) Comparative tissue distribution of mercury, cadmium and zinc in three species of pelagic seabirds. *Comp Biochem Physiol* 64C:61-67
- Ryan TA Jr, Joiner BL, Ryan RF (1976) *MINITAB Student Handbook*. Duxbury Press, North Scituate, Massachusetts
- Smith TG, Armstrong FAJ (1978) Mercury and selenium in ringed and bearded seal tissues from arctic Canada. *Arctic* 31:75-84
- Stout VF, Beezhold FL (1981) Chlorinated hydrocarbon levels in fishes and shellfishes of the Northeastern Pacific Ocean, including the Hawaiian Islands. *Mar Fish Rev* 43:1-12
- Turner JC, Solly SRB, Mol-Krijnen JCM, Shanks V (1978) Organochlorine, fluorine, and heavy-metal levels in some birds from New Zealand estuaries. *N Z J Sci* 21:99-102
- White DH, Finley MT (1978) Uptake and retention of dietary cadmium in mallard ducks. *Environ Res* 17:53-59

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