Effects of Dietary Mercury and Lead on Eggshell Thickness in Mallards

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In recent years, brown pelicans (Pelecanus occidentalis californicus) attempting to reproduce on Anacapa Island off the California coast have laid thin-shelled eggs, almost all of which have collapsed during early incubation (JEHL 1969, and RISEBROUGH et al. 1971). Laboratory exposure of other species to DDE (HEATH et al. 1969, LONGCORE et al. 1971, and WIEMEYER and PORTER 1970), the suspected cause of this phenomenon (HICKEY and ANDERSON 1968, KEITH et al. 1970, and RATCLIFFE 1967), has not produced the extreme eggshell thinning exhibited by Anacapa pelicans. The greater eggshell thinning in wild pelicans could be the result of this species being innately more sensitive to DDE as suggested by BLUS et al. (1971, 1972), or possibly it could be due to an additional effect on shell thickness of other pollutants in the marine environment. Two pollutants which may be capable of this type of synergistic or additive effect are mercury and lead compounds, which are biologically active and common in the environment (KLEIN and GOLDBERG 1970, KNAPP 1970, LAZRUS et al. 1970, and TEJNING 1967). The following tests were conducted to determine whether mercury and lead in the diet of captive mallards (Anas platyrhynchos) would increase the eggshell thinning above that caused by DDE alone.

Procedures

Sixty female mallards in their first reproductive season were stratified by weight into 10 classes and then randomly divided into six groups of ten females each, so that treatment groups would contain equal numbers of all weight classes. Each group was randomly assigned to a 10 x 14-foot pen containing a water trough, two feeders, and two nest boxes. One group of birds was fed untreated food, while each of the other five groups received food treated with one of the following: (a) 100 ppm of metallic lead made up of a combination of 43 ppm of lead carbonate, 37 ppm of lead oxide, and 49 ppm of lead sulfate (each compound made up 1/3 of the metallic lead); (b) 200 ppm of Ceresan M* (3.1% metallic mercury);

*Trade name of DuPont for N-(ethylmercury)-p-toluene sulfonanilide. Reference to trade names does not imply endorsement of commercial products by the Federal Government.

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(c) 40 ppm of p,p'-DDE; (d) 100 ppm of lead (same as treatment b) plus 40 ppm of DDE; and (e) 200 ppm of Ceresan M plus 40 ppm of DDE. The treatment levels of mercury and lead were chosen much higher than field levels in an effort to maximize possible effects on eggshell thickness. The diets were prepared by adding the compounds to dry commercial duck crumbles (without a carrier), mixing in a Y blender, and then pelleting. Except for the two groups receiving mercury, all diets were provided ad libitum from early February until the study was terminated in April. Mortality occurred among ducks in both of the mercury groups after 49 days of treatment, and their diets were changed to untreated food after 55 days. Neither clinical signs of intoxication nor mortality occurred among birds in other treatment groups.

To determine the effects of these treatments on eggshell thickness, all eggs laid on the 76th and 85th day of treatment were collected separately from each treatment group. Each bird was treated as the primary sampling unit, and the one-day samples assured that each egg came from a different bird. Eggs were opened at the equator, contents were removed, and eggshells were rinsed clean with warm water and dried at least 24 hours. Shell thickness, with the membrane intact, was measured at the equator with a micrometer to the nearest 5 microns. Eighty-nine days after the start of treatment, all the birds were asphyxiated, plucked, and prepared for residue analysis. Whole body, except head and appendages, and egg content samples were analyzed from all groups; in addition, brain samples were analyzed from the controls and the three DDE groups, eggshell and bone (femur) from the controls and the two lead groups, and kidney from the controls and the two mercury groups. Due to the presence of visceral in all birds, whole body residues could be slightly elevated above real values because of contamination from ingested food. In all cases, analyses were made from residues in one aliquot of a homogenized mixture of desired tissue from all birds in a treatment group. Egg analyses were of one aliquot of eggs laid by each group in a two-week period, starting on day 73 post-treatment.

Mercury residues were determined as described by OKUNO et al. (1972), and lead analyses were made following a modified method of BERMAN (1967). For DDE, sample preparation, extraction, residue purification, and analysis by gas chromatography were performed as follows: Sample materials were ground with anhydrous sodium sulfate. Aliquots of this were shaken with 20% (v/v) acetone in 2,2,4-trimethyl pentane (iso-octane). After centrifugation, aliquots of the extract were taken for lipid content determination and residue purification prior to GC analysis.

Purification was accomplished by liquid-liquid partition and the addition of activated Florisil to the final sample concentrate. Volumes of the sample concentrate were adjusted so that 1.0 µl of solution was equivalent to 0.5 mg of starting sample; this usually resulted in sample volumes ranging from < 0.5 to 5 µl. All identifications and quantitations (calculated from area of peaks) were based on readings from two or more dissimilar columns to assure that GC peaks were, in every case, due to single identifiable components. DC-200 and QF-1 columns were used with tritium-source electron-capture detectors; both columns had 5% loadings at 185-200° C (PETERSON, J. E.: Wildlife Research Center, U. S. Bureau of Sport Fisheries and Wildlife, Denver, Colorado; personal communication).

Differences among mean eggshell thickness were tested by analysis of variance, using methods of DUNCAN (1955) and KRAMER (1956).

Results and Discussion

DDE alone caused a 15% reduction in shell thickness, but neither the mercury nor the lead diets caused any significant eggshell thinning. There was no apparent synergistic or additive effect of these compounds when fed in combination with DDE as shell thinning was not significantly increased over that caused by DDE alone (Table 1). The fact that mercury did not cause eggshell thinning in captive mallards and, therefore, probably is not affecting wild avian species, is supported by PEAKALL AND LINCER (1972) who found no eggshell thinning in American kestrels (Falco sparverius) and ring doves (Streptopelia risoria) fed diets containing mercury compounds and SPANN et al. (1972) found no effects of mercury on pheasant eggshell thickness. In addition, BLUS et al (1971) have shown that increases in mercury residues in pelican eggs were not correlated with decreases in eggshell thickness.

The residues found in the various tissues analyzed are summarized in Tables 2a, 2b, and 2c. The mercury residues in egg contents for the mercury and the mercury + DDE groups were about 15 and 19 times higher than the average level (0.083 ppm) found in California brown pelican eggs (KEITH, J. O.: Wildlife Research Center, U. S. Bureau of Sport Fisheries and Wildlife, Denver, Colorado; personal communication). The lead concentrations in bone were about 30 and 100 times the mean lead concentrations (0.36 ppm) found in bones of Anacapa brown pelicans (ANDERLINI et al., in press).

TABLE 1

Effects of Treatments on Eggshell Thickness

Treatment	76th Day of Treatment				85th Day of Treatment			
	No. of Eggs	Mean* (Microns)	S.D.	% Difference from Control	No. of Eggs	Mean* (Microns)	S.D.	% Difference from Control
Control	8	370	36.4		7	374	29.8	
Lead	8	374	29.5	+1.1	8	383	12.8	+2.4
Mercury	3	352	50.1	-4.9	5	363	23.1	-2.9
DDE	9	315	17.3	-14.9	6	318	18.6	-15.0
Lead + DDE	7	318	21.4	-14.1	2	290	14.1	-22.5
Mercury + DDE	4	309	21.0	-16.5	4	304	32.2	-18.7

*Any means not connected by the same vertical line are significantly different from each other at $P \leq 0.05$.

TABLE 2a

DDE Residues (ppm wet weight)*

Treatment	Whole Body	Egg Contents	Brain
Control	0.1	0.1	0.1
DDE	78.2	61.6	4.1
Lead + DDE	78.8	67.0	4.4
Mercury + DDE[a]	41.0	39.0	2.5

[a]Treated diet discontinued after 55 days due to mortalities caused by the mercury.

TABLE 2b

Mercury Residues (ppm wet weight)*

Treatment	Whole Body	Egg Contents	Kidney
Control	0.011	0.012	0.016
Mercury	2.5	1.25	23.7
Mercury + DDE	4.0	1.62	19.4

TABLE 2c

Lead Residues (ppm wet weight)*

Treatment	Whole Body	Egg Contents	Eggshells	Bone
Control	0.88	0.88	0.52	0.64
Lead	3.7	1.34	2.5	9.6
Lead + DDE	3.4	.88	2.8	35.0

*All residues are based on a single analysis of one aliquot of the entire treatment group.

The threefold difference in bone lead residues between the lead treatment group and the lead + DDE treatment group supports the theory that DDE alters calcium mobilization in DDE-treated birds (OESTREICHER et al. 1971).

Neither lead nor mercury contributed significantly to mallard eggshell thinning, nor did these compounds appear to act synergistically with DDE. Although these results suggest that lead and mercury do not contribute to eggshell thinning in brown pelicans, it is possible that as a result of subtle differences in reproductive physiology between captive mallards and brown pelicans, mercury and lead could still act synergistically with DDE to cause the extreme degree of shell thinning experienced by the Anacapa brown pelicans. However, we believe it is more likely that either innate differences in reproductive physiology, or differences due to unknown contaminants or stresses, have contributed to the greater degree of shell thinning attributed to DDE in brown pelicans.

References Cited

ANDERLINI, V. C., P. G. CONNORS, R. W. RISEBROUGH, and J. H. MORTEN: Proc. Colloq. Conserv. of the 7th Continent, Antarctica, B. Parker, ed. (in press).

BERMAN, E.: Atomic Absorption Newsletter 6, 57 (1967).

BLUS, L. J., R. G. HEATH, C. D. GISH, A. A. BELISLE, and R. M. PROUTY: Bioscience 21, 1213 (1971).

BLUS, L. J., C. D. GISH, A. A. BELISLE, and R. M. PROUTY: Nature 235, 376 (1972).

DUNCAN, D. B.: Biometrics 11, 1 (1955).

HEATH, R. G., J. W. SPANN, and J. F. KREITZER: Nature 224, 47 (1969).

HICKEY, J. J. and D. W. ANDERSON: Science 162, 271 (1968).

JEHL, J. R., JR.: Environ. Southwest (June), 4 (1969).

KEITH, J. O., L. A. WOODS, JR., and E. G. HUNT: Trans. 35th North Am. Wildlife Nat. Resources Conf. 56 (1970).

KLEIN, D. H. and E. D. GOLDBERG: Environ. Sci. Technol. 4, 765 (1970).

KNAPP, C. E.: Environ. Sci. Technol. 4, 890 (1970).

KRAMER, C. Y.: Biometrics 12, 307 (1956).

LAZRUS, A. L., E. LORANGE, and J. P. LODGE, JR.: Environ. Sci. Technol. 4, 55 (1970).

LONGCORE, J. R., F. B. SAMSON, and T. W. WHITTENDALE, JR.: Bull. Environ. Cont. Toxicol. 6, 485 (1971).

OESTREICHER, M. I., D. H. SHYMAN, and C. F. WURSTER: Nature 229, 571 (1971).

OKUNO, I., R. A. WILSON, and R. E. WHITE: J. Assoc. Offic. Anal. Chem. 56, 06 (1972).

PEAKALL, D. B., J. L. LINCER: Bull. Environ. Cont. Toxicol. 8, 89 (1972).

RATCLIFFE, D. A.: Nature 215, 208 (1967).

RISEBROUGH, R. W., F. C. SIBLEY, and M. N. KIREN: Am. Birds 25, 8 (1971).

SPANN, J. W., R. G. HEATH, J. F. KREITZER and L. N. LOCKE: Science 175, 328 (1972).

TEJNING, S.: Oikos, Suppl. 8, 7 (1967).

WIEMEYER, S. N. and R. D. PORTER: Nature 227, 737 (1970).