

dose level, mean microbial counts, mean mutant counts, and mean mutant frequencies.

'Due to the voluminous nature of the dominant lethal reports, it will not be feasible to present all the data for each compound. We have chosen to present data on two indexes only: implantations per pregnant female and dead implantations per pregnant female. These comprise pre- and postimplantation loss and as such constitute the most relevant indexes.

As noted, in the protocols, toxicity (LD₅₀) was determined for each substance prior to testing. We have chosen for the sake of brevity to exclude this information also.

The Publication of these protocols by the FDA should not be viewed as an endorsement or recommendation for their use.

REFERENCES

- Ames, B. N. 1971. The detection of chemical mutagens with enteric bacteria. In *Chemical mutagens: Principles and methods for their detection*, ed. A. Hollaender, vol. 1, pp. 267-282. New York: Plenum Press.
- Armitage, P. 1955. Tests for linear trends in proportions and frequencies. *Biometrics* 11:375-386.
- Bateman, A. J. 1958. Mutagenic sensitivity of maturing germ cells in the male mouse. *Heredity* 12:213-232.
- Bateman, A. J. 1960. The induction of dominant lethal mutations in rats and mice with triethylenemelamine (TEM). *Genet. Res.* 1:381-392.
- Brusick, D. J. 1970. The mutagenic activity of ICR-170 in *Saccharomyces cerevisiae*. *Mutat. Res.* 10:11-19.
- Cattanach, B. M., Pollard, C. E. and Isaacson, J. H. 1968. Ethyl methanesulfonate-induced chromosome breakage in the mouse. *Mutat. Res.* 6:297-307.
- Datta, P. K., Frigger, H. and Schleiermacher, E. 1970. The effect of chemical mutagens on the mitotic chromosomes of the mouse *in vivo*. In *Chemical mutagenesis in mammals and man*, ed. F. Vogel and G. Rohrborn, pp. 194-213. Berlin: Springer.
- Gabridge, M. G. and Legator, M. S. 1969. A host-mediated microbial assay for the detection of mutagenic compounds. *Proc. Soc. Expt. Biol. Med.* 130:831-834.
- Legator, M. S. and Malling, H. V. 1971. The host-mediated assay: A practical procedure for evaluating mutagenic agents in mammals. In *Chemical mutagens: Principles and methods for their detection*, ed. A. Hollaender, vol. 2, pp. 569-589. New York: Plenum Press.
- Legator, M. S., Palmer, K. A., Green, S. and Petersen, K. W. 1969. Cytogenetic studies in rats of cyclohexylamine, a metabolite of cyclamate. *Science* 165:1139-1140.
- Mosteller, F. and Youtz, C. 1961. Tables of the Freeman-Tukey transformations for the binomial and Poisson distributions. *Biometrika* 48:433-440.
- Mrak, E. M., Chairman. 1969. *Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health*, pp. 565-653. Washington, D.C.: U.S. Department of Health, Education, and Welfare.
- Spizizen, J. 1958. Transformation in biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleic acid. *Proc. Natl. Acad. Sci. U.S.A.* 44:1072-1078.

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UBLETHAL EFFECTS OF CHRONIC LEAD INGESTION IN MALLARD DUCKS

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Mallard drakes (*Anas platyrhynchos*) fed 1, 5, or 25 ppm lead nitrate were bled and sacrificed at 3-wk intervals. No mortality occurred, and the pathologic lesions usually associated with lead poisoning were not found. Changes in hematocrit and hemoglobin concentration did not occur. After 3 wk ducks fed 25 ppm lead exhibited a 40% inhibition of blood δ -aminolevulinic acid dehydratase activity that persisted through 12 wk exposure. After 12 wk treatment similar enzyme inhibition was present in the ducks fed 5 ppm lead. At 3 wk there was a small accumulation of lead (less than 1 ppm) in the liver and kidneys of ducks fed 25 ppm lead; no further increases occurred throughout the exposure. No significant accumulation of lead occurred in the tibiae or wing bones. Groups of ducks fed 5 and 25 ppm diets for 12 wk were placed on clean feed and examined through a 12 wk posttreatment period. After 3 wk on clean diet δ -aminolevulinic acid dehydratase activity and lead concentrations in the blood had returned to pretreatment levels. Even though lead concentrations in the blood, soft organs, and bone were low, a highly significant negative correlation between blood lead and blood enzyme activity was obtained. This enzyme bioassay should provide a sensitive and precise estimate for monitoring lead in the blood for waterfowl.

INTRODUCTION

Interpretation of the biologic effects of environmental contamination by lead is controversial and identifying the source of contamination for wildlife species remains a major problem. Experimental studies with waterfowl, either as lead shot (Bates et al., 1968; Irwin and Karstar 1972; Longcore et al., 1974) or as inorganic lead salts (Coburn et al., 1951), resulted in the highest residues in bone, lower

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RESULTS

Analysis of lead-treated feed revealed means of 1.76, 5.13, and 21.63 ppm lead in the three respective diets (1, 5, and 25 ppm lead). Control feed contained a mean of 1.10 ppm lead, indicating rather high background levels of lead in feed material. These results are based on calculations using wet weights of feed that contained an average of 10.4% moisture and are within the range of detectability of the analytical methods used.

No mortality occurred during the study that could be attributed to lead ingestion. Body weights and food consumption did not differ among treatment groups or controls. During the 12-wk treatment period, ducks consumed an average of 119 g feed daily. Body weights of sacrificed birds averaged 1,162 g. Necropsy of sacrificed birds failed to reveal any tissue lesions commonly associated with lead poisoning in waterfowl. Ducks exhibited no muscular or liver atrophy and contained heavy fat deposits. Microscopic examination failed to reveal any acid-fast intranuclear inclusion bodies in kidney tubular cells of treated or control birds.

No differences were found in hemoglobin concentration or hematocrit in any treatment group when compared with controls. Means for erythrocyte ALAD activity and concentration of lead in the blood are given in Table 1. An overall mean of 172.6 units and 20 ppb blood lead was found in controls

TABLE 1. δ -Aminolevulinic Acid Dehydratase Activity^a and Lead Residues (ppb) in Blood of Mallard Ducks Fed Different Concentrations of Lead Nitrate

Lead in feed ($\mu\text{g/g}$)	Sampling interval (wk)			
	3	6	9	12
	ALAD enzyme activity (units)			
0	171.0 \pm 13.0	173.7 \pm 11.9	172.1 \pm 17.8	172.7 \pm 19.1
5	164.3 \pm 5.0	172.8 \pm 21.8	166.4 \pm 15.0	169.2 \pm a.
25	102.3 \pm 28.5* \pm 7.1	179.0 \pm 22.8 121.1 \pm 16.0'	170.0 \pm 27.7 126.5 \pm 21.7	110.8 \pm 11.2* 94.0 \pm 8.2*
	Lead concentration (ppb)			
0	c	20 \pm 0	22 \pm 2	20 \pm 0
1	27	20 \pm 0	28 \pm 6	22 \pm 2
5	\pm 7	41 \pm 10	26 \pm 6	66 \pm 13*
25	55 \pm 9* 138 \pm 22*	135 \pm 21*	142 \pm 14*	154 \pm 28*

^aUnit enzyme activity defined as 0.100 increased in absorbance/ml erythrocytes per hour (38°C) at 555nm with a 1.0-cm light path. Mean \pm SE, N=5.

^bAsterisk denotes significantly different from controls, $p < 0.01$, Student's *t* test.

^cBlood samples were not analyzed, values were chosen at random from the 15 control samples collected throughout the study to test for significant differences.

during the 12-wk treatment period. After 3 wk ducks fed 25 ppm lead exhibited a 40% decrease in blood ALAD activity and contained a mean of 138 ppb lead in the blood. This magnitude of inhibition and level of blood lead remained through the 12-wk treatment period. Ducks fed 5 ppm lead in the diet for 12 wk exhibited a similar degree of enzyme inhibition (36%).

Enzyme activity returned to normal within 3 wk following 12 wk of lead treatment. After 3 wk on clean feed ducks fed diets of 5 and 25 ppm lead exhibited normal ALAD levels of 161.3 and 192.7 units, respectively. These values were associated with blood lead levels of 66 and 46 ppb. ALAD activity remained normal throughout the 12-wk posttreatment period. Blood samples collected at 6, 9, and 12-wk intervals following lead treatment were not analyzed for lead.

The relationship between blood lead concentration and ALAD activity was examined further by comparing individual values obtained after 12 wk for all treatment levels and controls. When the variables are plotted on a logarithmic scale, a significant negative correlation ($p < 0.01$) was found between ALAD activity and lead levels (Fig. 1). Values shown range from 20 to 220 ppb lead and from 66.1 to 255.0 units of ALAD activity. It seems that ALAD activity is significantly reduced in ducks when blood lead approaches 100 ppb. This inverse relationship between erythrocyte ALAD activity and blood lead concentration is clearly illustrated in Fig. 2 when treatment means are plotted against dietary lead levels after 12 wk on lead diets.

Lead concentration in livers and kidneys was low for all treatment levels (Table 2). Residues ranged from less than 0.10 to 0.31 ppm in livers and from less than 0.10 to 2.67 ppm in kidneys. Feeding 1 and 5 ppm lead for 12 wk did not cause a significant accumulation of lead in the kidney or liver. Ducks fed the 25 ppm lead diet contained significantly higher ($p < 0.05$) lead residues in livers and kidneys after 3 wk when compared with controls or the two lower treatments. Residues did not significantly increase with the number of weeks birds were fed lead. Residues were not analyzed in organs of ducks fed clean feed following the lead treatment. Analysis of liver and kidney weights when expressed as a percentage of whole body weight revealed no significant difference among any treatment group for any sampling interval.

Lead residues in tibiae and radii-ulnae bones did not differ among treatment groups or with time (Table 3). Residues ranged from 0.87 to 14.43 ppm in tibiae and from 0.73 to 14.66 ppm in radii-ulnae bones. Regression analysis revealed a direct correlation between lead concentration in the tibiae and in the radii-ulnae ($r = 0.98$; $p < 0.01$). The higher tibiae residues were accompanied by equally high wing bone residues in all lead-treated ducks.

et al., 1973; Tomokuni, 1974). The value of this parameter as an indicator of environmental lead has also been demonstrated with pigeons and jack ducks. Ohi et al. (1974) reported that pigeons from urban areas had an average of 330 ppb lead in the blood, compared with only 29 ppb in birds from rural areas. The birds with higher lead levels exhibited 77% lower ALAD enzyme activity. Wild canvasback ducks exhibited a 75% decrease in ALAD enzyme activity when blood lead concentrations exceeded 200 ppb (Dieter et al., 1976). Experimental studies with mallards given sublethal levels of lead shot revealed that 200 ppb lead in the blood resulted in 50-75% inhibition of ALAD activity that remained up to 3 months following dosage (Dieter and Finley, 1975; Finley et al., 1976). In all of these studies enzyme inhibition preceded changes in hematocrit or hemoglobin values.

The maximum ALAD enzyme inhibition attained in this study was only 40%. No mortality or gross lesions usually associated with lead poisoning in waterfowl (Karstad, 1971; Locke et al., 1966) occurred. Treated ducks did not accumulate lead residues in bones when compared with controls, and only the group fed 25 ppm showed significantly increased lead concentrations in the liver and kidneys; these concentrations in soft organs seldom exceeded 1 ppm. Acute lead studies with waterfowl demonstrated that 6-20 ppm lead in the liver and 10-20 ppm in the kidney were required to cause pathologic changes (Longcore et al., 1974). Coburn et al. (1951) found that the critical lead level for mallard ducks was 6-8 mg/kg daily when dosed with lead nitrate. Those ducks exhibited characteristic signs of lead poisoning, including lowered hematocrits and hemoglobin concentrations. In our study ducks fed 25 ppm dietary lead consumed about 2.5 mg/kg lead daily, accumulating up to 210 mg lead within 12 wk. Our data show that ingestion of lead up to 25 ppm accompanied by a nutritionally adequate diet does not cause appreciable lead accumulation.

We have found that the only indirect way to detect the level of lead ingestion that approximates the amount encountered through the food chain is to measure blood ALAD enzyme activity. We obtained an excellent dose response after 12 wk on the low-lead diet, indicating the extreme sensitivity of this enzyme to lead concentrations in the blood. The rapidity of the enzyme response was evident in the recovery phase of the experiment, where after 3 wk on lead-free diets blood lead concentrations decreased to 50 ppb and ALAD enzyme activity returned to normal. Although it is difficult to distinguish the effects of chronic indirect lead accumulation through the food chain from that of acute direct lead accumulation after ingesting lead shot, ALAD enzyme inhibition from a recently ingested lead shot is usually unmistakable. Mallard ducks dosed with only one number 4 shot exhibited 88% ALAD inhibition (Finley et al., 1976) as compared with 40% in this study, and inhibition after lead shot dosage persisted for 3 months (Dieter and Finley, 1975) instead of only 3 wk. The sensitivity and rapid responsiveness of the ALAD enzyme bioassay should aid in distinguishing the source of environmental lead contamination in waterfowl surveys.

REFERENCES

- Barrett, M. W. and Karstad, L. H. 1971. A fluorescent erythrocyte test for lead poisoning in waterfowl. *J. Wildl. Manage.* 35:109-119.
- Bates, F. Y., Barnes, D. M. and Higbee, J. M. 1968. Lead toxicosis in mallard ducks. *Bull. Wildl. Dis. Assoc.* 4:116-125.
- Burch, H. B. and Siegel, A. L. 1971. Improved method for measurement of δ -aminolevulinic acid dehydratase activity of human erythrocytes. *Clin. Chem.* 17:1038-1041.
- Choi, D. D. and Richter, G. W. 1972. Lead poisoning: Rapid formation of intranuclear inclusions. *Science* 117:1194-1195.
- Coburn, D. R., Metzler, D. W. and Treichler, R. 1951. A study of absorption and retention of lead poisoning in wild fowl in relation to clinical evidence of lead poisoning. *J. Wildl. Manage.* 15:186-192.
- Dieter, M. P. and Finley, M. T. 1975. Kinetics of δ -aminolevulinic acid dehydratase (ALAD) enzyme response in ducks given lead shot. (In preparation).
- Dieter, M. P., Perry, M. C. and Mulhern, B. C. 1976. Lead and PCB's in canvasback ducks: Relationship between enzyme levels and residues in blood. *Arch. Environ. Contam. Toxicol.* 4(3).
- Finley, M. T., Dieter, M. P. and Locke, L. N. 1976. δ -Aminolevulinic acid dehydratase: Inhibition in ducks dosed with lead shot. *Environ. Res.* (In press).
- Haeger-Aronsen, B., Abdulla, M. and Fristedt, B. I. 1971. Effect of lead on δ -aminolevulinic acid dehydratase activity in red blood cells. *Arch. Environ. Health* 2x440-445.
- Haeger-Aronsen, B., Abdulla, M. and Fristedt, B. I. 1974. Effect of lead on δ -aminolevulinic acid dehydratase activity in red blood cells. II. Regeneration of enzyme after cessation of lead exposure. *Arch. Environ. Health* 29:150-153.
- Henry, R. J. 1964. Determination of blood hemoglobin, cyanmethemoglobin method. In *Clinical Chemistry*, p. 742. New York: Harper & Row.
- Hernberg, S., Nikkanen, J., Mellin, G. and Lilius, H. 1970. δ -Aminolevulinic acid dehydratase as a measure of lead exposure. *Arch. Environ. Health* 21:140-145.
- Irwin, J. C. and Karstad, L. H. 1972. The toxicity for ducks of disintegrated lead shot in a simulated-marsh environment. *J. Wildl. Dis.* 8:149-154.
- Jefferies D. J. and French, M. C. 1972. Lead concentrations in small mammals trapped on roadside verges and field sites: Lyön. *Pollut.* 3:147-156.
- Karstad, L. 1971. Angiopathy and cardiomyopathy in waterfowl after ingestion of lead shot. *Can. J. Med.* 35:355-360.
- Locke, L. N., Irby, H. D. and Bagley G. E. 1966. Acid-fast, intranuclear inclusion bodies in the kidneys of mallards fed lead shot. *Bull. Wildl. Dis. Assoc.* 2:127-131.
- Longcore, J. R., Locke, L. N., Bagley G. E., and Andrews, R. 1974. Significance of lead residues in mallard tissues. *Fish Wildl. Serv. Spec. Sci. Rep. Wildl.* 182:1-24.
- Martin, W. E. 1972. Mercury and lead residues in starlings—1970. *Pest. Monit. J.* 6:27-33.
- Ohi, G., Seki, H., Akiyama, K. and Yagyu, H. 1974. The pigeon, a sensor of lead pollution. *Bull. Environ. Contam. Toxicol.* 12:292-296.
- Secchi, G. C., Erba, L. and Cambiaghi, G. 1974. δ -Aminolevulinic acid dehydratase activity of erythrocytes and liver tissue in man. *Arch. Environ. Health* 28:130-132.
- Tanaka, S., Hernberg, S., Asp, S. and Nikkanen, J. 1973. Parameters indicative of absorption and biological effect in new lead exposure: A prospective study. *Br. J. Ind. Med.* 30:134-141.
- Tomokuni, K. 1974. δ -Aminolevulinic acid dehydratase test for lead exposure. *Arch. Environ. Health* 29:274-281.
- Williamson, P. and Evans, P. R. 1972. Lead: Levels in roadside invertebrates and small mammals. *Bull. Environ. Contam. Toxicol.* 8:280-288.

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