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Lead contamination of chicken eggs and tissues from a small farm flock

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Abstract. Twenty mixed-breed adult laying hens from a small farm flock in Iowa were clinically normal but had been exposed to chips of lead-based paint in their environment. These chickens were brought to the Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa, where the concentration of lead in blood, eggs (yolk, albumen, and shell), and tissues (liver, kidney, muscle, and ovary) from 5 selected chickens was determined over a period of 9 days. Blood lead levels ranged from less than 50 to 760 ppb. Lead contamination of the yolks varied from less than 20 to 400 ppb, and shells were found to contain up to 450 ppb lead. Albumen contained no detectable amount. Lead content of the egg yolks strongly correlated with blood lead levels. Deposition of lead in the shells did not correlate well with blood lead levels. Mean tissue lead accumulation was highest in kidneys (1,360 ppb), with livers ranking second (500 ppb) and ovarian tissue third (320 ppb). Muscle contained the lowest level of lead (280 ppb). Lead contamination of egg yolks and edible chicken tissues represents a potential public health hazard, especially to children repeatedly consuming eggs from contaminated family-owned flocks.

Lead is ubiquitous in the environment, persists indefinitely, and can be found at low levels in almost all living organisms.[3] Sources of lead contamination of air, water, and soil include internal combustion engines, oil burners, smelters, lead pipes, glass and alloy processing plants, incinerators, industrial effluents, and smokestack fallout.[11] Lead is found in the soil, plants and grains grown on contaminated soil, and tissues of animals that eat contaminated plants and feed grains.[2] Because of widespread environmental exposure, low levels of lead can be demonstrated in tissues of clinically normal birds and animals.[3] Lead toxicosis occurs when an animal or a bird inhales or ingests a concentrated source of lead. Concentrated lead sources include lead-based paint, lead arsenate crop sprays, lead plates in automotive batteries, fishing sinkers, lead shotgun pellets, drapery weights, sewage sludge, and lead mine tailings.[7]

Chickens are susceptible to lead intoxication. As little as 1.0 mg/kg lead in the diet can cause significant depression in the growth of broiler chickens and consistent reduction in blood δ-aminolevulinic acid dehydratase, an erythrocyte enzyme sensitive to lead.[1] Clinical signs of acute lead poisoning in chickens include muscle weakness, ataxia, and loss of appetite, followed by marked weight loss and eventual cessation of egg production. A severe anemia may develop. Young chickens are more susceptible than adult chickens.[9,12] Long-term lead intoxication of chickens results in degeneration of motor nerves in the spinal cord and loss of axons in peripheral nerves without demyelination. In addition, muscles show atrophy and degeneration of fibers.[6] Attempts to measure the effects of lead on the chicken's cell-mediated immune response, humoral immune response, and interferon production have yielded inconsistent results.[14,16]

Lead ingested by chickens is deposited in bones, soft tissues, and eggs and produces elevated blood lead levels.[1] Bone lead concentrations are by far the highest, followed by kidney and liver. The lowest concentration of lead is found in skeletal muscle.[14] Eggs accumulate lead in their shells, yolk, and albumen, with the highest concentration occurring in the shells.

A detailed evaluation of lead in blood, tissues, and eggs in a clinical case of lead toxicosis in laying hens is reported in this study. In April 2000, acute lead poisoning was diagnosed in a laying hen (blood lead = 1,500 ppb) from a small farm in northeast Iowa. This chicken showed clinical signs indicative of central nervous system dysfunction before submission. Laying hens remaining in the farm flock had been observed consuming chips of lead-based paint peeling from a farm building. All 20 remaining chickens were clinically normal and exhibited no clinical signs suggesting central nervous system disease. The chickens and a sample of the paint chips from this farm were brought to the Veterinary Diagnostic Laboratory at Iowa State University, Ames, Iowa, for further study. The objective of this study in laying hens was to examine the relationship between the concentration of lead in blood and lead levels found in eggs (shells, yolk, and albumen) and tissues (liver, kidney, pectoral muscle, and ovary).

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Table 1. Graphite furnace atomic absorption spectrophotometer conditions for lead determination in egg samples.

Temperature (C)	120	400	650	20	1,600	2,600
Ramp (sec)	15	1	1	1	0	0
Hold (sec)	45	20	15	15	0	0
Internal gas (ml/min)	300	300	300	300	0	300
Read (sec)					X	

Materials and methods

Experimental design

The 20 hens were housed separately in cages and randomly assigned a number (nos. 1–20). Eggs were collected for 9 consecutive days beginning on the day after chickens were removed from the farm. Blood lead levels of all 20 chickens were determined on days 2 and 9 of the experiment from samples collected in heparin tubes. Packed cell volume (PCV) was determined for day 2 blood samples to assess erythrocyte status. Five hens were selected for further study (hen nos. 3, 8, 10, 16, and 18) on the basis of day 2 blood lead levels that differed by increments of approximately 100 ppb and ranged from 240 to 645 ppb. One egg from each selected hen laid on days 1, 5, and 9 was separated into yolk, albumen, and shell, which were analyzed separately for lead. On day 9, blood samples were again collected from all 20 hens, all chickens were euthanized by intravenous pentobarbital injection and necropsied, and fresh tissue samples (liver, kidney, pectoral muscle, and ovary) were collected for lead analysis from each of the 5 hens under study.

Apparatus and reagents. Lead analysis was performed using a graphite furnace atomic absorption spectrophotometer with Zeeman correction equipped with an autosampler.[a] Flame atomic absorption was performed on a flame atomic absorption spectrophotometer.[b]

Sample lead analysis. Paint chips were wet digested in 20% magnesium nitrate in concentrated nitric acid, dry ashed at 500 C, and dissolved in 20% nitric acid. These samples were then analyzed for lead content by flame atomic absorption spectrophotometry and calculated against a standard curve.[13] Blood samples were analyzed for lead on a wet-weight basis using a graphite furnace atomic absorption spectrophotometer with Zeeman correction and calculated against a standard curve.[8] Tissues samples were dry ashed at 500 C, dissolved in 2 N HCl, and analyzed for lead on a wet-weight basis by flame atomic absorption spectrophotometry and calculated against a standard curve.[13]

Eggs were soaked in 0.1% Triton X-100 for 4 hr and brushed clean to eliminate external contamination. Eggs were thoroughly rinsed in distilled or deionized water and allowed to air-dry. Eggs were separated into albumen, yolk, and shell. Each albumen and yolk sample was thoroughly blended before weighing for analysis, and lead concentrations were determined on the basis of wet weights. Shells were rinsed and dried overnight at 103 C in a drying oven, and lead concentrations were determined on the basis of dry weights. Shells were blended to a diameter of 1 mm or less before weighing for analysis to obtain a uniform sample.

Control eggs were collected from similar breed chickens (with no known lead exposure) and treated and analyzed in a similar manner as experimental eggs. All egg samples were analyzed by the method of standard additions using 50, 100, and 200 ppb lead. The egg samples were wet digested using a modified Environmental Protection Agency 3050A (Jul 1992) procedure. The digestion used trace metal grade–concentrated nitric acid and 30% hydrogen peroxide mixture (20:1, v/v) using up to 6 ml hydrogen peroxide for complete digestion.[4] All samples were analyzed for lead using graphite furnace atomic absorption spectrophotometer with Zeeman correction. The furnace method[8] was modified as shown in Table 1 for the egg analysis.

A water control sample certified by the National Institute of Standards and Technology Standard Reference Material 3128 was run with each group of samples as a quality-control measure.

Results

Paint chips from the farm building were analyzed and found to contain 38.5% lead. No paint chips were found in the digestive tract when the chickens were necropsied on day 9 of the study.

Results of blood lead analyses on days 2 and 9 and PCVs on day 2 are presented in Table 2. The concentration of lead in the blood on day 2 ranged from less than 50 to 760 ppb, with a mean of 453 ppb. On day 9, the lowest lead level was 130 ppb, the highest was 800 ppb, and the average fell to 373 ppb. From days 2 to 9, levels of lead in the blood increased in 7 chickens and declined in 13 others. Packed cell volume varied from 22% to 47%. Normal PCV for domestic chickens is 24–43%.[10] Seventeen of 20 chickens had PCVs within the normal range.

Lead contamination of 3 eggs (laid on days 1, 5, and 9) from 5 selected hens was determined separately

Table 2. Blood lead on days 2 and 9 and PCV on day 2 from chickens after ingestion of lead-based paint chips.

Chicken number	Blood lead (ppb)*		Blood PCV (%)
	Day 2	Day 9	
1	<50	130	44
2	630	800	31
3	475	150	30
4	420	360	29
5	760	380	32
6	670	560	22
7	230	140	47
8	140	290	26
9	355	140	27
10	645	720	28
11	435	310	28
12	570	490	27
13	390	340	28
14	360	470	27
15	340	330	37
16	525	330	28
17	280	300	27
18	330	320	27
19	615	640	26
20	430	260	28
$\bar{x} \pm$ SD	453 ± 164	373 ± 190	30 ± 6
Control 1	<50	<50	
Control 2	<50	<50	
Control 3	<50	<50	

* Wet-weight basis.

for eggshells, albumen, and yolks. Varying levels of lead were deposited in the eggshells (Table 3), and there was no relationship between the concentration of lead in the blood and in the shells. From days 1 to 9, the amount of lead in eggshells declined for 3 hens and increased for 2 chickens. No measurable quantity of lead was found in the albumen of the eggs, with all analyses showing less than 10 ppb. Lead concentrations in yolks ranged from less than 20 to 400 ppb (Table 4). Yolk lead increased with time in eggs from each of the 5 hens. Mean lead concentrations on days 1, 5, and 9 were 170, 187, and 262 ppb, respectively.

Table 3. Shell lead on days 1, 5, and 9 in eggs from chickens after ingestion of lead-based paint chips.

Chicken number	Shell lead (ppb)*		
	Day 1	Day 5	Day 9
3	92	150	73
8	27	47	450
10	19	150	220
16	83	88	72
18	170	17	110
$\bar{x} \pm$ SD	78 ± 61	90 ± 60	185 ± 159
Control 1	<10	<10	<10
Control 2	17	17	17

* Dry-weight basis.

Table 4. Yolk lead on days 1, 5, and 9 in eggs from chickens after ingestion of lead-based paint chips.

Chicken number	Yolk lead (ppb)*		
	Day 1	Day 5	Day 9
3	120	250	340
8	<20	57	100
10	200	240	400
16	180	230	290
18	130	160	180
$\bar{x} \pm$ SD	170 ± 36	187 ± 81	262 ± 121
Control 1	<10	<10	<10
Control 2	12	12	12
Control 3	<10	<10	<10

* Wet-weight basis.

On each of the 3 days, the mean concentration of lead in eggs was highest in yolks, intermediate in shells, and lowest in albumen.

Most ovarian follicle development in chickens occurs within the last 7 days before ovulation.[4] A comparison of the concentration of lead in day 2 blood with lead levels found in day 9 yolks is presented in Fig. 1. Although the correlation between blood lead and yolk lead was not statistically significant because of small numbers of samples, a strong linear relationship was observed between the 2 values (R^2 = 0.927). As blood lead levels rise, yolk lead levels increase as well.

Liver, kidney, skeletal muscle, and ovarian tissue from the 5 selected hens all contained higher concentrations of lead than the corresponding tissues from control chickens (Table 5). Mean lead accumulation was highest in kidneys (1,360 ppb), with livers ranking second (500 ppb) and ovarian tissue third (320 ppb). Muscle contained the lowest levels of lead (280 ppb). Lead concentrations in the kidneys reached 2,500 ppb in 1 chicken, whereas the highest level found in the livers was 700 ppb. A positive linear relationship was observed between blood lead and lead concentrations in liver (R^2 = 0.645) and ovarian tissue (R^2 = 0.417).

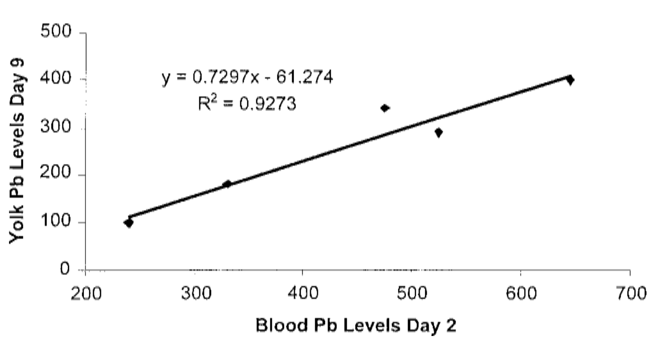

Figure 1. Blood lead (Pb) levels of day 2 versus yolk lead (Pb) levels on day 9.

Table 5. Tissue lead on day 9 from chickens after ingestion of lead-based paint chips.

Chicken number	Liver lead (ppb)*	Kidney lead (ppb)*	Muscle lead (ppb)*	Ovary lead (ppb)*
3	700	1,000	300	300
8	400	1,200	300	200
10	700	2,500	200	600
16	400	1,200	400	200
18	300	900	200	300
$\bar{x} \pm$ SD	500 ± 187	1,360 ± 650	280 ± 84	320 ± 164
Control 1	200	500	<100	<100
Control 2	100	100	100	100
Control 3	200	<100	100	<100

* Wet-weight basis.

There was no relationship between blood lead and lead levels in kidney or muscle.

Discussion

This study is the first report on concurrent lead measurements in the blood, tissues, and eggs of lead-exposed hens. The mean blood lead concentration of the 20 hens in this study (453 ± 164 ppb) was higher than that in samples from non–lead-exposed hens (<50 ppb) and confirmed that the hens in this study had ingested and absorbed significant quantities of lead (Table 1). Additional evidence for this conclusion was provided by elevated lead concentrations in tissues (liver, kidney, muscle, and ovary) from the 5 selected hens (Table 4). The presence of increased lead in ovarian tissue is of special interest because lead residues in the ovary are transferred into the yolk of developing eggs after ovulation.

Because of the potential health risk for lead exposure to people consuming eggs from lead-exposed hens, the concentration of lead in both the shell and edible portion of eggs laid by the 5 selected hens was measured on days 1, 5, and 9 of this study. The concentration of lead in the eggshells ranged from 17 to 450 ppb, with an overall mean shell lead for the 15 eggs of 117 ppb (Table 2). These concentrations are lower than those reported in a previous study of chronic lead poisoning in hens, which found the concentration of lead in eggshells to range from 2,560 to 16,180 ppm.[5]

Analysis of the albumen of eggs in this study showed that all samples contained less than 10 ppb lead. These results are consistent with previous reports of negligible lead residues in the albumen from eggs from lead-exposed hens.[2,3,5,15]

Concentrations of lead in the yolk of eggs were greatly elevated as compared with egg yolks from hens with no known lead exposure (Table 3). Mean yolk lead concentration of eggs laid on day 9 of this study was 262 ± 121 ppb. Previous studies reported variable concentrations of lead in egg yolk from lead-exposed chickens. A 329-day trial with feed levels of 2 and 5 ppm lead resulted in egg yolk lead levels of 100 ppb; however, a 198-day trial using 80 ppm lead in the feed for hens reportedly did not increase the lead concentration in egg yolk.[15] In another study, investigators fed a diet containing the equivalent of 7.8 ppm lead to hens for 34 days.[2] They found no increase in lead in yolk from eggs laid on days 7 and 124 of the trial but reported a lead concentration of 3.38 μg/g (dry weight) in the yolk of eggs laid on day 34 of the study. Yolk lead concentrations ranged from 400 to 1,080 ppb in eggs from hens with chronic lead poisoning.[5] A mean yolk lead concentration of 60 ppb was reported in 127 eggs collected in a survey of free-range hens that showed evidence of lead exposure as indicated by liver and kidney lead residues.[3]

This is the first report on concurrent blood and tissue levels from hens laying lead-contaminated eggs. The data show a strong positive correlation between blood lead and the concentration of lead in the yolk of eggs. Mean lead concentrations in edible tissues such as liver and muscle were greater than those found in egg yolk. Eggs and chicken tissues containing significant concentrations of lead are a potential human health hazard, especially to young children. Repeated consumption of contaminated eggs from a family-owned flock could provide a continuing dietary source of lead.

Sources and manufacturers

a. Perkin-Elmer 5100, Norwalk, CT.
b. Unicam, Thermal Elemental, Cambridge, UK.

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