Lead Poisoning In Chickens and the Effect of Lead on Interferon and Antibody Production

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ABSTRACT

The effect of aqueous lead acetate given per os to chickens for 35 consecutive days and the effect of lead on interferon and antibody production was investigated. Chickens were found to tolerate levels of lead as high as 160 mg/kg/day without exhibiting clinical signs or hematological changes in spite of very high levels of lead in the blood (6.2 ppm). It is apparent from these findings that chickens are more resistant to lead poisoning than humans, horses, dogs and wild fowl such as ducks.

Subclinical lead doses did not affect interferon induction in response to statolon and Newcastle Disease virus (NDV)-B_1. Interferon concentrations and duration in serum were markedly decreased in chickens which received lead at the 320 mg/kg level.

Long time lead exposure had no marked effect on antibody production to NDV in chickens. No consistent correlation was observed between blood lead concentration and antibody titer.

The results of these studies indicate that long term subclinical lead intake suppresses neither interferon nor antibody production in chickens.

RÉSUMÉ

Cette étude visait à déterminer l'effet de l'administration orale d'une solution aqueuse d'acétate de plomb à des poulets, pendant 35 jours consécutifs, ainsi que celui du plomb sur l'interféron et l'élaboration d'anticorps. Les poulets ingérèrent jusqu'à 160 mg/kg/jour de cet élément, sans manifester de signes cliniques ou d'altérations hématologiques, en dépit d'une forte teneur sanguine en plomb (6.2 ppm). Ces constatations révèlent que les poulets résistent mieux à l'empoisonnement par le plomb que l'homme, le cheval, le chien et le canard sauvage.

De faibles doses de plomb n'affectèrent pas l'élaboration d'interféron provoquée par le statolon et la souche B du virus de la pneumo-encéphalite aviaire. La teneur sérique en interféron et sa durée diminuèrent considérablement chez les poulets recevant du plomb, à raison de 320 mg/kg.

L'ingestion prolongée de plomb par les poulets ne produisit pas d'effet important sur l'élaboration d'anticorps contre le virus de la pneumo-encéphalite aviaire. On ne décela pas de relation soutenue entre la teneur du sang en plomb et en anticorps.

Les résultats de cette étude révèlent que l'ingestion prolongée de faibles doses de plomb ne supprime ni l'interféron, ni l'élaboration d'anticorps.

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INTRODUCTION

The interferon system appears to play a major role in recovery from viral infections for it is active early in infection, during viremia and before pathological changes occur (12). The quantities of interferon produced early in infection are sufficient to limit or prevent viral replication at the portal of entry. The appearance of measurable antibody is somewhat delayed by comparison and inhibits subsequent viral dissemination or limits reinfection (12).

Interferon response may be altered by various factors. Stress (27), hormones (19, 27), mycoplasma (26), arsenicals (11)

and chemical carcinogens (9) have suppressive effects. Among the natural substances that man concentrates in his immediate environment lead is one of the most ubiquitous (6). Even with the increased awareness of lead toxicity today it still continues to be a common toxicant of livestock, pets and man.

There is considerable evidence that lead exerts adverse effects on the resistance of the body to disease. Lead poisoning has been associated with the increased susceptibility of mice to salmonella infection (13) and subclinical doses of lead acetate increased sensitivity of the rat (24) and chicken (29) to endotoxin. Williams *et al* (34) suggested that lead may inactivate antibodies and thereby interfere with the immunity of man and animals to infectious diseases. Lead treated animals subsequently subjected to procedures of active immunization developed lesser quantities of gamma globulin, complement underwent progressive diminution and anti-typhoid antibody titers were reduced (8). Interference with the phagocytic activity of polymorphonuclear leukocytes (20) and reduction of lysozyme activity (8) were also reported in lead poisoning.

There is very little information in the literature on the effects of lead in chickens. Most published reports on lead poisoning in birds concern its toxicity in wild fowl (4, 7).

The objective of this study was to investigate the effect of aqueous lead acetate given *per os* to chickens for 35 consecutive days. The effect of long term lead exposure on antibody to Newcastle Disease virus (NDV)-B_1 and interferon production using statolon and NDV-B_1 as the inducers was studied.

MATERIALS AND METHODS

CELL CULTURES

Primary chicken embryo fibroblast (CEF) cells were used for vesicular stomatitis virus (VSV) propagation, titration and interferon assay. CEF cells were prepared from ten and 11 day old embryos using a previously described technique (23). Growth medium consisted of Eagle's basal medium (BME) with Earle's salts, supplemented with 5% fetal calf serum[1], 10% tryptose phosphate broth[1] and 0.11% sodium bicarbonate.

The Madin-Darby bovine kidney (MDBK) cells which were used for interferon characterization were grown and maintained in BME with Earle's salts, supplemented with 10% newborn calf serum and 0.11% sodium bicarbonate. The maintenance medium was the same except that the concentration of serum was reduced to 2%.

INTERFERON INDUCERS

Swine influenza virus, Shope strain (SIV) of undetermined passage level was propagated by allantoic cavity inoculation of 11 day old chicken embryos. The allantoic fluid was harvested at 96 hours postinoculation from both dead and live eggs. The pooled allantoic fluid has a titer of 640 chicken erythrocyte hemagglutinating (HA) units per ml.

Pseudorabies virus strain DR (PRV-DR) was isolated[2] on MDBK cells from the brain of a calf which died of pseudorabies. PRV-DR at the twelfth passage level on MDBK with a titer of 3.2×10^5 pfu/ml was used to induce interferon.

Newcastle disease virus, B_1 strain (NDV-B_1)[3] of undetermined passage level was propagated by allantoic-cavity inoculation of 11 day old chicken embryos. After embryonic death the allantoic fluid was harvested, pooled and the virus was partially purified and concentrated using a previously described centrifugation technique (30). The virus was titrated by the HA test.

Polyriboinosinic-polyribocytidylic acid (poly I:poly C)[4] (Serial number 76645) was used as the interferon inducer in chickens at the doses indicated in Table III.

Statolon[5] (lot 354-1080-B220) was weighed and suspended in distilled water at 50 or 500 mg/kg for use as an interferon inducer.

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[1]Grand Island Biological Company, Grand Island, New York.

[2]Dr. C. J. Maré, College of Veterinary Medicine, Iowa State University.

[3]Obtainable from Dr. M. S. Hofstad, Veterinary Medical Research Institute, Iowa State University.

[4]Microbiological Associates, Bethesda, Maryland.

[5]Obtained from Dr. W. J. Kleinschmidt, Eli Lilly and Co., Indianapolis, Indiana.

TABLE I. The Concentration[a] of Lead in the Blood of Chickens Given Lead *per os*

Daily Dose of Lead	Concentration of Lead (ppm)[b] Days After Treatment		
	10	21	30
Control (no lead)	0.04	0.05	0.05
20 mg/kg......	0.8	1.6	2.0
40 mg/kg......	1.1	1.8	2.8
80 mg/kg......	1.7	2.6	4.1
160 mg/kg......	—	5.9	6.2
320 mg/kg[c].....	4.75	—	12.0
640 mg/kg[d].....	10.5	—	—

[a]Pooled blood of 12 chickens
[b]ppm — parts per million
[c]One chicken died on the 11th day, five chickens died between days 21 and 30
[d]One chicken died on the sixth day, nine chickens died between days 11 and 21 and one died on day 28

Experimental Chickens and Interferon Induction

White Leghorn chickens of the Babcock strain were obtained from the specific-pathogen-free flock maintained at the Veterinary Medical Institute, Iowa State University. All the chickens for a given experiment were hatched together and subsequently held in wire floored battery brooders and in wire cages. Statolon, poly I:poly C and the viruses PRV-DR, SIV and NDV-B_1 were used as interferon inducers.

Nine groups consisting of three ten week old chickens per group were inoculated using different routes and doses of interferon inducers (Table III). Before inoculation and at specified times after inoculation blood samples were collected for interferon assay using the jugular and wing veins. Blood of the chickens from the same treatment group was pooled. After firm clots were formed at room temperature the blood was incubated for one to one and one half hours at 37°C and serum was harvested after centrifugation for 15 minutes at 2000 rpm. The sera were dialyzed in cellulose tubing at 4°C against 0.1M KC1-HC1 solution (pH 2) for 24 hours and then against phosphate buffer saline (PBS) (pH 7.5) for 24 additional hours. The sera were then filtered through 300 nm Millipore filters[6] and stored at –20°C until assayed.

Interferon Assay and Characterization

Interferon was assayed by the plaque reduction method following a previously described procedure (31) on CEF cells using vesicular stomatitis virus, Indiana strain (VSV-In) of unknown passage level. The second and third passage level on CEF cells was used for interferon assays. Crystal violet was used for plaque staining (33). The antiviral substance was characterized as being interferon by previously described techniques (31).

Lead Acetate

Lead acetate[7] dissolved in water was used as a lead source for chickens at the doses indicated in Table I.

Determination of Lead in Blood and Tissues

Analyses for lead in blood and tissues were performed by J. Hurd, Veterinary Diagnostic Laboratory, Iowa State University. Blood lead determinations were done using the procedure described by Hessel (14).

Unfixed tissues for chemical analysis were ashed in a furnace at 450-475°C for four to five hours after drying to constant weight. The ash was dissolved in a 2 N HCl and then diluted with water to an appropriate volume. The Perkin-Elmer atomic absorption spectrophotometer, Model 303, was used for all determinations. Instrument settings were those recommended in the Analytical Methods for Atomic Absorption Spectrophotometry (1).

Hematological Procedures

Total leukocyte counts were estimated using the Rees-Ecker method (16). Slide smears were air dried and stained with standard Wright's stain[8] for differential leukocyte counts. Erythrocyte counts were made using a Coulter counter (2). Packed cell volume (PCV) was determined by the

[6]Millipore Filter Corporation, Bedford, Massachusetts.

[7]Mallinckrodt Chemical Works, St. Louis, Missouri.

[8]Harleco, Philadelphia, Pennsylvania.

TABLE II. The Concentration of Lead in Tissues of Chickens[a] Given Lead *per os* for 35 Days

	Daily Dose of Lead *per os*					
	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg	320 mg/kg	640 mg/kg[b]
Tissue	Mean Concentration of Lead (ppm) in Tissues					
Brain	5.4	8.9	7.8	7.3	14.8	15.4
Lung	1.9	8.1	4.4	6.3	16.9	77.3
Muscle	1.3	5.1	3.1	4.2	10.6	13.7
Kidney	4.9	10.8	11.2	11.2	49.8	152.1
Liver	2.1	9.5	7.7	7.2	18.9	78.9
Bone	65.7	166.9	282.6	438.1	494.8	310.5

[a]Four chickens per treatment group
[b]Chickens of this treament group died between days 12 and 18 after initiation of lead treatment

micro hematocrit method using capillary tubes. The tubes were heat sealed and centrifuged at 15,000 rpm for three minutes and the percentage of packed erythrocytes was determined. Hemoglobin values were determined using the cyanmethemoglobin technique (2).

Histopathological Procedures

The tissues collected at necropsy were fixed in 10% buffered formalin. All tissues were dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin. Sections were cut at 6 μm and mounted on glass slides. All sections were stained with Harris' hematoxylin and eosin (H and E). Some selected tissues were also stained by Ziehl-Neelsen acid fast stain (17).

Lead Poisoning in Chickens

Six week old chickens of both sexes were divided at random into groups containing 12 chickens each. Chickens were given lead acetate dissolved in water individually *per os* daily for 35 consecutive days. Two separate trials were conducted. In trial one, the control group received no lead and the remaining three groups received 20 mg/kg, 40 mg/kg and 80 mg/kg respectively. In the second trial, the control group received no lead and the remaining three groups received 160 mg/kg, 320 mg/kg and 640 mg/kg of lead respectively. The chickens were bled on the tenth, 21st and 30th day after treatment initiation for the determination of lead levels in blood. Equal quantities of blood were obtained from all chickens within each group and the blood was pooled according to group. The blood of four chickens selected at random from each of the treatment groups was used for hematological studies. White and red blood cell counts, PCV, hemoglobin determinations and differential leukocyte counts were determined on the 15th and 30th days after lead treatment began. Four chickens selected at random from each of the treatment groups were necropsied at 35 days after lead administration and tissues were collected for the chemical and histopathological studies.

Effect of Lead on Interferon and Antibody Production

Statolon (500 mg/kg) and NDV-B_1 (1024 HA/Kg) were administered I.V. as interferon inducers. Twelve ten week old chickens from each group that previously received lead at the rate of 20 mg/kg, 40 mg/kg, 80 mg/kg or 160 mg/kg for 30 days *per os* were divided into two equal groups. Six chickens were inoculated with statolon and six with NDV-B_1. Only NDV-B_1 was used as an interferon inducer in the group of six chickens which received lead at the 320 mg/kg level for 30 days. Lead treatment was continued at the previous regimen until termination of this experiment. Chickens were bled at different times post induction (Table IV) and the blood of chickens from the same treatment group was pooled and serum assayed for interferon. The viral inhibitor produced in chickens was examined for dialysability, sensitivity to trypsin, acid and heat, sedimentation by centrifugation at 100,000 g for 60 minutes and species specificity. Chickens which were inoculated with NDV-B_1 were bled 14 days after virus inoculation. Blood samples were collected for lead determination and serum was tested for NDV antibodies using the hemagglutination-inhibition (HI) test.

RESULTS

Lead Poisoning in Chickens

Chickens tolerated daily levels of lead as high as 160 mg/kg for 30 days administered *per os* without exhibiting clinical signs or hematological changes in spite of very high levels of lead in the blood (Table I).

Chickens treated at a level of 320 mg/kg of lead exhibited early signs of lethargy, tiring and weakness. Anorexia, anemia and apparent loss of weight followed and marked peripheral paralysis was observed in most birds a day or two prior to death. One chicken in the 320 mg/kg treatment group died on the 11th day and five others at 21-30 days following initiation of lead treatment. All six surviving chicks of this group showed depression, anemia and loss of weight. A fall in hemoglobin concentration was noted in some chickens after 15 days of treatment and all chickens had decreased levels of hemoglobin after 30 days of treatment. Examination of blood smears indicated increased numbers of immature erythrocytes.

Chickens at the 640 mg/kg lead level showed a high mortality rate. The first chicken died on the sixth day after lead administration started and the last one on day 34. The clinical signs were similar to those described above. The number of leukocytes was slightly increased as compared to the control chickens. Hemoglobin concentration was markedly decreased in all chickens with one chicken showing a low of 2.1 gm/100 ml. Hemoglobin concentrations in control chickens were 8.6-10.1 gm/100 ml. Blood smears revealed approximately 12% immature erythrocytes, some of which were in various stages of mitotic division. At necropsy, chickens were found to have severe anemia and emaciation and all of them weighed 150 to 250 g in comparison to 750 to 1,000 g for the control group.

Chemical analysis of tissue specimens revealed that significant quantities of lead were present in the tissues of lead treated chickens. Generally the mean lead content of tissues was higher in all tissues of chickens which were given higher doses of lead. Few exceptions were observed. The mean lead concentration in bone (femur) tissue of chickens at the 640 mg/kg level was considerably lower than in chickens which were given 320 mg/kg and 160 mg/kg lead levels. The results are summarized in Table II.

Histopathological examination of kidney, liver and lung stained with H and E as well as acid fast stain did not reveal any specific lesions which could be attributed to lead poisoning.

Effect of Lead on Interferon and Antibody Production

Lead treatment of chickens at the 20 mg/kg, 40 mg/kg, 80 mg/kg and 160 mg/kg levels did not affect interferon production by statolon. Lead treatment of chickens at the 160 mg/kg level slightly decreased interferon concentration induced by NDV-B_1.

All of the chickens in the 320 mg/kg groups showed some signs of lead poisoning at the time of inoculation of NDV-B_1. Interferon concentrations and duration in serum were markedly decreased in chickens of this group. The results are summarized in Table IV.

Long term lead exposure at doses ranging from 20 mg/kg/day to 320 mg/kg/day had no marked effect on antibody production to NDV-B_1 in chickens. No consistent correlation was observed between blood lead concentration and antibody titers. Three chickens out of six which were on the 320 mg/kg lead treatment died between days 35 and 40 after initiation of lead treatment. All three surviving chickens showed signs of lead poisoning and only one chicken had a markedly lower antibody titer than the untreated chickens. The results are summarized in Table V.

DISCUSSION

There is very little published information on the effects of lead in chickens. Signs of poisoning including drowsiness, thirst and loss of appetite ascribed to the feeding of lead arsenate were described in 1932 (28). Salisbury *et al* (22) reported lead poisoning in adult chickens caused by feeding a grit which contained lead oxide. These authors observed a marked loss of weight in all birds, cessation of egg production, severe anemia and high mortality. Most of the chickens were found to have a severe

TABLE III. Induction of Interferon in Chickens by Various Interferon Inducers

Inducer	Dosage	Route	Interferon Titer Hours Postinoculation				
			0	2	6	24	48
Poly I:poly C	0.1 mg/kg	I.V.	0	—	8	8	< 8
	0.5 mg/kg	I.V.	0	—	8	< 8	< 8
	1 mg/kg	wing web	0	8	< 8	< 8	< 8
	5 mg/kg	wing web	0	—	< 8	< 8	< 8
Statolon	50 mg/kg	I.V.	0	—	8	< 8	< 8
	500 mg/kg	I.V.	0	—	256	128	8
PVR-DR[a]	1 ml/kg	I.V.	0	—	< 8	< 8	< 8
SIV	640 HA/kg	I.V.	0	—	8	< 8	< 8
NDV-B_1	2048 HA/kg	I.V.	0	—	256	256	32

[a]PRV-DR titer 3.2 x 10^5 pfu/ml

TABLE IV. Effect of Lead on Interferon Production in Chickens

Inducer	Daily Dose of Lead for 30 Days	Interferon Titer Hours Postinoculation						
		0	2	6	24	48	72	100
Trial I								
Statolon	Control (no lead)	0	64	64	16	8	4	0
500 mg/kg I.V.	20 mg/kg	0	16	64	32	8	4	0
	40 mg/kg	0	128	64	16	4	2	0
	80 mg/kg	0	64	128	8	8	4	0
NDV-B_1								
1024 HA/kg I.V.	Control (no lead)	0	—	64	128	32	8	4
	20 mg/kg	0	—	128	64	64	4	4
	40 mg/kg	0	—	32	—	32	16	0
	80 mg/kg	0	—	64	256	64	8	4
Trial II								
Statolon	Control (no lead)	0	256	64	16	32	4	4
500 mg/kg I.V.	160 mg/kg	0	256	128	8	16	4	2
NDV-B_1								
1024 HA/kg I.V.	Control (no lead)	0	—	128	512	256	32	16
	160 mg/kg	0	—	64	256	32	4	4
	320 mg/kg	0	—	16	64	8	0	0

TABLE V. Effect of High Lead Levels on Antibody Titer to NDV-B_1 in Chickens

Chicken No.	Daily Dose of Lead	HI Antibody Titer[a]	Led Concentration in Blood (ppm)[b]
1	Control (no lead)	128	0.25
2		128	—
3		128	—
4		128	—
5		64	0.20
6		256	—
7	160 mg/kg	128	6.55
8		128	—
9		256	5.25
10		256	6.40
11		128	5.70
12		64	11.05
13	320 mg/kg	128	9.00
14		128	10.85
15		32	13.64

[a]On the 14th day after NDV-B_1 inoculation
[b]After 44 days of lead administration *per os*

necrosis of the gizzard lining. Simpson *et al* (25) described abnormalities of erythrocytes and renal tubules of chickens poisoned with high doses of lead. They observed increased numbers of immature erythrocytes some of which were in various stages of mitotic division. By light microscopy, necrosis of the epithelium of the proximal tubules of the kidney was observed.

Our findings show that chickens can tolerate levels of lead as high as 160 mg/kg without exhibiting clinical signs or hematological changes in spite of very high levels of lead in the blood.

Analysis of lead in the blood is generally considered to be the best single test for the antemortem diagnosis of lead poisoning. Lead concentrations of pooled samples of 12 chickens which were given 160 mg/kg of lead for 30 days was 6.2 ppm. It is apparent from these data that chickens are more resistant to lead poisoning than humans, horses, dogs and wild fowl such as ducks. The upper limit for lead in human blood is subject to some disagreement. Some believe it to be approximately 0.8 ppm but others believe it to be less, especially in young children (6, 21). Lead poisoning has been confirmed in cattle having blood lead levels as low as 0.35 ppm in both experimental and naturally-occurring cases (5). Horses appear more susceptible to lead poisoning than cattle (3). The upper limit for blood lead in dogs is 0.35 ppm (35). Wild water fowl are very susceptible to lead poisoning. Daily administration of aqueous lead nitrate solution *per os* at the dosage level of 12 mg/kg killed all experimental ducks within a month (7). No information was found in the literature describing blood lead levels in gallinaceous birds.

Hemoglobin concentration was markedly decreased in all chickens of the 320 mg/kg and 640 mg/kg lead treatment groups. Examination of blood smears revealed increased numbers of immature erythrocytes, some of which were in various stages of mitosis. Poikylocytosis, anisocytosis and binucleated or non-nucleated cells were common. This finding is in agreement with previous findings (25). Since lead interferes with heme synthesis (6, 32) anemia is the functional effect of lead poisoning. The decrease in heme synthesis leads at first to a decrease in the life span of erythrocytes and the amount of hemoglobin per cell (32). In compensation the blood forming tissues step up their production of RBC and immature forms of cells appear in the circulation (6, 32).

Chemical analysis of tissue specimens revealed that significant quantities of lead are present in tissues of lead treated chickens. Deposition of lead in the tissues showed variation in chickens which were in the same lead treatment group as well as within the same chicken. The highest lead concentrations were found in bone (femur). The next highest lead levels were in kidney. The lowest concentrations of lead were found in muscle (gastrocnemius). Generally, the mean lead content was higher in all tissues of chickens which were given higher doses of lead. However, the mean lead concentration of femur in chickens receiving the 640 mg/kg lead dose was lower than in chickens which were given 320 mg/kg and 160 mg/kg of lead. Lead concentrations in liver, kidney and lung were at least three times higher in the 640 mg/kg groups than in the 320 mg/kg treatment group. It was reported (4) that the degree of acute exposure to lead is indicated by the concentration of the metal in liver while chronic exposure is reflected by relatively higher concentrations of lead in bone. Apparently the concentrations of lead in the bone of 320 mg/kg and 160 mg/kg chickens reflect the longer exposure to lead. Tissues of chickens from these groups were collected after 35 days of lead treatment whereas chickens of the 640 mg/kg group died of acute lead poisoning between 12 and 18 days after initiation of lead treatment.

A number of different interferon inducers were used in this study. Poly I:poly C at the several doses and routes of inoculation used induced very low titers of circulating interferon. This finding agrees with reports in the literature (10, 18).

Swine influenza and PRV viruses were not effective in inducing interferon in chickens at the doses used. In contrast, statolon at the 500 mg/kg dose level and NDV-B_1 I.V. were effective in producing high levels of circulating interferon of long duration.

In this study long term lead treatment of chickens at the lead dosage levels used did not affect interferon response to statolon. Neither interferon concentration nor duration of detectable interferon in serum were changed as compared with the controls. Lead treatment of chickens at the 160 mg/kg level slightly decreased blood interferon concentration induced by NDV. The duration of circulating interferon was not affected. This could be due to variation in the

ability of individual chickens to produce interferon. Only NDV-B_1 was used as an interferon inducer in the group of chickens which received lead at the 320 mg/kg level for 30 days. Interferon concentrations and duration in serum were markedly decreased in chickens of this group. However, all chickens in this group showed some clinical signs of lead poisoning at the time of interferon stimulation. The decrease in interferon concentration and duration observed in chickens of this group could be due to lead interference with many biological functions in the body. Lead decreases blood levels of nicotinic acid and many amino acids, inhibits biosynthesis of DNA and RNA and interferes with the activities of numerous enzymes (8). Interference with any of these biologically important functions may lead to suppression of interferon production. Gainer (11) reported that arsenicals inhibited both the synthesis and action of interferon. It is possible that the antiviral action of interferon could be suppressed or inhibited by lead poisoning since the mechanism of antiviral action is an involved process and requires synthesis of a new protein (15). Low and high doses of lead had no marked effect on antibody production to NDV in chickens. No consistent correlation was observed between blood lead concentration and antibody titer.

The results of these studies indicate that long term subclinical lead intake suppresses neither interferon nor antibody production in chickens. However, chickens are very resistant to lead poisoning as shown in these studies and thus these findings may not be applicable to other species.

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