

needed before a more complete picture of the role of sediments in the kinetics of aqueous chemical reactions can be formulated.

#### CONCLUSIONS

Neutral hydrolysis studies show that for three organophosphorothioate esters hydrolysis occurs in the sediment-sorbed phase of sediment-water samples. The hydrolysis reactions in the sediment-sorbed phase has the same neutral hydrolysis rate constant (within experimental error) as that in the aqueous phase of the sediment-water systems. Thus, in pollutant fate modeling, the neutral hydrolysis process can be simulated by assuming equilibrium between the aqueous and sediment phase with hydrolysis occurring in both phases with the same disappearance rate constant.

In contrast, the alkaline hydrolysis of chlorpyrifos does not occur in the sediment-sorbed phase with a rate constant comparable to that of the aqueous phase. For describing the fate of organophosphorothioate in sediments, a model with partitioning of the ester to the sediment with reaction in the aqueous phase only is adequate. Further studies with these esters are needed, however, to evaluate the effect of pH and incubation time on this process.

#### ACKNOWLEDGMENT

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**Registry No.** Chlorpyrifos, 2921-88-2; diazinon, 333-41-5; ronnel, 299-84-3.

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## Transfer of Sludge-Borne Cadmium through Plants to Chickens

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Corn hybrids (*Zea mays* L.) and soybean cultivars (*Glycine max* L.), selected to accumulate high levels of Cd in grain and beans, were grown on strip-mine spoil amended with about 200 Mg/ha (dry weight) of sewage sludge. The corn grain and soybean meal were used to formulate starter, developer, and layer diets for White Leghorn chickens. The diets contained  $0.09 \pm 0.05$  (low Cd),  $0.57 \pm 0.11$  (medium Cd), or  $0.97 \pm 0.14$  (high Cd) mg/kg biologically incorporated Cd. Each of the diets was fed to replicated groups of birds, starting as chicks to the end of their productive lives as laying hens. The highest dietary Cd level did not alter the concentration of the metal in the brain, breast muscle, leg muscle, or eggs. There was no indication that the highest dietary level of Cd affected feed consumption, body weight gains, the rate of mortality, egg production, egg quality, or the absorption of essential inorganic nutrients. The body of spent hens retained about 1.3% of the highest level of Cd ingested as a constituent of feed, of which about 60% was found in kidneys.

There is very little information concerning the effect of low-level Cd ingestion by domestic animals for long periods

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of time. Most studies on Cd toxicity have involved animals [see Doyle (1977), for a review] challenged with unnaturally high doses of soluble Cd salts (Supple, 1961; Sell, 1975; Leach et al., 1979; Grote and Speck, 1981). But, excessive levels of heavy metals do not normally enter the higher trophic levels of natural food chains as soluble metal salts. However, some heavy metals, especially Cd, may be accumulated at abnormal high concentrations in food-chain plants grown on contaminated soils. Concentrations of Cd,

Cu, Ni, and Zn are frequently higher than background levels in crop plants grown on soils amended with municipal sewage sludges. The transfer of metals grown on sludge-amended soils to various animals have been investigated by several researchers. Health effects resulting from metal accumulations in the tissues of pheasants (Hinesly et al., 1976), swine (Hansen et al., 1976; Hansen and Hinesly, 1979; Lisk et al., 1982), sheep (Haschek et al., 1979; Heffron et al., 1980), and poultry (Damron et al., 1980) maintained either wholly or partially on feedstuffs produced on sludge-amended soils have been investigated. These studies involving feedstuffs containing enhanced concentrations of biologically incorporated Cd and other heavy metals were short-term relative to the total life span of the animals. We report here the results from a long-term study in which rations containing enhanced levels of biologically incorporated Cd were fed to laying hens throughout their productive life. Since laying hens consume more dry food per unit of body weight than other domestic food animals, they are more likely to exhibit adverse health and performance effects from the absorption and accumulation of Cd. A major objective of this study was to evaluate the potential hazard to human food chains where municipal sewage sludges are used to produce animal feedstuffs.

#### MATERIALS AND METHODS

On the basis of data from a previous screening study (Hinesly et al., 1978), single crosses of corn (*Zea mays* L.) were selected to accumulate high (Mo17 × FR14A) and intermediate (R802 × R806) concentrations of Cd in grain. Both crosses were grown on sewage sludge amended strip-mine spoil to obtain sufficient quantities of corn grain containing 0.71 (high cross) and 0.35 mg of Cd/kg (intermediate cross) for formulating poultry diets with high and intermediate levels of Cd. Commercial corn grain containing less than 0.06 mg of Cd/kg was used in low-Cd rations. Soybeans (*Glycine max* L., Woodward and Harosoy 63) were also selected for their capacities to accumulate Cd and grown on sludge-amended strip-mine spoil to yield a processed meal that contained 2.38 mg of Cd/kg. This meal was used to formulate the high-Cd rations. The intermediate-Cd rations were formulated by using a half and half mixture of the high-Cd soybean meal and a commercial soybean meal containing less than 0.06 mg/kg Cd. Only the commercial meal was used in the low-Cd rations. Composition of the experimental diets is shown in Table I. Mineral and vitamin supplements were added to rations in amounts and from sources that are generally used in commercial poultry operations. Mean concentrations of Zn, Cd, Cu, Mn, Fe, Pb, Cr, Ni, Se, Mg, Ca, and P in low-, medium-, and high-Cd diets (LCd, MCd, and HCd) are presented in Table II. Each time a new batch of rations was prepared samples were collected for analysis.

Three hundred commercial hybrid pullet chicks (Hyline W36) were brooded in lots of 25 each in Petersime brooder batteries. At 6 weeks of age they were transferred by lots to growing batteries. From 20 to 80 weeks of age they were housed two birds per cage in 25.4 × 45.7 × 40.6 cm laying cages. Starter, developer, and layer diets were fed ad libitum from 0 to 8, 8 to 20, and 20 to 80 weeks of age, respectively. Distilled and deionized water was provided ad libitum in stainless steel or plastic waterers throughout the experiment.

The birds were housed in environmentally controlled quarters maintained at 16–27 °C with heaters and air conditioners as needed. A standard step down–step up lighting schedule was provided following the first 5 days

during which lighting was continuous.

Feed intake and body weights were determined biweekly from 0 to 8 weeks of age and at 4-week intervals thereafter. Egg production was measured daily, and egg weight and specific gravity were determined from a 3-day collection of eggs taken at the end of each 4-week period starting during the 28th week.

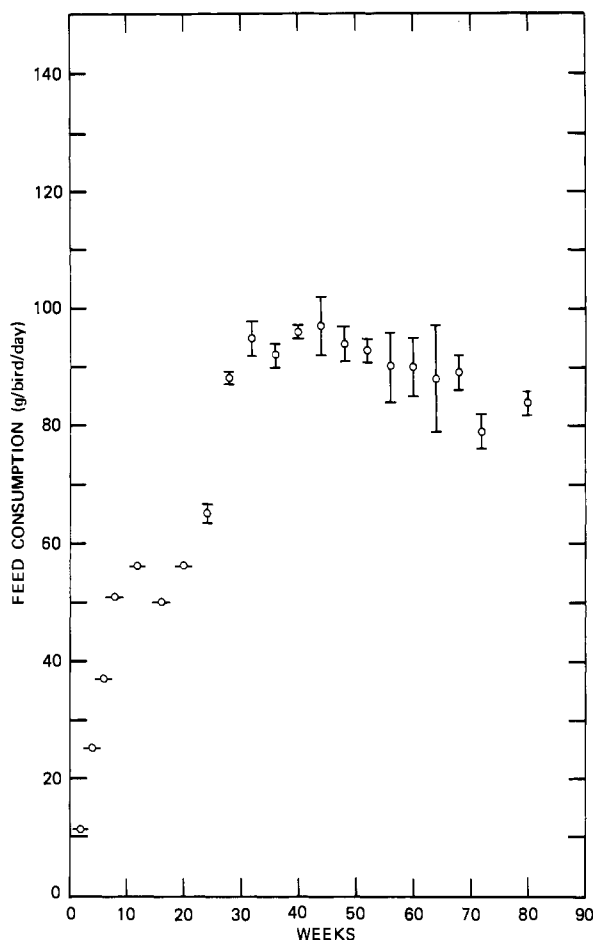
The experiment was initiated with four replications of 25 birds on each of the LCd, MCd, and HCd diets (Tables I and II). Random samples of birds were terminated at 8, 20, 50, 72, and 80 weeks. At 8 and 50 weeks, four birds were sampled from each of the four replications and tissues collected for chemical analyses and pathological examination. The four samples were pooled within each replicate. Sampling protocol at 20 weeks was the same, except only three birds per replication were sacrificed. At the end of 50 weeks, two of the four replications of hens fed LCd diets were switched to HCd diets and vice versa. At the 72- and 80-week sampling periods, five birds from each of the remaining two replications per treatment were randomly selected and tissues from each bird were analyzed separately. Egg samples were pooled by replication.

Birds were dispatched by cervical dislocation and bled by decapitation prior to dissection. Dissection began with removal of breast muscle from the most anterior point of the keel bone and extended posteriorly and laterally about 2.54 cm toward the rib cage for the entire depth of tissue. The heart, lung, pancreas, spleen, brain, liver, and kidney were removed and blotted with Kimwipe towels to remove excess blood and fat. Crop, proventriculus, gizzard, and duodenum were removed, slit longitudinally, irrigated with 0.95% NaCl solution, and blotted dry. Also removed were the femur bone (either right or left), leg muscle around the femur bone, and a sample of the primary flight feathers. Samples were weighed, placed in sealed plastic bags (femur bone, feathers), or screw-top plastic bottles (all other tissues), and stored at –25 °C.

After defrosting, tissue samples were dried to constant weight at 60 °C. Samples of breast muscle, leg muscle, gizzard, gizzard muscle, kidney, liver, heart, and pancreas were fat extracted by refluxing with ethyl ether for 4 h in a Soxhlet apparatus, evaporated to dryness, and ground in a Wiley mill. Two-gram subsamples were digested in 10 mL of concentrated HNO<sub>3</sub> until fuming ceased. After being heated to dryness, samples were digested in 10 mL of 30% H<sub>2</sub>O<sub>2</sub> until a clear liquid was obtained. This liquid was heated at 90 °C on a hot plate to dryness, and residues were dissolved in 25 mL of 0.5 N HNO<sub>3</sub> for subsequent atomic absorption analysis. Samples of brain, crop, duodenum, feathers, gizzard lining, lung, proventriculus, and spleen were prepared in an identical fashion except for the following: fat was not removed; feathers were washed in doubly distilled water prior to drying at 60 °C; only 1 g of dry tissue was available for analysis in some instances. Gizzard linings were left attached to gizzard muscle for the 8- and 20-week samples. These tissues were separated for the 50-, 72-, and 80-week samples and analyzed accordingly.

Leg bones were first autoclaved to remove extraneous tissue and then prepared for analysis by the procedures used for non-fat-extracted tissues. Eggs were washed in doubly distilled water, boiled in plastic cooking bags, separated into shells, whites, and yolks, dried at 60 °C, and ground in a Wiley mill. Yolks were fat-extracted; shells and whites were not. Remaining procedures were the same as those employed for other samples.

Tissues were analyzed for Zn, Cd, Cu, Mn, Fe, and Pb by atomic absorption spectrophotometry with appropriate



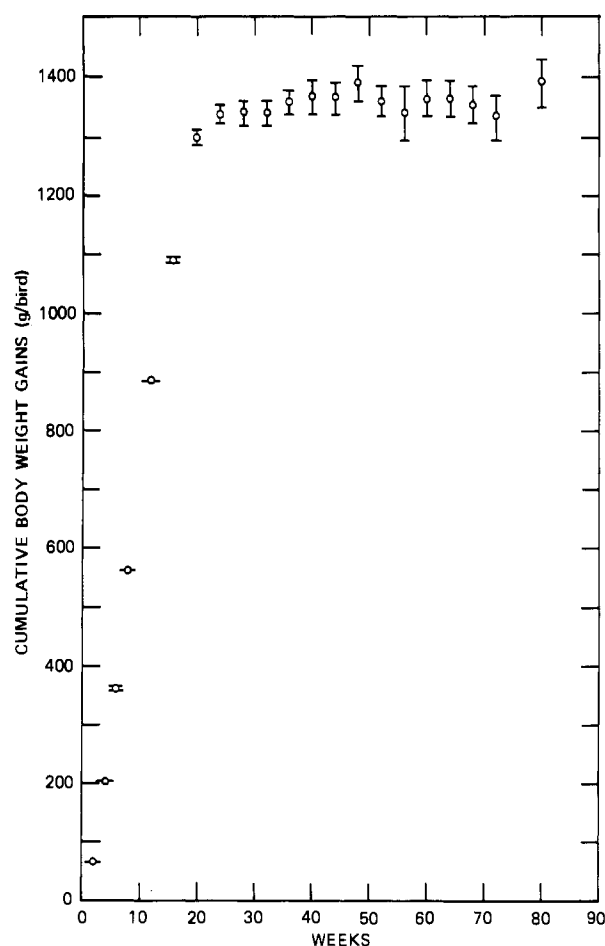
**Figure 1.** Mean daily consumption of high-Cd feed by pullet chicks determined biweekly from 0 to 8 weeks and at 4-week intervals thereafter to the end of their productive lives as layers.

background correction. All tissues were analyzed at the 50- and 80-week sampling periods. Only breast muscle, gizzard (muscle and lining), kidney, leg muscle, and liver were analyzed at the 8-, 20-, and 72-week periods. Eggs were analyzed only for Cd.

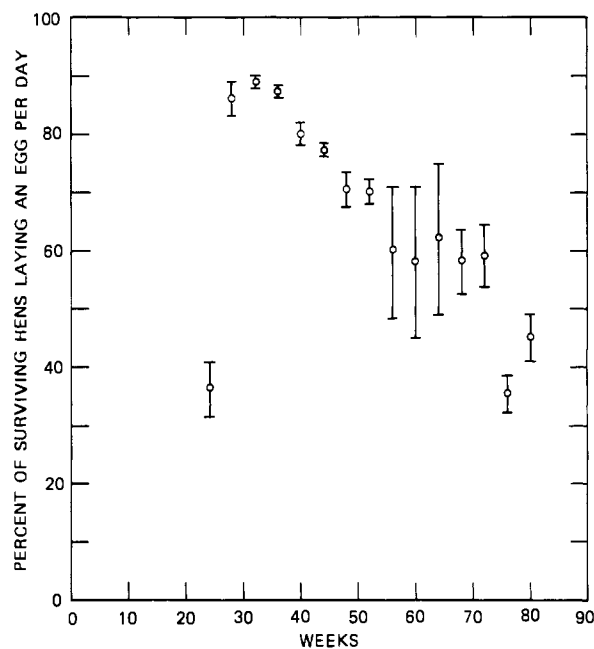
An analysis of variance was performed to test the effect of dietary Cd levels on concentrations of the above metals in various tissues. Where metal concentrations in tissues were significantly different, the least significant difference (LSD) was calculated according to procedures outlined by Rickmers and Todd (1967).

## RESULTS

**General Health and Performance.** Consumption of feed, cumulative gain in body weight, and rate of lay data are presented in Figures 1, 2, and 3, respectively, for chicks, pullets, and hens fed the high-Cd diet. These data are representative for the entire experiment since there were no significant differences in any of these parameters associated with concentrations of Cd in the diets. Both feed intake and weight gains remained fairly constant after 24 weeks. There was a normal decrease in egg production associated with increasing age. The rate of mortality, normally about 1% each 4-week period, was well below expectations for all diets. Cadmium in diets had no effect on egg weight, shell quality, and concentrations of Cd in egg parts. The data presented in Table III show that egg whites and yolks generally contained Cd concentrations that were below the detectable limits of 0.062 mg/kg. Egg shells contained measurable amounts of Cd, and these amounts increased with age and as the rate of egg lay decreased. But shell Cd concentrations were not influenced by differences in dietary levels of Cd.



**Figure 2.** Mean weight changes of chickens on high-Cd feed as determined biweekly from 0 to 8 weeks and at 4-week intervals thereafter to the end of their productive lives as layers.



**Figure 3.** Mean rate of egg lay for hens on high-Cd diets.

**Tissue Cd Concentrations.** Concentrations of Cd in samples of various chicken tissues collected at 8, 20, 50, and 80 weeks of age are presented in Table IV. These data show that concentrations of Cd in brain, breast muscle, and feathers were unaffected by dietary levels of Cd and age of birds. Some doubts exist as to whether or not increased dietary levels of Cd increased concentrations of

Table I. Percent Composition of Experimental Poultry Diets

ingredient <sup>a</sup>	low-Cd diet (LCd)			medium-Cd diet (MCd)			high-Cd diet (HCd)		
	starter	developer	layer	starter	developer	layer	starter	developer	layer
high-cadmium corn (12.1% P)							71.63	86.63	69.10
medium-cadmium corn (10.6% P)				56.59	67.58	53.70			
low-cadmium corn (9.6% P)	69.90	82.60	64.50	13.27	15.85	12.60			
high-cadmium soybean meal (46.4% P)				11.62	6.46	11.60	21.47	9.72	20.40
low-cadmium soybean meal (51.4% P)	23.20	13.75	25.00	11.62	6.46	11.60			
corn gluten meal (60% P)	2.00			2.00			2.00		
alfalfa meal (17% P)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-methionine	0.05			0.05			0.05		
dicalcium phosphate	2.20	1.00	1.25	2.20	1.00	1.25	2.20	1.00	1.25
ground limestone	1.00	1.00	7.50	1.00	1.00	7.50	1.00	1.00	7.50
iodized salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
manganese sulfate (27% Mn)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
choline chloride (50%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
vitamin mixture <sup>b</sup>	0.10	0.10	0.25	0.10	0.10	0.25	0.10	0.10	0.25
tylosin	0.05	0.05		0.05	0.05		0.05	0.05	

<sup>a</sup> High-, medium-, low-cadmium corn contained 0.71, 0.35, and <0.06 mg of Cd/kg. High- and low-cadmium soybean meal contained 2.38 and <0.06 mg of Cd/kg. Dicalcium phosphate and limestone contained 5.55 and 0.55 mg of Cd/kg, respectively. All other ingredients contained negligible (<0.06 mg/kg) levels of Cd. P denotes protein content. <sup>b</sup> Provided per kg of starter and developer diet: vitamin A, 2000 IU; D<sub>3</sub>, 1000 IU; K, 1 mg; niacin, 27 mg; calcium pantothenate, 11 mg; B<sub>12</sub>, 9 µg; biotin, 100 µg; riboflavin, 3.6 mg. Provided per kg of layer diet: vitamin A, 4400 IU; D<sub>3</sub>, 1000 IU; K, 1.1 mg; niacin, 22 mg; calcium pantothenate, 11 mg; B<sub>12</sub>, 12.5 µg; E, 2.2 mg; riboflavin, 4.4 mg; chlortetracycline, 20 µg.

Table II. Mean Concentrations ± SD of Selected Elements in Experimental Poultry Diets

diet <sup>a</sup>	chemical element											
	Zn	Cd	Cu	Mn	Fe	Pb	Cr	Ni	Se	Mg	Ca	P
	mg/kg											
	%											
starter LCd (N = 2)	25.4 ± 2.3	0.13 ± 0.00	6.22 ± 0.81	198 ± 11	456 ± 13	<0.62	3.35 ± 0.23	4.03 ± 0.00	<0.02	0.19 ± 0.00	1.25 ± 0.04	1.44 ± 0.44
starter MCd (N = 2)	38.6 ± 6.5	0.76 ± 0.16	5.88 ± 0.04	184 ± 35	540 ± 181	<0.62	2.57 ± 0.18	5.52 ± 0.43	0.03 ± 0.02	0.23 ± 0.06	1.30 ± 0.36	1.08 ± 0.32
starter HCD (N = 2)	36.4 ± 3.5	1.08 ± 0.13	6.56 ± 1.61	185 ± 37	448 ± 30	<0.62	2.47 ± 0.51	5.56 ± 0.54	0.03 ± 0.01	0.16 ± 0.01	1.24 ± 0.11	1.25 ± 0.31
developer LCd (N = 3)	25.2 ± 3.0	0.08 ± 0.05	3.91 ± 0.12	155 ± 11	217 ± 22	<0.62	1.34 ± 0.22	2.62 ± 0.09	<0.02	0.18 ± 0.01	0.77 ± 0.04	0.68 ± 0.08
developer MCd (N = 3)	37.8 ± 2.5	0.46 ± 0.03	5.01 ± 0.95	172 ± 20	236 ± 18	<0.62	1.73 ± 0.36	3.98 ± 0.27	0.04 ± 0.02	0.16 ± 0.01	0.81 ± 0.15	0.80 ± 0.10
developer HCD (N = 3)	38.6 ± 2.4	0.77 ± 0.11	4.01 ± 0.30	152 ± 20	248 ± 54	<0.62	1.43 ± 0.59	3.62 ± 0.56	0.04 ± 0.01	0.13 ± 0.02	0.83 ± 0.10	0.98 ± 0.49
layer LCd (N = 9)	31.7 ± 2.3	0.09 ± 0.05	5.78 ± 0.70	177 ± 37	438 ± 31	<0.62	2.28 ± 0.30	3.50 ± 0.32	<0.02	0.18 ± 0.02	3.42 ± 0.25	0.78 ± 0.45
layer MCd (N = 9)	47.8 ± 2.9	0.56 ± 0.04	6.17 ± 1.73	167 ± 24	421 ± 35	<0.62	2.22 ± 0.25	4.49 ± 0.53	<0.02	0.19 ± 0.01	3.60 ± 0.54	0.75 ± 0.10
layer HCD (N = 9)	54.5 ± 3.3	1.01 ± 0.07	6.65 ± 1.97	174 ± 35	408 ± 42	<0.62	2.40 ± 0.80	5.19 ± 0.60	0.03 ± 0.02	0.16 ± 0.04	3.59 ± 0.52	0.90 ± 0.13

<sup>a</sup> N = number of samples examined.

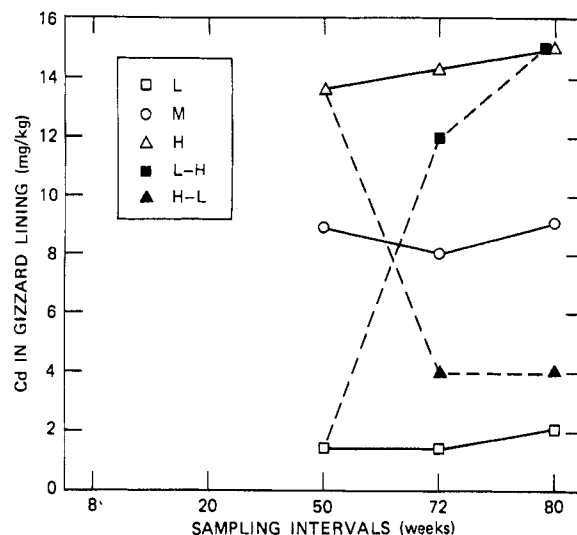
**Table III. Mean Cd Concentrations in Egg Constituents Sampled at Various Intervals during a 54-Week Period (mg/kg, Dry Weight)**

no. of weeks after laying began	Cd			
	treatment	white	yolk	shell
6	low	<0.062	<0.062	0.114
	medium	<0.062	<0.062	0.149
	high	<0.062	<0.062	0.148
	LSD	n.s. <sup>a</sup>	n.s.	n.s.
15	low	<0.062	0.068	0.068
	medium	<0.062	<0.062	0.071
	high	<0.062	0.111	0.090
	LSD	n.s.	n.s.	n.s.
24	low	<0.062	<0.062	0.101
	medium	<0.062	<0.062	0.124
	high	<0.062	<0.062	0.088
	LSD	n.s.	n.s.	n.s.
32	low	<0.062	<0.062	0.352
	medium	<0.062	<0.062	0.365
	high	<0.062	<0.062	0.294
	LSD	n.s.	n.s.	n.s.
41	low	0.317	<0.062	0.456
	medium	0.144	<0.062	0.320
	high	0.125	<0.062	0.340
	LSD	n.s.	n.s.	n.s.
54	low	<0.062	<0.062	0.303
	medium	<0.062	<0.062	0.325
	high	<0.062	<0.062	0.347
	LSD	n.s.	n.s.	n.s.

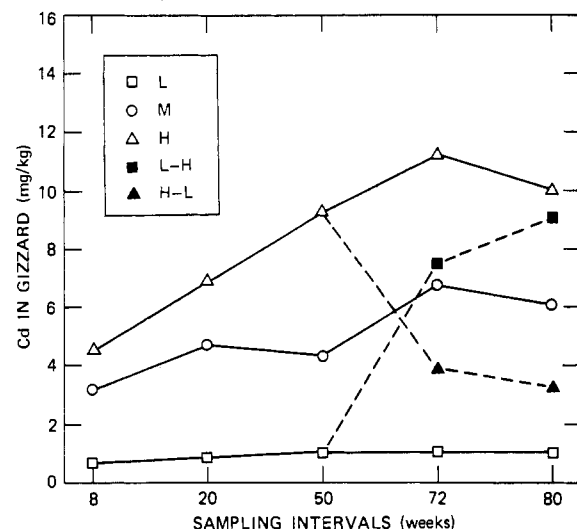
<sup>a</sup> n.s. = nonsignificant difference between Cd concentrations.

the metal in heart, leg muscle, and lung tissues, because the significant difference in tissues from 80-week-old hens depended on the use of half of the lowest detectable level for the statistical analysis. Furthermore, there was no indication that Cd concentrations in these tissues increased with age. Higher dietary Cd levels were reflected by higher Cd concentrations in the duodenum, pancreas, spleen, and gizzard lining but remained at a rather stable concentration in these tissues as hen ages increased from 50 to 80 weeks. Compared to effects of lower dietary levels, the highest dietary level of Cd caused higher concentrations of the metal in the proventriculus and femur bones of 50- and 80-week-old hens. Also, tissues from hens in the oldest group contained significantly higher levels of Cd than those from 50-week-old hens. Concentrations of Cd were increased in crop, gizzard muscle, liver, and kidney tissues in concert with higher dietary levels of Cd. Regardless of dietary level, Cd concentration tended to increase in these four tissues with age of hens, except at the lowest dietary Cd level. Although there was a trend for higher Cd concentrations in crop and gizzard muscle with hen age at the lowest dietary-Cd level, it was not statistically significant. At the highest dietary level of Cd, the small increases of Cd concentrations that occurred in liver and gizzard muscle tissues as the longevity of hens increased from 50 to 80 weeks were not statistically significant. This suggests that Cd concentrations in these two tissues were approaching an equilibrium or saturated condition near 50 weeks of age. After 50 weeks, marked enhancement of Cd concentrations occurred only in kidney tissues. In tissues from 80-week-old hens fed the HCd diet, concentrations of Cd were lower, by order of listing, in kidney, gizzard lining, gizzard muscle, liver, pancreas, duodenum, proventriculus, spleen, crop, lung, femur bone, feathers, leg muscle, heart, brain, and breast muscle. In comparison to cumulations of Cd in the first four, concentrations in the last four tissues were of little consequence.

There were significant changes in Cd concentrations in several tissues from hens who, at 50 weeks of age, were changed from high to low dietary Cd levels or conversely. These changes are illustrated in Figures 4-7 and include



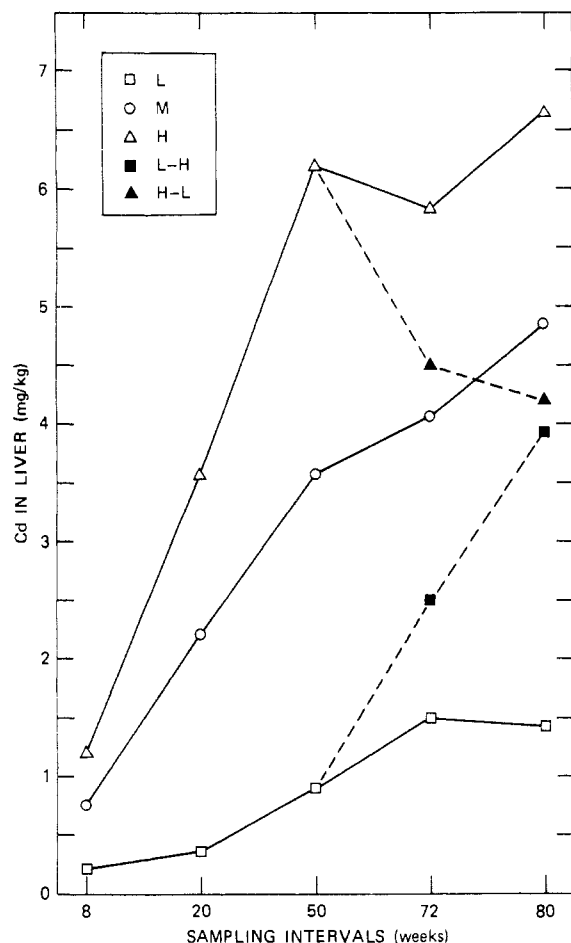
**Figure 4.** Mean concentrations of Cd in gizzard linings of 50-, 72-, and 80-week-old hens after continuous lifetime exposure to low (□), intermediate (○), or high (△) dietary levels of Cd. At 50 weeks some hens were switched from low to high (■) dietary levels of Cd and conversely (▲) for others.



**Figure 5.** Mean concentration of Cd in whole chicken gizzard at 8, 20, 50, 72, and 80 weeks of age during a lifetime exposure to low (□), intermediate (○), or high (△) dietary levels of Cd. At 50 weeks some hens were switched from low to high (■) dietary levels of Cd and conversely (▲) for others.

data from an additional 72-week sampling period. Cadmium concentrations in gizzard linings (Figure 4) and gizzard muscle tissue (Figure 5) suggested a trend toward complete reversal. Although the changes were not as pronounced as in gizzard tissues, Cd concentrations in liver (Figure 6) were significantly reversed in concert with reciprocal changes in dietary levels of the metal. In kidney tissues (Figure 7), Cd burden continued to increase for both HCd to LCd and LCd to HCd groups albeit at lower and higher rates, respectively.

Zinc concentrations ranged from a low of 16-24 mg/kg in breast muscle to a high of 206-268 mg/kg in femur bone. Kidney was the only tissue in which Zn concentrations increased with increasing levels of dietary Cd. This finding was not entirely unexpected because the corn grain and soybeans grown on sludge-amended strip-mine spoil also contained enhanced levels of Zn. Concentrations of Zn were increased only in kidney and liver tissues with longevity. At the highest dietary level of Cd, Zn increased from 110 mg/kg in 8-week-old chicks to 155 mg/kg in 80-week-old hens. During the same time span, Zn in-



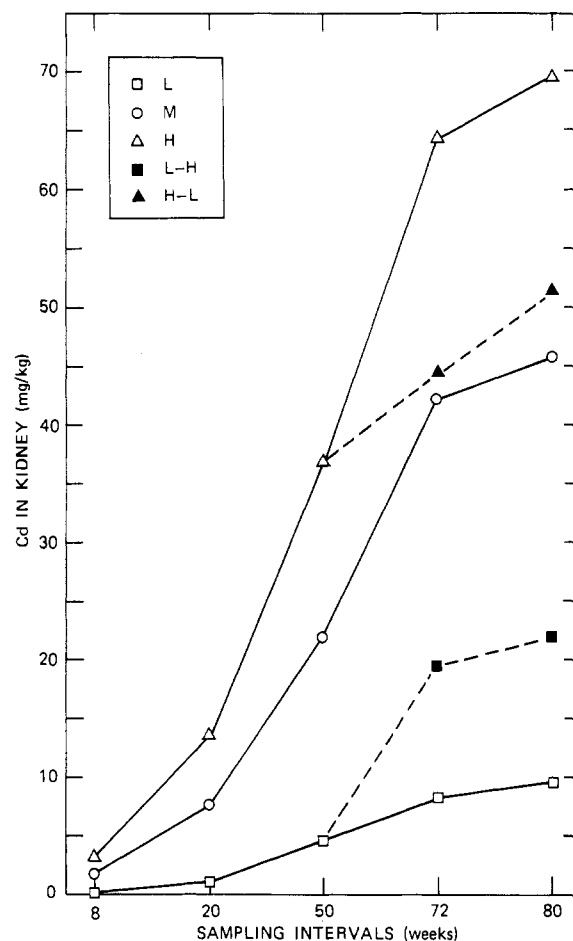
**Figure 6.** Mean concentrations of Cd in livers of chickens at 8, 20, 50, 72, and 80 weeks of age during a lifetime exposure to low (□), intermediate (○), or high (△) dietary levels of Cd. At 50 weeks of age some hens were switched from low to high (■) dietary levels of Cd and conversely (▲) for others.

creased from 103 to 177 mg/kg in livers. Copper and Mn concentrations remained fairly constant in all tissues throughout the lifetime of chickens, and the few differences that seemed to be attributable to differences in dietary Cd levels were not consistent from one sampling period to another. Iron concentrations tended to increase in all tissues up to 50 weeks of age, after which they remained fairly constant. There were no statistically significant differences in tissue Fe concentrations that could be attributed to differences in diets. To the greatest extent, Pb concentrations were below detectable limits of 0.625 mg/kg in all tissues, except feathers. In feathers, Pb concentrations ranged from 4.4 to 13.2 mg/kg but were not influenced by differences in dietary Cd levels. Since all diets contained less than 0.62 mg Pb/kg, differences in tissue Pb concentrations were not expected.

It is of some interest that gizzard muscle from 50- and 80-week-old hens had higher concentrations of Zn and Fe and lower concentrations of Cd, Cu, and Mn than did the gizzard lining. In samples from 80-week-old hens fed HCD diets, concentrations of Zn and Fe were respectively 6.4 and 2.4 times higher in gizzard muscle than in gizzard lining. Conversely, concentrations of Cd, Cu, and Mn were respectively 1.5, 6.9, and 3.2 times higher in gizzard lining than in gizzard muscle.

#### DISCUSSION

The concentrations of biologically incorporated Cd present in the corn grain and soybean meal used in this feeding study represent levels that are not likely to be



**Figure 7.** Mean concentrations of Cd in kidneys of chickens at 8, 20, 50, 72, and 80 weeks of age during a lifetime exposure to low (□), intermediate (○), or high (△) dietary levels of Cd. At 50 weeks of age some hens were switched from low to high (■) dietary levels of Cd and conversely (▲) for others.

exceeded in similar feedstuffs on soils amended with agronomic rates of sewage sludge. Thus, this study involved a lifetime exposure of chickens to the highest dietary level of biologically incorporated Cd that is likely to be encountered if seeds from healthy plants are used to formulate rations. The results from this study suggest, and others have shown (Buck et al., 1979), that Cd biologically incorporated in seeds was as available for adsorption by chickens as that from Cd salts. Conclusions based on results from poultry feeding studies employing soluble Cd salts appear to be valid where concentrations of the metal did not exceed maximum levels that could be accumulated in seeds of healthy plants and where proper attention was given to maintaining a balance between added Cd and essential nutrients. Hill et al. (1963) reported antagonist interaction of Cd with the absorption of Cu, Zn, and Fe. They concluded that Cd replaced Cu and Zn at active metabolic sites. Fox (1979) concluded that Cd toxicity in animals was altered by dietary levels of protein, ascorbic acid, vitamin D, Ca, Fe, Mn, Se, and Zn. Although this study was not designed to test for interaction effects, there was no indication that Cd interfered with the absorption and metabolism of essential inorganic elements other than perhaps Fe. Inconsistent and nonlinear effects on Fe metabolism have been noted previously in sludge-exposed swine (Hansen and Hinesly, 1979). Dietary levels of essential inorganic elements were adequate, and none that were measured were present in excessive concentrations. If they affected the absorption and distribution of Cd in the various tissues, the effect was consistent with a natural

**Table IV. Concentrations of Cd in Tissues of Chickens Collected at Various Ages: Included Are Cd Concentrations in Tissues from Subgroups of Hens That Were Switched at 50 Weeks of Age from High- to Low-Cd Diets and Vice Versa**

diet	mg/kg of fat-free dry weight at weeks after hatching					diet	mg/kg of fat-free dry weight at weeks after hatching				
	8	20	50	80	LSD		8	20	50	80	LSD
	Brain						Heart				
low			<0.062	0.101	n.s. <sup>c</sup>	low			<0.062	<0.077	n.s.
medium			<0.062	<0.092	n.s.	medium			<0.062	<0.077	n.s.
high			0.070	<0.092	n.s.	high			0.188	0.114	n.s.
low-high				<0.092		low-high				<0.077	
high-low				<0.092		high-low				0.090	
LSD			n.s.	n.s.		LSD			n.s.	0.051**	
	Breast Muscle						Kidney				
low	<0.062	<0.062	0.134	<0.062	n.s.	low	0.30	0.99	4.6	9.5	2.07**
medium	<0.062	0.091	0.095	<0.062	n.s.	medium	1.76	7.64	21.8	45.8	4.41**
high	<0.062	0.109	0.112	<0.062	n.s.	high	3.21	13.60	36.8	69.5	7.07**
low-high				<0.062		low-high				21.8	
high-low				<0.062		high-low				51.3	
LSD	n.s.	n.s.	n.s.	n.s.		LSD	0.81**	1.82*	4.8**	9.67**	
	Crop						Leg Muscle				
low			<0.062	0.243	n.s.	low	0.078	0.093	0.111	<0.062	n.s.
medium			0.198	0.400	0.131** <sup>b</sup>	medium	<0.062	0.074	0.110	0.113	n.s.
high			0.329	0.528	0.140**	high	<0.062	0.121	0.163	0.131	n.s.
low-high				0.294		low-high				<0.062	
high-low				0.458		high-low				0.115	
LSD			0.100**	0.078**		LSD	n.s.	n.s.	0.039*	0.045**	
	Duodenum						Liver				
low			0.43	0.52	n.s.	low	0.176	0.35	0.90	1.43	0.19**
medium			1.31	1.23	n.s.	medium	0.746	2.17	3.57	4.86	0.72**
high			2.18	1.98	n.s.	high	1.170	3.55	6.18	6.66	0.91**
low-high				1.33		low-high				3.93	
high-low				0.61		high-low				4.20	
LSD			0.74**	0.65**		LSD	0.226**	0.78**	0.89**	1.64**	
	Feathers						Lung				
low			0.170	0.073	n.s.	low			<0.062	<0.098	n.s.
medium			0.165	0.100	n.s.	medium			0.128	0.149	n.s.
high			0.218	0.155	n.s.	high			0.319	0.277	n.s.
low-high				0.118		low-high				0.126	
high-low				0.108		high-low				0.234	
LSD			n.s.	n.s.		LSD			0.140**	0.154**	
	Femur Bone						Pancreas				
low			<0.062	0.145	n.s.	low			0.30	0.37	n.s.
medium			<0.062	0.152	n.s.	medium			1.85	1.86	n.s.
high			0.073	0.221	0.089*	high			2.90	2.78	n.s.
low-high				0.162		low-high				1.11	
high-low				0.176		high-low				2.11	
LSD			n.s.	0.046*		LSD			0.59**	0.61**	
	Gizzard Muscle <sup>a</sup>						Proventriculus				
low	0.72	0.87	1.03	1.11	n.s.	low			0.23	0.29	n.s.
medium	3.19	4.69	4.27	6.06	1.09*	medium			0.80	1.16	n.s.
high	4.54	6.90	9.34	9.96	1.20**	high			1.32	1.83	0.30*
low-high				9.00		low-high				0.85	
high-low				3.22		high-low				1.30	
LSD	1.23**	1.14**	3.33**	2.33**		LSD			0.32**	0.39**	
	Gizzard Lining						Spleen				
low			1.39	2.08	n.s.	low			0.35	0.36	n.s.
medium			8.78	9.14	n.s.	medium			1.58	1.19	n.s.
high			13.60	15.00	n.s.	high			1.56	1.49	n.s.
low-high				14.90		low-high				0.97	
high-low				3.96		high-low				1.41	
LSD			5.79**	2.82**		LSD			0.98*	0.46*	

<sup>a</sup>Included gizzard lining during the 8- and 20-week sampling periods. <sup>b</sup>(\*) and (\*\*) = significant at 0.05 and 0.01, respectively. <sup>c</sup>n.s. = nonsignificant difference between Cd concentrations.

situation involving the feeding of materials produced on sludge-amended soils.

Measurements relevant to the potential manifestation of Cd toxicosis (clinical chemistry, hematology, and histopathology) made throughout the study will be presented in a subsequent publication. A preliminary evaluation of these data confirms the conclusion inferred from the data presented here that a dietary level of about 1 mg/kg or

less of biologically incorporated Cd will have little if any effect on the health and performance of poultry.

If the enhanced concentration of Cd in corn grain and soybeans presents a potential hazard to human food chains as a result of feeding these materials to poultry, it must be a nominal one. Concentrations of Cd in eggs and muscle tissues were unaffected by corn grain and bean Cd concentrations. A potential health hazard would exist only

if internal organs from hens were continuously consumed over a long period of time. On a fresh weight basis (including fat and water), the highest concentration of Cd in liver of hens was 1.45 mg/kg and well within the upper range of concentrations that Kreuzer et al. (1977) found in the livers of swine produced under normal farm management conditions. At the age of 80 weeks, gizzards (lining removed) from hens on high-Cd diets contained 9.96 mg of Cd/kg on a dry weight basis, which translates to a wet weight concentration of about 3.3 mg of Cd/kg. Shellfish and crustaceans used for human foods frequently contain concentrations of Cd that are as high or higher than those found in the gizzard of hens (Ministry of Agriculture, Fisheries, and Food, 1973; Fassett, 1975), and thus the latter may present about the same potential human health hazard. But Cd contents of gizzards could be markedly reduced by switching the hens to a low-Cd diet a few weeks before they are marketed. The kidneys are removed from carcasses of spent hens before they are further processed and would not impact human food chains.

Lifetime ingestion of biologically incorporated Cd was about 3634, 21 687, and 36 949  $\mu\text{g}/\text{bird}$  for chickens on low-, intermediate- and high-Cd diets, respectively. On the basis of amounts in those tissues that accumulated Cd, it was estimated that total amounts of the metal retained in 80-week-old hens were 47.6, 212.6, and 298.3  $\mu\text{g}$  for those on low-, intermediate-, and high-Cd diets, respectively. This corresponds to a retention of 1.31, 0.98, and 0.81% of the total ingested Cd, respectively, by the several organs from hens on diets containing  $0.095 \pm 0.05$ ,  $0.57 \pm 0.11$ , and  $0.97 \pm 0.14$  mg of Cd/kg. The decrease in retention of Cd at higher contents in diets may indicate that either one or more of the organs was approaching saturation with the metal and more was excreted or it was translocated to other tissues whose contents were not considered in the calculation. At the highest dietary level, there was some indication that Cd was perhaps translocated to bones and lungs.

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## Crude Oleic Acid Volatiles

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Commercial-grade crude oleic acid has been shown to be a coyote attractant and may be effective in predator control. Pure oleic acid was found to lack attractancy; therefore, a study of the volatiles associated with the crude oleic mixture was made in an effort to identify the most active compounds. Volatiles obtained via steam distillation-extraction were separated into basic, neutral, and acidic fractions and analyzed by GC-MS. The total number of compounds identified was 132.

Coyotes (*Canis latrans*) cause considerable damage to livestock, especially sheep. A cooperative project between the U.S. Department of Agriculture and the University of

California, Davis, has resulted in the investigation of various materials that could be useful as lures in coyote trapping (Lorenz et al., 1983; Teranishi et al., 1977). Crude oleic acid volatiles but not pure oleic acid have shown promise as an attractant (Teranishi et al., 1981).

There are numerous literature references to the flavor of cooked meats (Buttery et al., 1977; Caporaso et al., 1977; Mussinan et al., 1974) but none on crude oleic acid. Wa-

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