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## Effects of Dietary Nickel on Survival and Growth of Mallard Ducklings

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**Abstract.** Mallard (*Anas platyrhynchos*) ducklings were fed nickel sulphate in their diet from day one to 90 days of age. Ducklings fed 1,200 ppm nickel began to tremor and show signs of paresis after 14 days of dosage (age) and 71% of this group died within 60 days of age. Birds fed 1,200 ppm nickel weighed significantly less ( $P < 0.05$ ) at 28 days of age than birds fed the other diets. Weights of ducklings fed untreated food or dietary dosages of 200 and 800 ppm nickel diets were not significantly different ( $P > 0.05$ ). The weight/length ratio of the humerus (an expression of bone density) from the 800 ppm diet females was significantly lower ( $P < 0.05$ ) than the control fed females at 30 and 60 days and for all ducklings fed 1,200 ppm at 30 days of age. The organ-weight/body-weight ratios for heart, liver, and gizzard did not differ from controls or between any dosage group. Liver nickel residues from ducklings that died during this study ranged between 1.0 to 22.7 ppm and kidney residues ranged between 2.7 to 74.4 ppm. Liver and kidney tissues from all ducklings that survived to 90 days of age contained less than 1.0 ppm nickel.

Environmental contamination by nickel occurs in local areas as a result of mining, smelting, combustion of fossil fuels, and industrial activities such as nickel plating and alloy manufacturing. Nickel may enter natural waterways from wastewater because it is poorly removed by the treatment process (Barth *et al.* 1965). Near the Copper Cliff smelter in Sudbury, Ontario, nickel concentration in the Wanapitei River water averaged 42 ppb, and 826 ppm in the algal periphyton and 690 ppm in the leaves of *Potamogeton* sp. (Hutchinson *et al.* 1975). Young surface feeding ducks (Subfamily Anatinae) eat periphyton, zooplankton, aquatic invertebrates, and *Potamogeton* during their development. The potential for waterfowl to concentrate nickel through their food is supported by the fact that elevated nickel levels were found in feathers from mallard and black ducks (*Anas rubripes*) collected in the nickel smelting areas of the Sudbury district of Ontario (Ranta *et al.* 1978). Little is known about the

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effects of dietary nickel on young animals. Nickel chloride injected into chicken eggs at two days of incubation produced many embryonic malformations and up to 96% mortality (Gilani and Marano 1980). As chicks ingested increasing amounts of nickel the body weight decreased (Weber and Reid 1968) and as little as 300 ppm dietary nickel reduced growth rates in chickens (Ling and Leach 1979). Dietary nickel also depressed weight gains in growing rats and accumulated to the greatest extent in the kidney tissue (Whanger 1973). Our objectives were to measure the effects of dietary nickel on the growth of mallard ducklings and to determine the residue levels in the liver and kidney tissues.

### Materials and Methods

Two hundred fertile mallard duck eggs were artificially incubated at the Patuxent Wildlife Research Center. One hundred and forty-four ducklings were weighed, sexed, web tagged, and placed in 12 vinyl-coated wire cages (0.9 m by 0.9 m by 0.6 m). Each cage of 12 ducklings (6 males and 6 females) was provided with flowing water, a food tray, a heat lamp, and a fluorescent light source set for a 13L:11D photoperiod. The temperature in the laboratory varied between 20° and 30°C. At 14 days of age the ducklings were separated into 24 cages (3 males and 3 females per cage) and the heat lamps were disconnected, because young waterfowl begin thermoregulation around 10 days of age (Cain 1972; Koskimies and Lahti 1964). The ducklings at day one were provided duck starter mash, *ad libitum*, for 90 days that had nickel sulfate dissolved in distilled water mixed into the food to produce dietary nickel at 0, 200, 800, or 1,200 ppm. Each of the 6 cages of 6 ducklings per treatment was randomly assigned the diet. At approximately 30-day intervals, the ducklings were weighed to the nearest gram, the bill length was measured in mm, and a male and a female from each cage (12 birds from each diet) were randomly selected and sacrificed. At 28 days, all ducklings were weighed and measured but necropsy, of randomly selected ducklings from the surviving ducklings, was delayed for two days. The heart, gizzard, liver, and right humerus were weighed to the nearest 0.1 g and the humerus length was measured to the nearest mm. All measurements and the necropsy were performed on the same day at 60 and 90 days of age. Liver and kidney tissues were placed in nitric-acid cleaned glass jars with teflon liners in the lid, frozen and later sent to the Analytical Bio Chemistry Laboratory for nickel analysis. Each liver or kidney sample was allowed to thaw and then physically pulverized by hand. An aliquot of the homogenized sample was weighed into a 100-ml micro-kjeldahl flask and digested with nitric-perchloric acid to the fumes of perchloric acid. The digested sample was transferred to a 10-ml volumetric flask, diluted with deionized water, and injected into a Perkin Elmer 305 B unit with D<sub>2</sub> background correction and the nickel content determined by the flame atomic absorption method. The lower limit of quantification was 1 ppm with an average recovery of 96%. Residues, expressed as ppm wet weight, were not corrected for recovery. Data on the body weights, bill length, organ-weight/body-weight ratio, and the humerus length/weight ratio were analyzed by t-tests for any significance between all treatment levels. Two-way ANOVA tests were used to determine any difference due to sex and confirm overall treatment effects.  $P < 0.05$  was necessary for significance between all comparisons.

### Results and Discussion

Nickel in the feed samples averaged 2.3, 176, 774, and 1,069 ppm nickel for the 0, 200, 800, and 1,200 ppm nickel diets respectively. The food was not dried before analysis. The 36 ducklings fed 1,200 ppm nickel began to tremor and show signs of developing paresis and ataxia at 14 days of age. The first duckling died at 29 days of age and 12 ducklings died at 30 days of age (Table 1). Six females at this high dose level survived and 5 of these were sacrificed and necropsied at 60 days of age. Ducklings fed 800 ppm nickel began to tremor at four weeks of age but only 2 of these ducklings died before the scheduled

Table 1. Effect of dietary nickel on survival of laboratory-raised mallard ducklings

Dietary nickel (ppm)	Age (days)		
	30	60	90
Control	36 <sup>a</sup> - 36 <sup>b</sup> - 12 <sup>c</sup>	24 - 24 - 12	12 - 12 - 12
200	36 - 36 - 12	24 - 24 - 12	12 - 12 - 12
800	36 - 36 - 12	24 - 22 - 12	12 - 10 - 10
1200	36 - 24 - 5	24 - 6 - 5	12 - 1 - 1

<sup>a</sup> Ducklings expected to be alive if no nickel related deaths had occurred

<sup>b</sup> Ducklings actually alive at indicated age and used for measurements

<sup>c</sup> Ducklings sacrificed at these scheduled necropsy intervals

necropsy at 60 days of age (Table 1). All ducklings fed 800 ppm and the one that survived on 1,200 ppm nickel continued to tremor throughout the 90-day experimental period.

Nickel toxicity in pigeons, and rats was expressed by unsteadiness while walking or by a constant chorea-like tremor of the whole body (Stuart 1883). Nickel ions are known to cause a 15-fold increase in the action potential of the nodes of Ranvier in the vagus nerve of the cat (Spyropoulos and Brady 1959); and nickel had been reported to prolong the active state of contraction in the sartorius muscle of the frog (Sandow and Isaacson 1966). These effects could cause the tremors and paresis as seen in this study. The major effects of nickel on excitable tissues (nerve, nerve-muscle junctions, and the central nervous system) increased the duration of the action potential, and was competitive and imitative of calcium (National Research Council 1975). In this study, pronounced edema in the toes and leg joints was also characteristic of all ducklings that developed tremors and paresis.

### Body Weight

The body weight of pen-raised mallards fed dietary nickel at 200 or 800 ppm did not differ significantly ( $P > 0.05$ ) from the controls at 90 days of age (Table 2); however, nickel at 1,200 ppm significantly ( $P < 0.05$ ) reduced the body weight of both sexes at 28 days of age. The average body weights for ducklings fed 0, 200, and 800 ppm nickel were significantly greater ( $P < 0.05$ ) than the weight of ducklings fed 1,200 ppm and these differences were not sex related. Males appeared more susceptible to the effect of 1,200 ppm nickel than females, because no males survived to 60 days of age whereas 6 females survived. A sex difference to nickel toxicity in rats was suggested by Schroeder and Mitchner (1971), because fewer males than females were born in a laboratory population given 5 ppm nickel in their drinking water for three generations. Growth rates of chickens fed a graded diet of 300 to 1,100 ppm dietary nickel for the first three weeks of age declined linearly (Ling and Leach 1979). Dietary nickel at 700 ppm also reduced chicken growth and the effect of nickel was in addition to reduced feed intake (Weber and Reid 1968). Growth rate reduction in weanling rats fed 500 ppm nickel (Whanger 1973) or 225 ppm in their drinking water (Clary 1975) also suggested that a major effect of nickel toxicity was reduced growth in young animals.

Table 2. Average body weights of ducklings fed dietary nickel for 90 days

Dietary nickel (ppm)	Age (days)				
	1	14	28	60	90
<b>Males</b>					
Control	41 (18) <sup>a</sup>	165 (18)	445 (18)	1079 (12)	1254 (6)
200	39 (18)	173 (18)	505 (18)	1098 (12)	1271 (6)
800	39 (18)	159 (18)	445 (18)	1017 (10)	1193 (4)
1200	41 (18)	137 (18)	284 (18) <sup>b</sup>	—	—
<b>Females</b>					
Control	40 (18)	198 (18)	524 (18)	1079 (12)	1120 (6)
200	39 (18)	179 (18)	532 (18)	1023 (12)	1119 (6)
800	40 (18)	178 (18)	469 (18)	1028 (12)	1040 (6)
1200	41 (18)	185 (18)	383 (18) <sup>b</sup>	837 (6) <sup>b</sup>	856 (1)

<sup>a</sup> First number is average body weight in grams for all ducklings alive at the indicated ages. Number in parenthesis is the sample size

<sup>b</sup> These values are significantly different ( $P < 0.05$ ) from all values above in the same column within the sex group. Sexes do not differ from each other except at 90 days of age

Table 3. Average bill length of mallard ducklings fed dietary nickel

Dietary nickel (ppm)	Age (days)				
	1	14	28	60	90
<b>Males</b>					
Control	14 (18) <sup>a</sup>	28 (18)	38 (18)	52 (12)	53 (6)
200	14 (18)	29 (18)	40 (18)	52 (12)	53 (6)
800	14 (18)	28 (18)	39 (18)	51 (10)	52 (4)
1200	14 (18)	28 (18)	37 (18) <sup>b</sup>	—	—
<b>Females</b>					
Control	14 (18)	28 (18)	39 (18)	47 (12)	51 (6)
200	14 (18)	27 (18)	39 (18)	50 (12)	51 (6)
800	14 (18)	28 (18)	39 (18)	50 (12)	51 (6)
1200	14 (18)	29 (18)	38 (18)	49 (6)	51 (1)

<sup>a</sup> First number is bill length in mm; number in parenthesis is sample size

<sup>b</sup> This value is significantly different ( $P < 0.05$ ) from all the values above it in this column

### Bill Length

Dietary nickel at 800 ppm or lower levels did not significantly affect the bill length of growing mallards (Table 3). Males fed 1,200 ppm nickel had significantly shorter bills ( $P < 0.05$ ) at 28 days of age than males fed the lower levels of nickel. This difference was not significant in the females. The average bill length of all mallards surviving to 90 days of age (Table 3) was similar to that reported by Bellrose (1976).

### Humerus

The weight of the right humerus was divided by its length to determine if dietary nickel affected this ratio. A decrease in the ratio would suggest that the

Table 4. Weight:length ratio of humerus from mallard ducklings fed dietary nickel

Dietary nickel (ppm)	Age (days)		
	28-30	60	90
<b>Males</b>			
Control	0.036 (6) <sup>a</sup>	0.083 (6)	0.076 (6)
200	0.039 (6)	0.078 (6)	0.075 (6)
800	0.041 (6)	0.070 (6)	0.075 (4)
1200	0.024 (6) <sup>b</sup>	—	—
<b>Females</b>			
Control	0.049 (6)	0.083 (6)	0.074 (6)
200	0.046 (6)	0.073 (6) <sup>b</sup>	0.069 (6)
800	0.036 (6) <sup>b</sup>	0.067 (6) <sup>b</sup>	0.074 (6)
1200	0.023 (6) <sup>b</sup>	0.063 (6) <sup>b</sup>	0.063 (1)

<sup>a</sup> First number is humerus weight (g) to length (mm) ratio; number in parenthesis is sample size  
<sup>b</sup> These values are significantly different ( $P < 0.05$ ) from all values above in the same sex group. Sexes do not differ from each other

bone is lighter per unit length. There was a significant decrease ( $P < 0.05$ ) in the ratio for 30-day old male ducklings fed 1,200 ppm and for female ducklings of the same age fed 800 and 1200 ppm compared to the ducklings fed 200 ppm or the control diet (Table 4). In the female ducklings, the decrease (i.e., lighter bones) was significant at 60 days of age for all the treated birds. At 90 days of age the difference was not significant between the females fed 800 ppm and the controls. These data suggest that dietary nickel at 800 ppm or higher affected the right humerus weight of birds up to 60 days of age; however, the effect was not evident at 90 days of age. Nickel substitutes for calcium in the excitation-contraction coupling of skeletal muscle (Fischman and Swan 1967) and competes with calcium in nervous tissue (National Research Council 1975). Perhaps an interference with calcium into skeletal bone is a possible effect that warrants further investigation.

#### *Heart, Liver, and Gizzard*

Smaller ducklings at all dietary levels had smaller organs, so the weight of each organ removed from all ducklings was divided by the fresh-killed body weight of that animal. There was no significant difference ( $P > 0.05$ ) in the organ-weight/body-weight ratio between the sexes or the treatment levels. Dietary nickel at the levels tested apparently had no effect on the wet weight of the heart, liver, or gizzard relative to the body weight of these mallard ducklings.

#### *Liver and Kidney Residue Levels*

At 30 days of age, only 2 of the 12 ducklings sacrificed from the 200 ppm diet and 3 of the 5 from the 1,200 ppm group had nickel residues above the 1 ppm detection level in their liver and kidney tissues (Table 5). Nickel was not detected in liver tissue from control ducklings or those fed 800 ppm at 30 or 60 days of age. Nickel residues were below the detection level of 1 ppm in all of

Table 5. Mean body weight and mean detectable nickel residues in liver and kidney tissue from ducklings fed dietary nickel

	Age (days)	Dietary nickel (ppm)			
		0	200	800	1200
	30				
Sample size		12	12	12	5
Body weight (g)		452	465	436	253
Liver nickel residue (ppm)		nd <sup>a</sup>	1.2	nd	3.7
No. with residue		0	2	0	3
Kidney nickel residue (ppm)		nd	1.6	1.9	11.6
No. with residue		0	2	11	5
	60				
Sample size		12	12	12	5
Body weight (g)		975	1054	1024	812
Liver nickel residue (ppm)		nd	nd	nd	2.1
No. with residue		0	0	0	2
Kidney nickel residue (ppm)		nd	nd	1.0	4.3
No. with residue		0	0	3	5
	90				
Sample size		12	12	10	1
Body weight (g)		1187	1195	1121	856
Liver nickel residue (ppm)		nd	nd	nd	nd
No. with residue		0	0	0	0
Kidney nickel residue (ppm)		nd	nd	nd	nd
No. with residue		0	0	0	0

<sup>a</sup> nd = not detected at the 1.0 ppm wet weight level

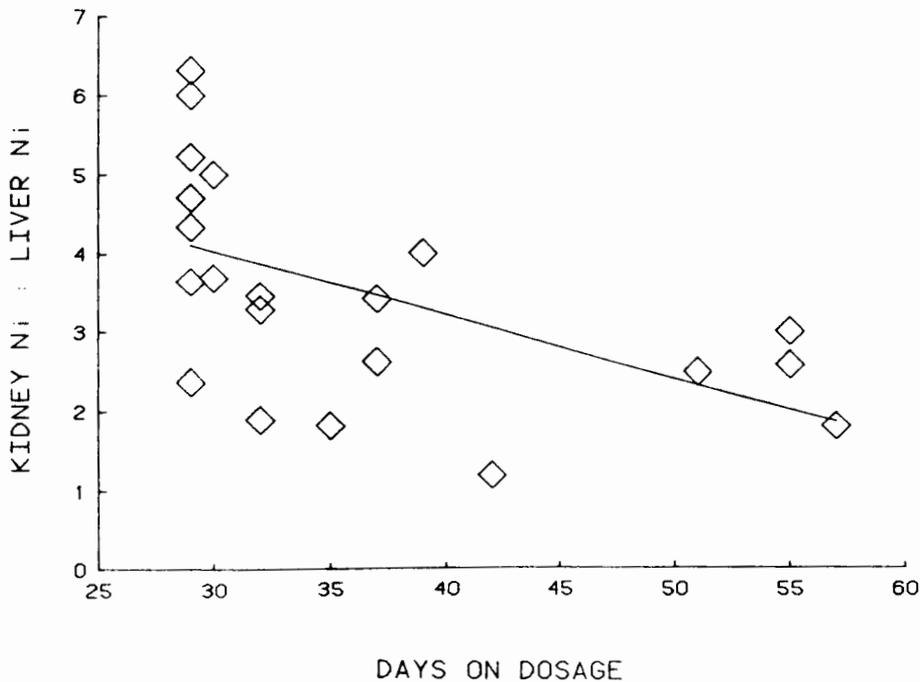
the liver samples from the 35 ducklings that survived to 90 days of age (Table 5). Ducklings that died before scheduled necropsy had high nickel residues in the liver tissue (Table 6). These residue levels were not sex dependent although more males than females died (Table 2). Nickel at 1,100 ppm was so toxic to young chickens that 11 of 16 chicks died before 21 days of age (Ling and Leach 1979) and the 5 that survived to 21 days had a mean liver residue level of 1.43 ppm. Nickel accumulated in the kidney during the first 30 days of growth, reaching a mean of 11.6 ppm in the ducklings fed 1,200 ppm (Table 5). By 60 days of age, the mean level had declined to 4.3 ppm in the ducklings necropsied; the one female fed 1,200 ppm that survived to 90 days of age had less than 1.0 ppm nickel in the kidney. Nickel residues in kidney also declined in the ducklings fed 800 ppm (Table 5). Nickel levels were high in the kidney and liver tissues from ducklings that died during dosage of 1,200 ppm (Table 6). Nickel averaged 20.4 ppm in the kidney tissue from 10 ducklings fed 1,200 ppm nickel and died on or before 30 days of age. This is in contrast to 11.6 ppm in the 5 ducklings on 1200 ppm dosage that were sacrificed at 30 days of age (Table 5). Nickel levels in the kidneys from 21 of 25 ducklings that died on the 1,200 ppm diet were about three times those in the livers (Figure 1). The relationship of this ratio to age was linear ( $Y = 6.43 - 0.08 X$ ,  $r^2 = 0.30$ ). The large variation in this ratio at 29 days of age (Figure 1) probably accounts for the low  $r^2$  value in the equation. The equation suggests the ratio will decrease as the ducklings age and this is in line with the residue data after 60 days of age (Table 5). Nickel

**Table 6.** Nickel residues in liver and kidney tissues from 24 of 27 ducklings that died before scheduled necropsy after dietary nickel exposure

Nickel in diet (ppm)	Age in days at death	Males		Females	
		Liver	Kidney	Liver	Kidney
Control	— <sup>a</sup>	—	—	—	—
200	—	—	—	—	—
800	43	2.6 <sup>b</sup>	5.5	—	—
800	56	1.5	20.7	—	—
1,200	29	11.5	41.8	7.2	33.9
	29	5.7	24.7	10.0	23.7
	29	2.7	12.7	1.8	9.4
	29	1.0	6.0	3.5	22.1
	30	2.5	9.2	4.1	20.5
	32	22.7	74.4	4.9	16.9
	32	—	—	5.4	10.2
	35	7.3	13.3	—	—
	37	13.6	35.8	5.8	19.8
	39	4.7	18.8	—	—
	42	6.0	7.2	—	—
	51	4.5	11.3	—	—
55	7.5	22.6	4.2	10.9	
57	1.5	2.7	—	—	

<sup>a</sup> No duckling at this age or dietary nickel treatment (—) died

<sup>b</sup> Nickel residues in ppm wet weight



**Fig. 1.** Relationship of the ratio of the kidney/liver nickel residues (ppm wet weight) from 21 ducklings that died after dietary nickel exposure of 1200 ppm ( $Y = 6.43 - 0.08 X$ ,  $r^2 = 0.30$ )

levels in the livers of all but five of the 11 ducklings that survived to necropsy were below the 1.0 ppm detection level (Table 5).

Data from this study indicate that mallard ducklings feeding on diets that contain at least 800 ppm nickel would be affected adversely. Tremors and paresis that were noted after 14 days of age probably would increase mortality in wild ducklings that were restricted to a nickel-contaminated feeding area. Most waterfowl begin to fly between 42 and 56 days of age and thus would remain in the same area past the time period that recorded a 71% mortality with the 1,200 ppm diet. The data indicate that analysis of liver and kidney tissue from mallard ducklings 30 days of age may not lead to the detection of serious nickel exposure. Ducklings that were fed 800 ppm nickel for 30 days did not have nickel residues in their livers at the 1.0 ppm level. If, however, residues of nickel greater than 1.0 ppm are found in both liver and kidney tissues, the exposure to dietary nickel may have been substantial and may be at a critical level in the food chain of the ducklings.

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