# Mercury Levels in the Tissues of Ring-necked Pheasants Fed Two Mercurial Fungicides

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## INTRODUCTION

The widespread use of mercurial fungicides as seed dressings has been a common practice since the 1940's. A number of investigators have pointed out that a reduction in the reproductive potential could theoretically occur if pheasants (*Phasianus colchicus*) consumed seed grain treated with alkylmercury compounds (BORG et al. 1969, FIMREITE et al. 1970, FIMREITE 1971, ADAMS and PRINCE 1972, SPANN et al. 1972). Removal of the alkylmercurials as seed dressings has apparently corrected these problems.

Although data are available on relative toxicity of mercury compounds to pheasants, the relative distribution of mercury in the tissues is not well understood. BORG et al. (1969) reported on the distribution of mercury in pheasant tissues while distribution of mercury compounds in chickens (*Gallus gallus*) have been studied by KIWIMAE et al. (1969); MILLER et al. (1961); SWENSSON and ULFVARSON (1968); SWENSSON and ULFVARSON (1969); SMART and LLOYD (1963); and TEJNING and VESTERBERG (1964); and in quail by BACKSTROM (1969).

The purpose of this study is to describe the relative distribution, excretion, and mercury concentrations in body tissues resulting from the consumption of methylmercury dicyandiamide and phenylmercuric acetate.

## MATERIALS AND METHODS

Two mercurial fungicides (NOR-AM Agricultural Products, Inc.) were used, Panogen 15$^R$, containing 2.2 percent methylmercury dicyandiamide, and Panomatic$^R$, containing 3.4 percent phenylmercuric acetate.

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Adult hen and cock pheasants were fed Purina game breeder pellets *ad libitum* treated with varied levels of the mercurial fungicides. The birds were divided into two groups, those fed methylmercury dicyandiamide and those fed phenylmercuric acetate. These groups were subdivided into those fed treated food every day and those fed treated food every third day. There was an array of 12 concentrations of each compound for the daily treatment and 5 concentrations of each compound for the treatment given every third day. There was one hen per concentration and one cock with each hen in all treatments except the hens fed treated food daily which had one cock for each three hens. These cocks were rotated between the hens on a daily basis and were allowed to feed on treated food of the same concentrations as the hens they were with. The amount of food consumed and mg of mercury was recorded daily for each bird during the experiment. The concentrations of mercury in the diet ranged from zero to 30.7 ppm methylmercury dicyandiamide and from zero to 43.0 ppm phenylmercury acetate. The concentrations selected for each compound correspond to the range of concentrations recommended for seed treatment and are based on mg/kg of mercury rather than mg/kg of the respective compounds. The concentration of mercury in the diet of each bird was obtained by spraying a predetermined amount of mercurial compound on the food in a rotating jar. The various diets were made singly in one kg quantities, thoroughly mixed, and stored in sealed plastic bags unitl used.

The experiment was terminated on day 74 and all birds living at this time were sacrificed for mercury analysis. At least 10 grams of breast muscle and liver and the entire kidney, gonad, and brain were collected and frozen until analysis. Similar samples were collected from all birds that died during the experiment. Fecal material was collected on day 10, 18, and 24 of the experiment from the hens fed methyl- and phenylmercury daily.

All tissues and fecal samples were prepared for analysis by cold acid digestion employing trichloroacetic acid and sulfuric acid in an ice bath. Final digestion and clearing of the samples was done with concentrated hydrogen peroxide. The samples were analyzed by flameless atomic absorption as described by HATCH and OTT (1968) and UTHE et al. (1970) following minor modifications for analysis on a Jarrell Ash atomic absorption spectrophotometer with a detection limit of one ppb in solution. All values are reported as total mercury and are given on a wet weight basis as mg/kg (ppm). Standard statistical procedures (variance and correlation) were used (STEEL and TORRIE 1958). All values were tested at the 0.05 and 0.01 probability levels and unless otherwise specified the term significance applies to the 0.05 level. Variation about the mean is denoted by the standard error.

## RESULTS

The mean concentration of mercury in the tissues of the control birds ranged from 0.027 ± 0.006 mg/kg in the brain tissue to 0.090 ± 0.042 mg/kg in the breast muscle (Figure 1). There were no significant ($p < .01$) changes in the mercury concentrations of 4 control hens sacrificed at the start and 7 hens at the conclusion of the experiment and mercury levels were not significantly ($p < .01$) different between the sexes of control birds. The greatest concentration of mercury occurred in the breast muscle and the kidney with somewhat lower quantities in the ovary and brain. The liver values were between the two groups.

Tissues from 36 experimental birds were analyzed for total mercury content (Table 1). The concentration of mercury in the tissues of 7 of 9 hens which had consumed an average of 24.7 ± 0.8 mg of methylmercury dicyandiamide prior to death showed a significant ($p < .01$) increase from the control hens and ranged from 12.33 ± 1.97 mg/kg in the gonad to 53.62 ± 6.39 mg/kg in the kidney (Figure 2). The breast muscle contained significantly less mercury than the kidney and liver, but more than either the ovary or brain. The hens fed methylmercury treated food every third day showed essentially the same distribution pattern as the hens fed daily.

The amount of methylmercury dicyandiamide consumed by all cocks fed methylmercury dicyandiamide ranged from 3.42 to 45.3 mg. The cock which consumed the largest amount died on day 41 of the experiment. There is a positive correlation between the amount of methylmercury dicyandiamide consumed and the mercury content of the kidney ($r = 0.96$), breast muscle ($r = 0.99$), and gonad ($r = 0.94$). Insufficient data were available to include liver or brain tissue in this analysis. The highest rate of increase of mercury in the tissues with the quantity consumed was in the kidney followed by the breast muscle, liver and gonad.

The concentration of mercury in hens (in all tissues except the breast muscle) continued to increase as additional phenylmercuric acetate was consumed (Figure 3). The correlation coefficient between the concentration in the breast muscle and the amount of phenylmercury consumed was not significant ($r = 0.49$). The average concentration in the breast muscle was 0.53 ± 0.17 mg/kg with the highest concentration being 1.61 mg/kg after 191.2 mg of phenylmercuric acetate had been consumed. The greatest accumulation of mercury occurred in the kidney followed by the liver and gonad, breast muscle, and brain. Consumption of 10 mg of phenylmercuric acetate resulted in significant increases in mercury concentrations of all tissues analyzed except brain when compared to control birds. Consumption of approximately 100 mg of phenylmercuric acetate resulted in mercury levels in the brain that were higher than control birds.

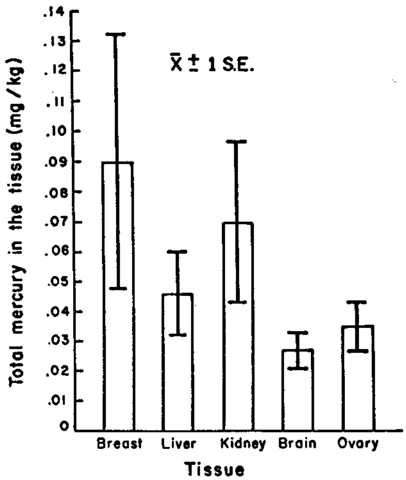


Figure 1. Distribution of mercury in the tissues of 11 control birds.

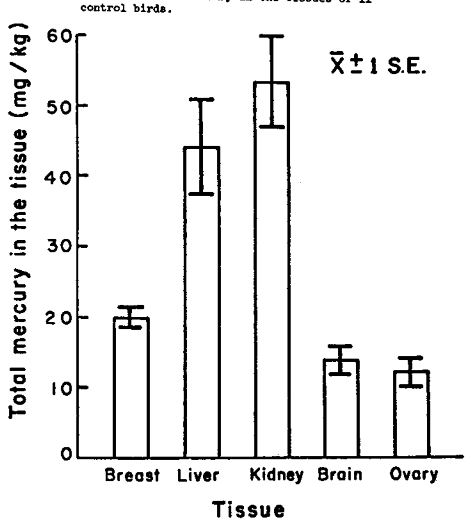


Figure 2. Distribution of mercury in the tissues of 7 hens that died from consumption of methylmercury dicyandiamide.

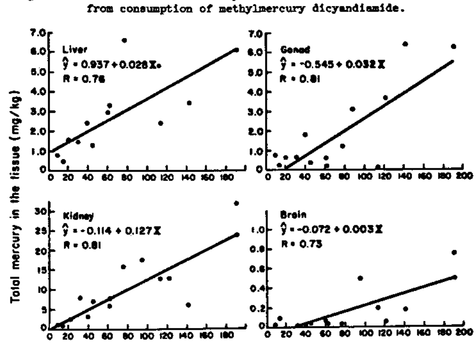


Figure 3. Distribution of mercury in the tissues of female pheasants as related to the total consumption of phenylmercuric acetate.

TABLE 1

Mercury concentrations in the tissues of male and female pheasants fed methylmercury dicyandiamide and phenylmercuric acetate.

| Treatment | Sex (F or M) | Concentration of Hg on the food (mg/kg) | Amount of Hg Consumed (mg) | Fate | Mercury in the Tissue (mg/kg) Breast Muscle | Liver | Kidney | Brain | Gonad |
|---|---|---|---|---|---|---|---|---|---|
| Methyl- | F | 2.6 | 11.2 | Sacrificed | 6.32 | 10.50 | 11.89 | 5.13 | 1.74 |
| mercury | F | 5.1 | 22.5 | " | 31.36 | 15.50 | 19.51 | 7.35 | 3.67 |
| dicyandia- | F | 10.2 | 25.4 | Died | 12.72 | 44.48 | 46.10 | 9.43 | 10.90 |
| mide fed | F | 12.8 | 20.2 | " | 19.34 | -- | 57.45 | -- | 13.08 |
| daily | F | 15.4 | 21.9 | " | 19.06 | -- | 50.16 | -- | 10.64 |
| | F | 17.9 | 25.7 | " | 25.18 | -- | 88.59 | -- | 22.25 |
| | F | 20.5 | 26.0 | " | 20.79 | 38.32 | 47.57 | 18.54 | 8.24 |
| | F | 25.6 | 24.8 | " | 22.73 | 31.27 | 34.46 | 12.36 | 6.33 |
| | F | 30.7 | 26.7 | " | 19.75 | 62.93 | 51.04 | 16.14 | 14.86 |
| | M | 0-7.7 | 18.0 | " | 11.77 | 8.15 | 11.42 | 0.90 | 1.23 |
| | M | 20.5-30.7 | 45.3 | " | 27.25 | -- | 96.83 | -- | 19.11 |
| Methyl- | F | 5.1 | 7.6 | Sacrificed | 4.46 | 8.21 | 7.22 | 2.26 | 0.24 |
| mercury | F | 10.2 | 14.3 | " | 7.83 | 12.82 | 12.01 | 3.86 | 0.36 |
| dicyandia- mide fed | M | 5.1 | 3.4 | " | 5.09 | 9.35 | 3.29 | -- | 1.03 |
| every | M | 10.2 | 4.6 | " | 8.16 | 14.35 | 4.72 | -- | 1.94 |
| third day | M | 20.5 | 11.8 | " | 9.08 | 10.88 | 10.38 | -- | 2.67 |
| | F | 3.6 | 15.3 | " | 0.13 | 0.52 | 1.19 | 0.09 | 0.31 |
| Phenyl- | F | 7.2 | 32.3 | " | 0.21 | 1.46 | 8.10 | 0.03 | 0.62 |
| mercuric | F | 10.7 | 45.0 | " | 0.41 | 1.29 | 7.04 | 0.06 | 0.40 |
| acetate | F | 14.3 | 62.0 | " | 0.15 | 3.28 | 8.02 | 0.04 | 0.22 |
| fed daily | F | 17.9 | 78.1 | " | 0.08 | 6.58 | 15.80 | 0.04 | 1.22 |

TABLE 1. (Continued)

| Treatment | Sex (F or M) | Concentration of Hg on the food (mg/kg) | Amount of Hg Consumed (mg) | Fate | Mercury in the Tissue (mg/kg) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Breast Muscle | Liver | Kidney | Brain | Gonad |
| | F | 21.5 | 95.7 | Sacrificed | 0.12 | -- | 17.38 | 0.50 | -- |
| | F | 25.1 | 113.5 | " | 0.12 | 2.43 | 12.67 | 0.20 | 0.14 |
| | F | 28.7 | 122.3 | " | 0.50 | 5.26 | 12.83 | 0.07 | 3.66 |
| | F | 32.2 | 142.3 | " | 1.08 | 3.46 | 6.18 | 0.19 | 6.37 |
| | F | 43.0 | 191.2 | " | 1.61 | 6.11 | 31.82 | 0.76 | 6.26 |
| | M | 0-10.7 | 23.7 | " | 2.27 | 2.51 | 2.44 | 0.07 | 0.31 |
| | M | 14.3-25.1 | 86.6 | " | 0.12 | 1.13 | 8.46 | 0.05 | 0.21 |
| | M | 28.7-43.0 | 152.7 | " | 2.25 | 5.72 | 12.67 | 0.20 | 0.19 |
| Phenyl-mercuric acetate fed every third day | M | 7.2 | 4.4 | " | 0.79 | -- | 1.23 | -- | 0.28 |
| | M | 14.3 | 8.6 | " | 2.97 | 6.11 | 4.02 | -- | 0.21 |
| | M | 28.6 | 17.3 | " | 1.14 | -- | 5.49 | -- | 0.05 |
| | F | 7.2 | 10.0 | " | 0.18 | 0.82 | 1.47 | 0.04 | 0.80 |
| | F | 14.3 | 20.6 | " | 0.34 | 1.58 | 2.78 | -- | 0.68 |
| | F | 28.6 | 39.6 | " | 0.44 | 2.53 | 3.03 | 0.02 | 1.86 |
| | F | 43.0 | 60.7 | " | 1.87 | 2.99 | 5.80 | 0.08 | 0.60 |

The hens fed phenylmercuric acetate every third day tended to show higher concentrations of mercury in the breast muscle, liver and gonad and lower concentrations in the kidney than the hens fed similar amounts of phenylmercury acetate daily (Table 1). The concentrations in the brain and the relative distribution among the tissues showed no differences for the two treatments.

The amount of phenylmercuric acetate consumed by 6 cocks, all living at the termination of the experiment, ranged from 4.4 to 152.7 mg. The highest concentration of mercury occurred in the kidney which showed a positive correlation ($r = 0.95$) between increased consumption of phenylmercuric acetate and levels of mercury in the kidney tissue. This correlation was not significant for any other tissues. The concentration of mercury in the liver, breast muscle, gonad and brain was 3.86 ± 1.22 mg/kg, 1.60 ± 0.44 mg/kg, 0.21 ± 0.04 mg/kg and 0.11 ± 0.05 mg/kg, respectively.

Although there was a trend for the total mercury levels in the feces to increase as the concentration of mercury on the food increased, only 3 of the 6 sample groups had significant correlation coefficients. The correlation coefficients (r) for the samples collected on day 10, 18 and 24 from hens fed methylmercury dicyandiamide and phenylmercuric acetate daily were 0.77*, 0.57, 0.49 and 0.08, 0.67* and 0.98*, respectively. Significant correlation coefficients are denoted by an asterisk. The average concentration of mercury in the feces collected on day 10, 18 and 24 from hens fed methylmercury dicyandiamide and phenylmercuric acetate daily was 0.34 ± 0.06 mg/kg, 0.98 ± 0.03 mg/kg, 1.30 ± 0.33 and 7.89 ± 1.27 mg/kg, 11.49 ± 3.49 mg/kg and 6.21 ± 1.34 mg/kg, respectively. The concentration of the total mercury in the feces from hens fed methylmercury dicyandiamide was significantly less than in the feces from the hens fed phenylmercuric acetate, however, it tended to increase with time whereas the concentration of mercury in the feces from hens fed phenylmercuric acetate did not tend to increase with time.

## DISCUSSION

A comparison of total mercury concentrations in the tissues and feces of pheasants fed methylmercury dicyandiamide and phenylmercuric acetate indicates there are distinct differences between the toxicity (ADAMS and PRINCE 1972) and rates of assimilation and elimination of these compounds. The concentration of total mercury in the tissue was related to the relative toxicity of the two compounds. The highest mercury levels were in the tissues of birds that died from methylmercury dicyandiamide. Although the birds consumed greater quantities of phenylmercuric acetate without dying, up to 191.2 mg, relatively low concentrations of total mercury were present in all tissues with brain and breast muscle tissues showing the smallest increase over levels in the control birds. Investigations on chickens and quail by SWENSSON and ULFVARSON (1969) and BACKSTROM (1969) have also shown that alkylmercury compounds do accumulate in greater quantities than arylmercury compounds.

The proportional increase of total mercury in the tissues as the total quantity of mercury consumed increased was more rapid for methylmercury dicyandiamide and was reflected by smaller concentrations of total mercury in the feces. The opposite of this was observed in the birds fed phenylmercuric acetate. The relative distribution of total mercury was similar for both compounds. Similar results have been reported for pheasants by BORG *et al*. (1969) and by TEJNING and VESTERBERG (1964) with chickens using methylmercury dicyandiamide and by MILLER *et al*. (1960) and SWENSSON and ULFVARSON (1969) with chickens using phenylmercuric acetate.

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