# Residues of Mercury in Tissues and Eggs of Chickens Given Oral Doses of Panogen 15[1]

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## INTRODUCTION

Seed is commonly treated with fungicides to reduce loss from plant diseases caused by organisms associated with the seed or present in the soil. Fungicides containing mercury have been used for this purpose in the past, and one of these fungicides is Panogen 15® (methyl mercury dicyandiamide)[3]. On March 9, 1970, the USDA suspended alkyl mercury compounds, such as Panogen 15, from use as seed treatments. Panogen 15 was still registered for use on cotton as a liquid or granular formulation applied in-furrow and covered at planting or for non-grazed grass areas. As of March 22, 1972, all uses of alkyl mercury compounds were cancelled or suspended by the Environmental Protection Agency.

Although most mercurial compounds are toxic to domestic animals, it has been shown that adult chickens are usually not affected by consuming large quantities of seed treated with Panogen 15 (TEJNING and VESTERBERG 1964, TEJNING 1967). Residues of mercury have been determined in 6-week-old chickens given Panogen 15 by oral capsule (WRIGHT et al. 1973) or fed treated seed (HANKO et al. 1970), and have been determined in eggs and certain tissues of chickens that were accidentally fed Panogen-treated seed (HOWELL 1969) or purposely fed the treated seed (SMART and LLOYD 1963, TEJNING and VESTERBERG 1964, TEJNING 1967) at levels that might be consumed if accidentally exposed to seed treated with the fungicide as recommended.

The objectives of our study were to determine the toxic effects on, and the residues of mercury in tissues and eggs of, individual chickens dosed daily by oral capsule for an extended period with Panogen 15 at a specific mg/kg dosage that chickens might be expected to consume if exposed to seed treated with the fungicide in the previously recommended manner.

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1/ This paper reflects the results of research only. Mention of a proprietary product or a pesticide in this paper does not constitute an endorsement or a recommendation of this product by the USDA.

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3/ 2.2% methyl mercury dicyandiamide and 1.5% mercury equivalent, Nor-Am Agricultural Products, Inc., Chicago, Ill.



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## MATERIALS AND METHODS

One group of 3 roosters and 10 hens received daily oral doses of 0.5 mg/kg of mercury as Panogen 15. The dose was equivalent to 33 mg/kg per day of the Panogen 15 formulation. Another group of 3 roosters and 10 hens were sham-treated and served as a control. All chickens were 8-month-old White Leghorns individually caged with feed and water supplied ad libitum. Daily dosing continued until death or until 100 doses had been given. New dosages for each chicken were calculated weekly to account for weight changes.

Semen was collected from the roosters and was pooled within each group. The hens were artificially inseminated three times a week with pooled semen from roosters in the same group.

The daily feed consumption and egg production and weekly weight of each chicken were recorded. Means were examined for significance by Student's t test. Eggs collected from each group of hens were combined into weekly groups; some were analyzed for residues of mercury, and the remainder were used in hatchability studies. Eggs that failed to hatch were opened, and embryos were examined for gross defects. Some of the chicks hatched from eggs laid by dosed hens were killed for tissue analysis.

Collection and Preparation of Samples. The eggs were broken, the yolk and albumin separated, and the yolk was washed with saline and carefully dried by rolling on a paper towel. Samples of yolk and albumin were pooled according to group and week laid, mixed well, and frozen at -20°C until analyzed.

Chickens were slaughtered at days 1, 15, 50, and 100 after completion of the 100 daily doses, and samples of tissue were collected, ground in an electric meat grinder, mixed well, and frozen until analyzed. Samples were also collected from the two hens that died during the experiment, on days 42 and 90 after initiation of treatment. Tissues of chicks hatched from eggs of treated hens were pooled within each tissue type and age group and stored as above until analysis.

Digestion and Analysis of Samples. Samples of albumin and tissue were digested and analyzed for mercury according to the modified atomic absorption procedure (ANALYTICAL METHODS COMMITTEE 1960, DALTON 1969, WILLIS 1962) used earlier (WRIGHT et al. 1973). Because of the high concentration of mercury present in some tissues, samples of 1.0 - 5.0 g were routinely analyzed.

The wet-acid digestion procedure used for the tissues and albumin could not be used for yolk because the lipid portion of the yolk charred and made recovery of mercury erratic. Therefore, a modified

procedure for the determination of submicro quantities of mercury in biological materials was used to digest the samples of yolk (MALAIYANDI and BARETTE 1970). In this procedure, 5.0 g of yolk, 6.0 ml concentrated nitric acid, and 0.25 g of vanadium pentoxide were heated in a reflux apparatus until foaming started. (In original procedure, a reflux temperature of 75-80°C and a dry ice cold-finger condensor were used. We used a temperature of 110-115°C and a water-cooled condensor). At the time of foaming, 2.0 ml of concentrated sulfuric acid was added dropwise to help control foaming and temperature. Finally, 12.0 ml of a 1:1 mixture of concentrated nitric acid and concentrated sulfuric acid was added dropwise. After addition of the acids was complete, the sample was refluxed for 15 min at 110-115°C and then allowed to cool. Hydrogen peroxide (5 drops of 30%) was added, and then a 3% solution of sulfuric acid was used to wash down the apparatus. The lipid contents were filtered off by pouring the cooled solution through prewashed glass wool, and the sample was brought to volume in a 100-ml volumetric flask with 3% sulfuric acid.

The mercury present in the digested yolk was quantitated by the flameless mercury technique (HATCH and OTT 1968) of atomic absorption spectrophotometry.

In order to determine the recovery of mercury in the procedures used in this study, we fortified samples of tissue, albumin, and yolk with known amounts of mercury as Panogen 15. These fortified samples were digested and analyzed for mercury as previously described. The percentage recovery of mercury from the samples ranged from 81 to 116%. Average recovery of mercury from the tissues and albumin was about 91% and from yolk by the flameless mercury technique was 103%. The results reported in Tables I-III have not been corrected for recovery.

## RESULTS AND DISCUSSION

Residues. Residues of mercury in tissues of chickens given daily oral doses of 0.5 mg/kg of mercury as Panogen 15 were greatest in liver and kidney (Table I). In these chickens, residues of mercury in the kidney were generally higher than the residues in the liver, whereas earlier studies (WRIGHT et al. 1973) showed the reverse. The difference may be due to the variance in the dose level (0.075 and 0.15 mg/kg of mercury per dose in the earlier study (WRIGHT et al. 1973) to 0.5 mg/kg of mercury per dose in this study) or to age and physiological state of the chickens. The chickens in our study were 8 months old and those in the earlier study (WRIGHT et al. 1973) were 6 weeks old. The residues of mercury in muscle followed the same pattern in both studies, i.e., residues were greater in breast muscle than in leg muscle.

TABLE I

Residues of mercury (ppm) in tissues of chickens given Panogen 15 in daily oral doses (0.5 mg/kg mercury) for 100 days[a]

| | PPM at Indicated Days after Initial Dose | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 42 | 90 | 100[b] | | 115 | 150 | | 200 | |
| Tissue | ♀ | ♀ | ♀ | ♂ | ♀ | ♀ | ♂ | ♀ | ♂ |
| Liver | 114.0 | 67.0 | 23.0 | 38.0 | 50.9 | < 0.1 | 10.7 | 1.5 | < 0.1 |
| Kidney | 192.0 | 78.0 | 22.0 | 52.0 | 50.9 | < 0.1 | 15.8 | 1.6 | 3.3 |
| Spleen | 72.0 | 28.6 | 13.8 | 19.3 | 18.3 | 1.1 | 5.9 | 0.6 | < 0.1 |
| Gizzard | 34.0 | 25.5 | 11.5 | 15.5 | 11.1 | < 0.1 | 1.0 | 0.1 | < 0.1 |
| Heart | 23.3 | 14.5 | 6.1 | 6.9 | 10.4 | < 0.1 | 1.6 | < 0.1 | < 0.1 |
| Breast Muscle | 31.2 | 30.0 | 13.6 | 18.2 | 23.0 | 1.9 | 4.4 | < 0.1 | 1.0 |
| Leg Muscle | 25.8 | 24.2 | 9.2 | 10.0 | 8.7 | 0.6 | 2.6 | < 0.1 | 0.2 |
| Brain | 25.3 | 19.0 | 9.7 | 10.6 | 11.1 | 1.6 | 2.3 | < 0.1 | 0.1 |
| Ovary | 31.2 | 14.7 | 8.5 | -- | 8.2 | 0.8 | -- | 2.3 | -- |
| Oviduct | -- | 37.0 | 37.5 | -- | 18.5 | < 0.1 | -- | < 0.1 | -- |
| Testicle | -- | -- | -- | 2.8 | -- | -- | 0.7 | -- | 0.5 |

[a] Determinations on pooled sample from two hens except days 42 and 90 represent single samples of hens that died of apparent mercury poisoning. Data for males represents single sample.

[b] Treatment stopped after 100 days.

The residues of mercury in the tissues analyzed in this study agree favorably with those in earlier work of TEJNING and VESTERBERG (1964); kidney had greatest residues of mercury, followed by liver, breast muscle brain, heart, and ovaries. After dosing had stopped in this study, the residues of mercury in tissues of both hens and roosters decreased to less than 4.0 ppm at 100 days. Residues in controls were < 0.3 ppm throughout the study.

The residues of mercury in eggs collected from treated chickens were much higher in albumin than in yolk (Table II). An average of about 90% of the total mercury in the egg was found in the albumin and the remainde in the yolk. These results agree well with those of SMART and LLOYD (196 who found 91% of the total mercury in the egg in the albumin fraction. The highest percentage of mercury in the yolk in this study was near the end (12% at weeks 14 and 15). The highest percentage of mercury in the albumin (94%) was found in eggs collected during the first week after initiation of treatment. Residues of mercury reached a plateau at about 0.8 mg/egg by 7 weeks after initiation of treatment and remained near this level throughout the feeding period. Residues in eggs of control hens were < 0.3 ppm throughout the study.

TABLE II

Mercury (ppm) in albumin and yolk of eggs collected from hens given Panogen 15 in daily oral doses (0.5 mg/kg mercury) for 100 days

| Week Collected | PPM | | Total Mercury in Egg (mg)[a] |
|---|---|---|---|
| | Albumin | Yolk | |
| 1 | 7.7 | 0.9 | .277 |
| 2 | 12.4 | 2.3 | .461 |
| 3 | 13.6 | 3.2 | .517 |
| 4 | 18.2 | 4.2 | .690 |
| 5 | 18.1 | 3.6 | .677 |
| 6 | 19.8 | 4.1 | .743 |
| 7 | 24.0 | 4.0 | .884 |
| 8 | 23.8 | 4.4 | .884 |
| 9 | 20.9 | 3.6 | .772 |
| 10 | 23.0 | 4.7 | .862 |
| 11 | 26.4 | 4.6 | .976 |
| 12 | 26.7 | 4.9 | .991 |
| 13 | 20.5 | 5.0 | .782 |
| 14 | 20.6 | 5.6 | .796 |
| 15[b] | 21.6 | 6.1 | .838 |

[a] Calculated figure assuming average weight of egg to be 56.0 g, with egg shell weight 5.0 g, yolk 17.0 g, and albumin 34.0 g.

[b] Dosing stopped at 100 days.

The residues of mercury found in tissues of chicks hatched from eggs of treated chickens are given in Table III. The tissues of 1-day-old chicks hatched from eggs collected during the sixth week of dosing showed greatest residues in kidney and liver, as expected. Residues of mercury in the tissues of 14-day-old chicks hatched from eggs collected during the eighth week of dosing had decreased sharply because of the fast growth of chicks at this age and the possible loss of residues through excretion. Greatest residues were again found in the kidney and liver. Residues of mercury were not detected in tissues of chicks hatched from eggs of control chicken.

TABLE III

Mercury (ppm) in tissues of chicks hatched from eggs laid by hens given Panogen 15 in daily oral doses (0.5 mg/kg mercury) for 100 days

| Tissue[a] | PPM at Indicated Week Egg Collected | |
|---|---|---|
| | 6[b] | 8[c] |
| Liver | 31.4 | 3.6 |
| Kidney | 34.3 | 3.9 |
| Spleen | 5.5 | < 0.1 |
| Gizzard | 16.1 | 1.2 |
| Heart | | 0.8 |
| Breast Muscle | 16.5 | 1.3 |
| Leg Muscle | 13.3 | 1.2 |
| Brain | 15.6 | 2.6 |

[a] Pooled tissues from two to five chicks.

[b] Chicks were 1 day old at slaughter.

[c] Chicks were 14 days old at slaughter.

Toxicologic Effects. The average weekly intake of mercury by chickens in this study was 5.6 mg and 7.4 mg for hens and male chickens, respectively. The two hens in the group given mercury that died after 42 and 90 doses became completely anorexic for 3 days prior to death. Subsequently, they developed leg weakness and were unable to stand in cage units or upon a flat solid surface, and as a result both hens rested continuously on their sternums during this period. At necropsy, both hens showed severe weight loss. No clinical signs of toxicosis were manifest in any of the other birds given mercury.

About 50% of the baby chickens hatched after the third week of the study manifested clinical signs of mercurial poisoning at hatch or developed clinical signs of toxicity 3-5 days after hatch. Affected baby chickens had or developed weakness, incoordination, and tremors of peripheral musculature.

No gross defects were observed in baby chicks hatched from eggs of treated hens or in embryos from eggs that failed to hatch. Hatchability of fertile control eggs averaged 80%, but by the third week hatchability of fertile eggs of treated hens had declined to 30%. This low level of hatchability continued throughout the remainder of the study. No attempts were made to correlate the low hatchability with loss of fertility in either the hens or roosters.

No significant differences occurred in mean feed consumption between hens given mercury and control hens, but mean feed consumption for roosters given mercury was significantly lower ($P < 0.05$) than that for control males. For the first and last week of the study, mean body weights of treated hens and roosters were not significantly different from those of the respective controls.

Egg production of the treated hens was 73-90% of pretreatment production during the first 7 weeks. After that time egg production gradually declined to 19% of pretreatment production at 14 weeks. Mean egg production for hens given mercury was significantly lower ($P < 0.001$) than that for the controls. The dosed hens which exhibited relatively high egg production generally had relatively lower residues of mercury in their tissues, which indicates that eggs are a possible pathway for the elimination of mercury.

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