SURVIVAL, REPRODUCTION, AND MERCURY LEVELS IN THE TISSUES OF RING-NECKED PHEASANTS CONSUMING TWO MERCURIAL FUNGICIDES

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

WILLIAM J. ADAMS

1971

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ABSTRACT

SURVIVAL, REPRODUCTION, AND MERCURY LEVELS IN THE TISSUES OF RING-NECKED PHEASANTS CONSUMING TWO MERCURIAL FUNGICIDES

By

William J. Adams

Female pheasants (Phasianus colchicus) were fed methyl mercury dicyandiamide and phenyl mercuric acetate for 74 days during the reproductive period. Twelve concentrations of each fungicide were fed daily and five concentrations were fed every third day. A cumulative consumption of more than 4 mg of methyl mercury dicyandiamide significantly reduced hatchability of eggs at a rate of 6.8 ± 0.5 percent per mg increase in methyl mercury dicyandiamide. Egg production and food consumption declined and death occurred after consumption of 15.8 mg ± 0.9 mg, 17.0 mg ± 3.0 mg, and 24.7 mg ± 0.8 mg, respectively. Phenyl mercuric acetate had no effects on egg production, food consumption, or survival. Hatchability was reduced after consumption of 20 mg at a rate of 0.20 ± 0.06 percent per mg increase in phenyl mercuric acetate.

Based on recommended dosages for seed treatments a wild pheasant would consume 4 mg of methyl mercury dicyandiamide from treated wheat and corn in approximately 6.5 and 0.8 days, respectively. Quantities consumed in excess of 4 mg would be capable of reducing pheasant numbers.

The concentration of total mercury in the tissues was related to the relative toxicity of the two compounds. The highest mercury levels were in the tissues of birds that died from methyl mercury dicyandiamide. Greater quantities of phenyl mercuric acetate were consumed, but relatively low concentrations of total mercury were present in all tissues with brain and breast tissues showing the smallest increase over levels in control birds. The relative distribution of total mercury was similar for both compounds with the greatest concentrations in the kidney and liver followed by breast muscle, and brain and gonad. The proportional increase of total mercury in the tissues as the total quantity of mercury consumed increased was more rapid for methyl mercury dicyandiamide than for phenyl mercuric acetate. This was reflected by smaller concentrations of mercury in the feces of the birds fed methyl mercury dicyandiamide. These results indicate that the use of methyl mercury dicyandiamide for seed treatment presents a much greater threat to pheasant populations than phenyl mercuric acetate.

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By

William J. Adams

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

The widespread use of mercurial fungicides on cereal grains and other agricultural crops has been a common practice since the 1940's. The use of alkyl-mercury fungicides has been recently restricted and the use of aryl-mercury compounds has been encouraged. The effects of mercury compounds on pheasants (Petoskey 1948, Leedy and Cole 1950, and Carnaghan and Blaxland 1957) and chickens (Heuser 1956, and Smart and Lloyd 1963) have not been considered serious until recently when some of these compounds were linked to cases of mercury poisoning in Swedish wildlife, including pheasants (Borg et al. 1969, and Tejning 1967a).

There is a paucity of data on relative distribution, excretion, and lethal mercury concentrations in tissues of pheasants. Borg et al. (1969) studied the effects of methyl mercury dicyandiamide on the survival of male and female pheasants and the distribution and retention of mercury in the tissues. Other members of the order Galliformes have been studied more intensely than pheasants. The distribution and excretion of mercury compounds in chickens have been studied by Kiwimae et al.

(1960), Miller et al. (1960), 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 determine the effects of two mercurial fungicides, methyl mercury dicyandiamide, and a common subsitute, phenyl mercuric acetate, on survival and reproduction of hen pheasants and to determine the relative distribution, excretion, and mercury concentrations in body tissues resulting from the consumption of the two compounds.

EXPERIMENT I: SURVIVAL AND REPRODUCTION OF RING-NECKED PHEASANTS CONSUMING TWO MERCURIAL FUNGICIDES

Materials and Methods

Treatments

Two mercurial fungicides were used, Panogen 15, containing 2.2 percent methyl mercury dicandiamide, and Panomatic, containing 3.4 percent phenyl mercuric acetate (Table 1). Adult pheasant hens were fed Purina game breeder pellets ad libitum treated with varied levels of the two mercurial fungicides. The birds were divided into two groups, those fed treated food every day and those fed treated food every third day. There was an array of twelve concentrations of each compound for the daily treatment and five concentrations of each compound for the hens fed every third. There was one hen per concentration in all treatments.

The mercury compounds were sprayed with an aspirator on food pellets in a rotating jar. The food was treated in one kilogram quantities under a forced air hood.

Table 1. The experimental design.[a]

Treatment	Mercury Compounds (mg/kg)	
	Methyl Mercury Dicyandiamide	Phenyl Mercury Acetate
Daily	0.0	0.0
	2.6	3.6
	5.1	7.1
	7.7	10.7
	10.2	14.3
	12.8	17.9
	15.4	21.5
	17.9	25.1
	20.5	28.7
	23.0	32.2
	25.6	35.8
	30.7	43.0
Every 3rd Day	0.0	0.0
	5.1	7.1
	10.2	14.3
	20.5	28.7
	30.7	43.0

[a]One hen per concentration.

Management

The birds were placed in outdoor pens and given three weeks to acclimate prior to receiving mercury treated food. Each female occupied a 3 x 4 meter pen containing natural vegetation. A male was placed with each hen for a 24-hour period every fourth or fifth day for insemination. New food was given each day with the previous day's food being removed and weighed to obtain a measure of consumption.

Eggs were collected daily and stored at 10° C for no longer than seven days. They were incubated at 37.5° C, 67 percent humidity, and rotated eight times daily. The eggs were candled and transferred to individual hatching trays on the 21st day of incubation. All chicks were weighed, banded, and placed in brooder houses. The contents of all eggs that were candled and removed or did not hatch were classified by the scheme developed by Hamburger and Hamilton (1951) and Labisky and Opsahl (1958).

Statistical Analysis

Standard statistical procedures (chi square and regression analysis) were used (steel and Torie 1958) and all values were tested at 0.05 level. Variation about the mean is denoted by the standard error.

Results

Survival of Adult Females

All birds given untreated food survived the 74-day trial. One of 11 hens fed phenyl mercury daily died. Hens given methyl mercury began to die on the 25th day with 9 of 11 fed daily and 1 of 4 fed every third day dying. Two of 4 males died that consumed methyl mercury treated food each day. Using a logarithmic transformation of the data, there was a linear decrease of survival time as the concentration of methyl mercury on the food increased (Fig. 1). The cumulative amount of methyl mercury consumed prior to death was 24.7 mg $\pm$ 0.8 mg (Fig. 2). The total amount of methyl mercury consumed by the two hens surviving the daily treatment was 11.2 mg and 22.5 mg suggesting they had not yet consumed a lethal quantity. Although the hen that consumed 22.5 mg of methyl mercury had not died, symptoms of acute mercury poisoning were visible when the experiment terminated.

The first visible indications of poisoning occurred after the hens had consumed a total of 13 to 17 mg of methyl mercury. This was usually about 2 weeks prior to death and there was also cessation in egg production at this time. Obvious changes in behavior of the hens typically included ruffed head feather, tameness, refusal to attempt flight, and slight incoordination in walking. This progressed into acute ataxia until the

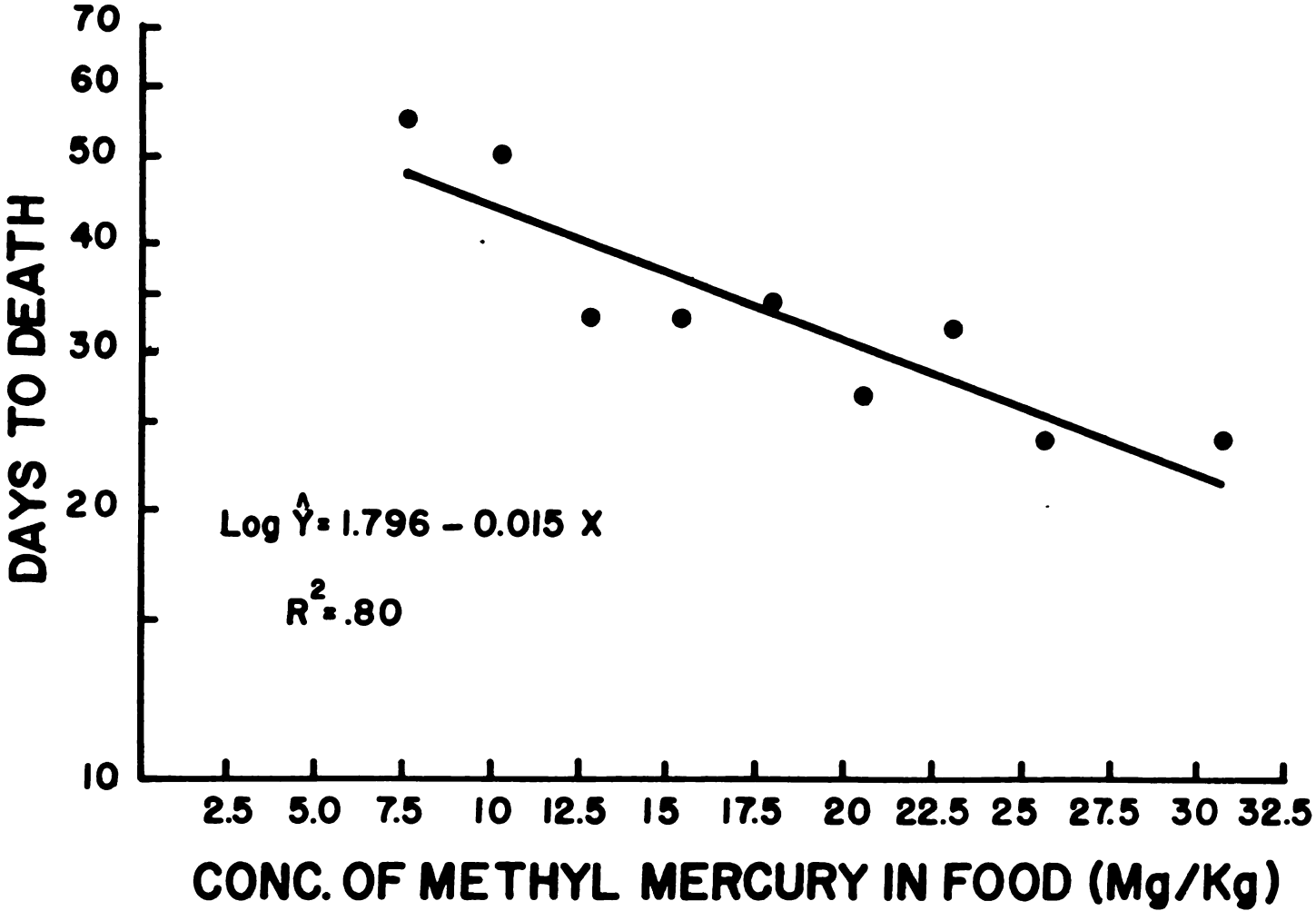

Fig. 1. The effects of varied concentrations of methyl mercury dicyandiamide fed daily on survival time of nine pheasant hens.

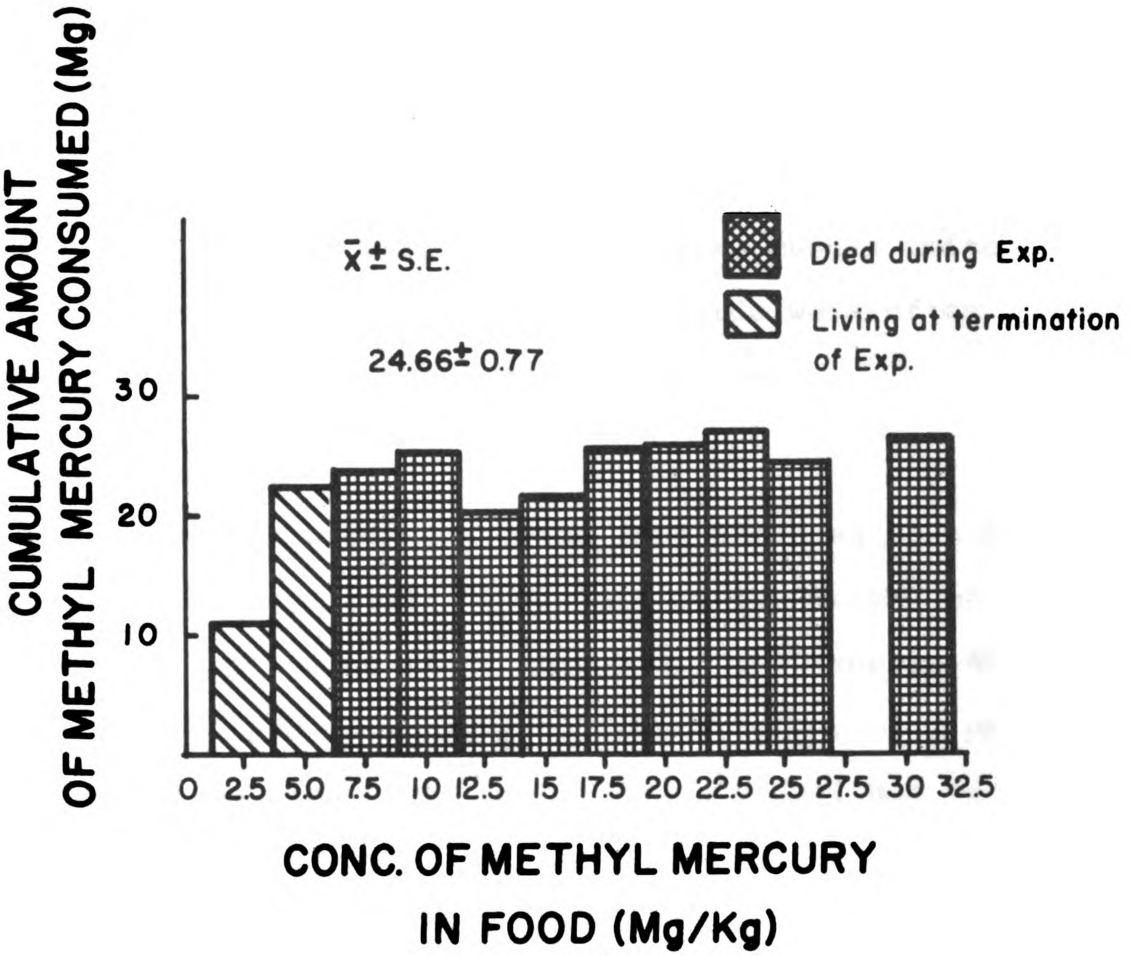

Fig. 2. The total weight of methyl mercury dicyandiamide consumed by eleven pheasant hens given treated food each day.

birds were unable to fly and walking became difficult. Food consumption diminished from about 58 g per day prior to a 15 mg culminative total consumption of methyl mercury until nothing was eaten during the last two or three days prior to death (Fig. 3). In the most advanced stages of poisoning the birds were unable to walk, muscle twitching of wings and legs occurred, and the birds were often found lying on their chests in a comatose state.

Reproduction

The hens given untreated food produced eggs during the entire 74-day trial. Fertility of the treatment and control groups was constant throughout the experiment and averaged 96.1 and 90.4 percent, respectively. Egg production of the hens given methyl mercury declined during the second week of the experiment with hens on the higher concentrations going out of production first. There was a linear decrease in the number of production days after logrithmic transformation as the concentration of methyl mercury on the food increased on the daily and three-day treatments (Fig. 4). No decline in the number of egg production days was observed for either of the phenyl mercury treatments.

The total number of eggs laid during the period of treatment by hens fed methyl mercury daily followed the same pattern of decline as the number of days in production (Fig. 5). This is not surprising since

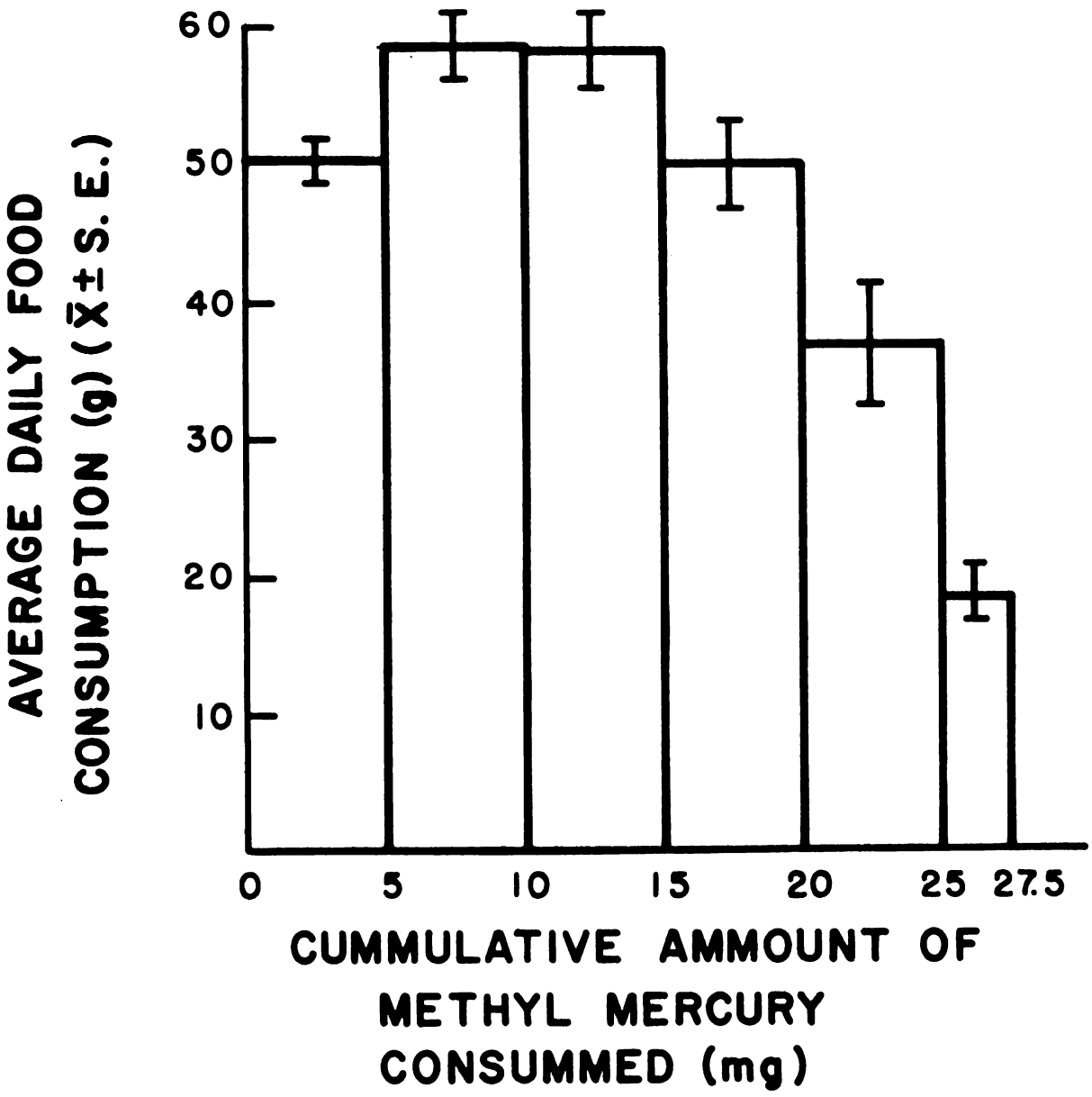

Fig. 3. Average daily food consumption of hen pheasants influenced by the cumulative amount of methyl mercury dicyandiamide eaten.

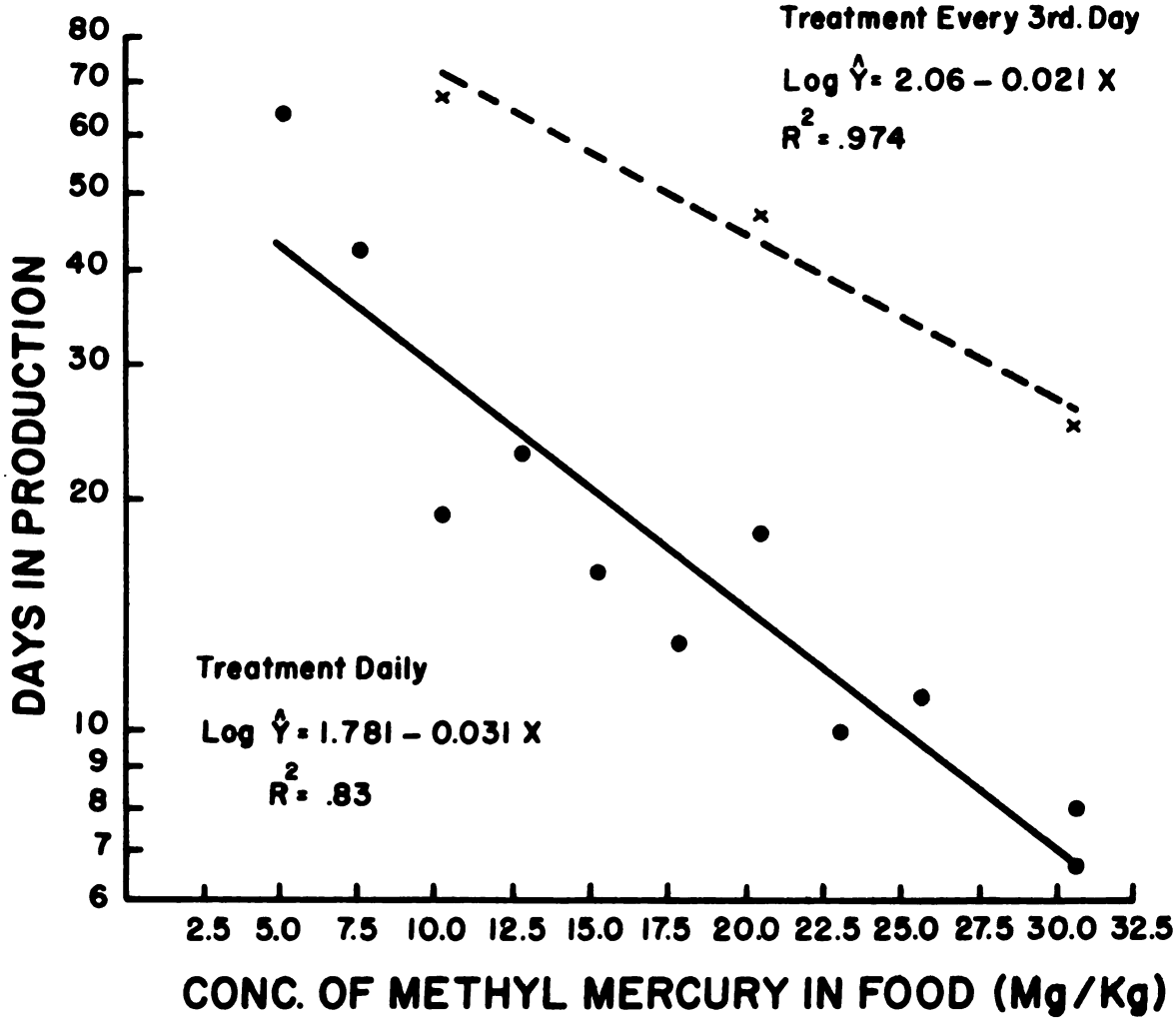

Fig. 4. The relationship of the number of days in production to the concentration of methyl mercury dicyandiamide on the food consumed daily and every third day.

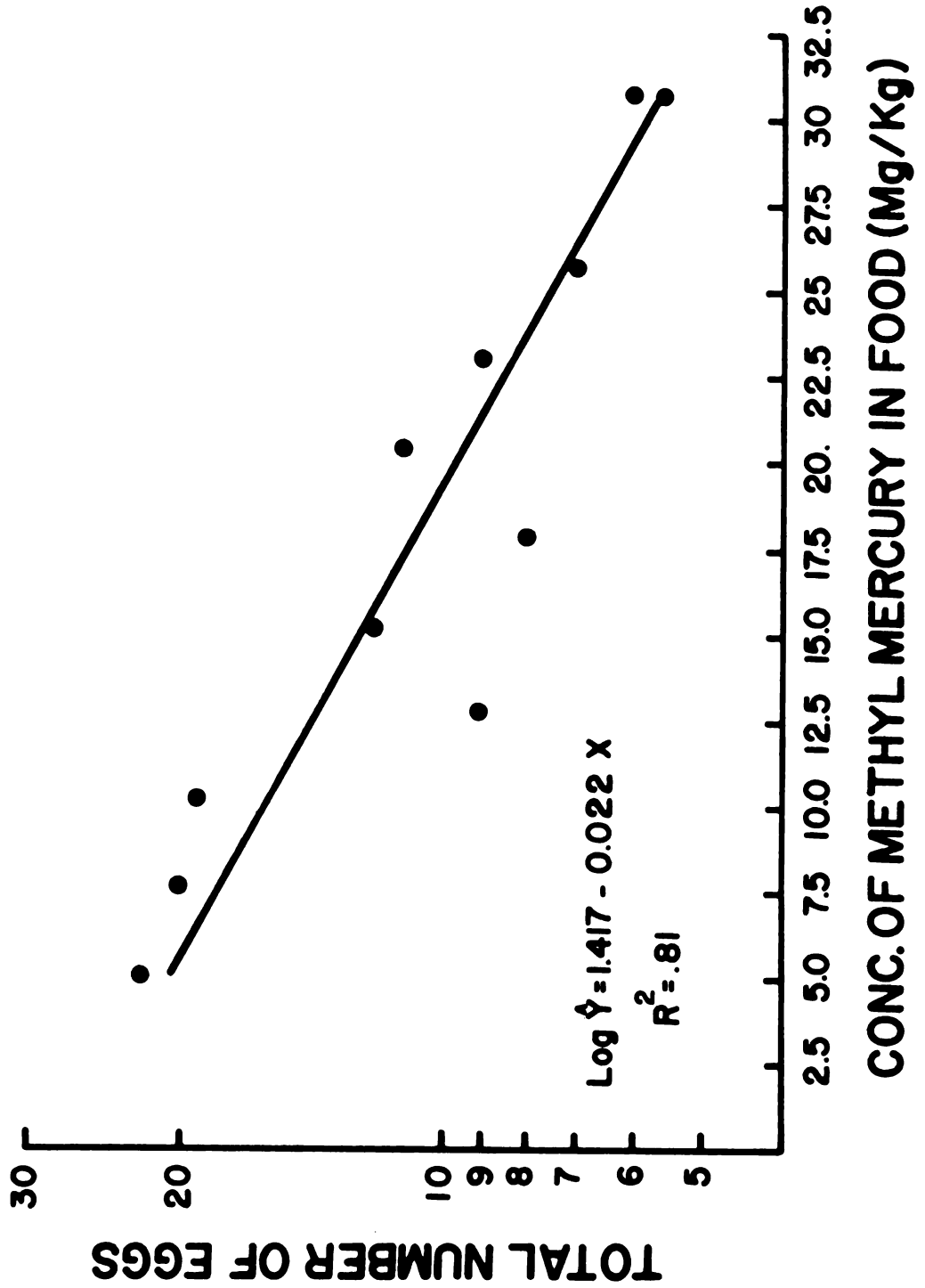

Fig. 5. The relationship of the number of eggs produced to the concentration of methyl mercury dicyandiamide on the food consumed each day.

pheasants will lay an egg per 1.25 days (Labisky and Jackson 1969). Hens fed higher concentrations of methyl mercury laid fewer eggs and went out of production sooner than those on lower concentrations. There was a decrease from a high of 40 eggs to a low of 10 eggs for the hens fed methyl mercury every third day.

The total amount of methyl mercury consumed prior to the last egg laid by those hens on a daily treatment was 15.8 mg $\pm$ 0.9 mg and 15.1 mg $\pm$ 1.3 mg by the hens fed treated food every third day. Egg production stopped after the consumption of approximately 16 mg of methyl mercury, even though this occurred over a period of 10-58 days.

There seems to be no reduction in egg production due to any of the phenyl mercury treatments within the limits of this experiment.

Both methyl and phenyl mercury compounds affected hatchability (Fig. 6). Hatchability of the eggs laid by hens fed methyl mercury daily increased significantly above the control value of 74 percent to 93 percent until about 3.5 mg of methyl mercury had been eaten. Then hatchability decreased at a rate of 6.8 $\pm$ 0.5 percent for each additional mg of methyl mercury eaten (Fig. 7). Hatchability decreased at a rate of 0.20 $\pm$ 0.06 percent for each mg of phenyl mercury consumed on a daily basis.

Embryonic mortality in the control and treatment groups was greatest during the first and last parts of

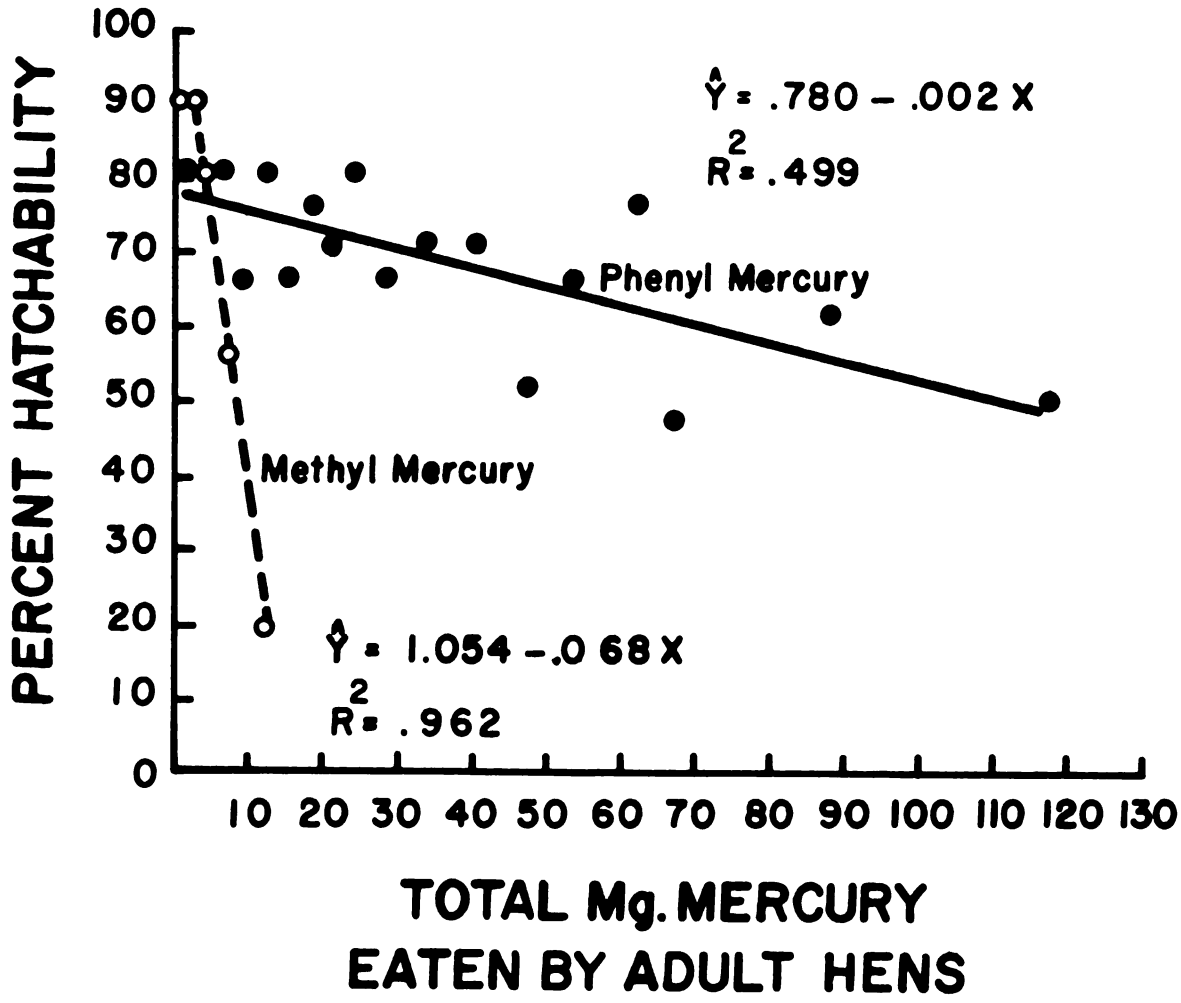

Fig. 6. Hatchability of eggs related to total amount of methyl and phenyl mercury consumed by hen pheasants.

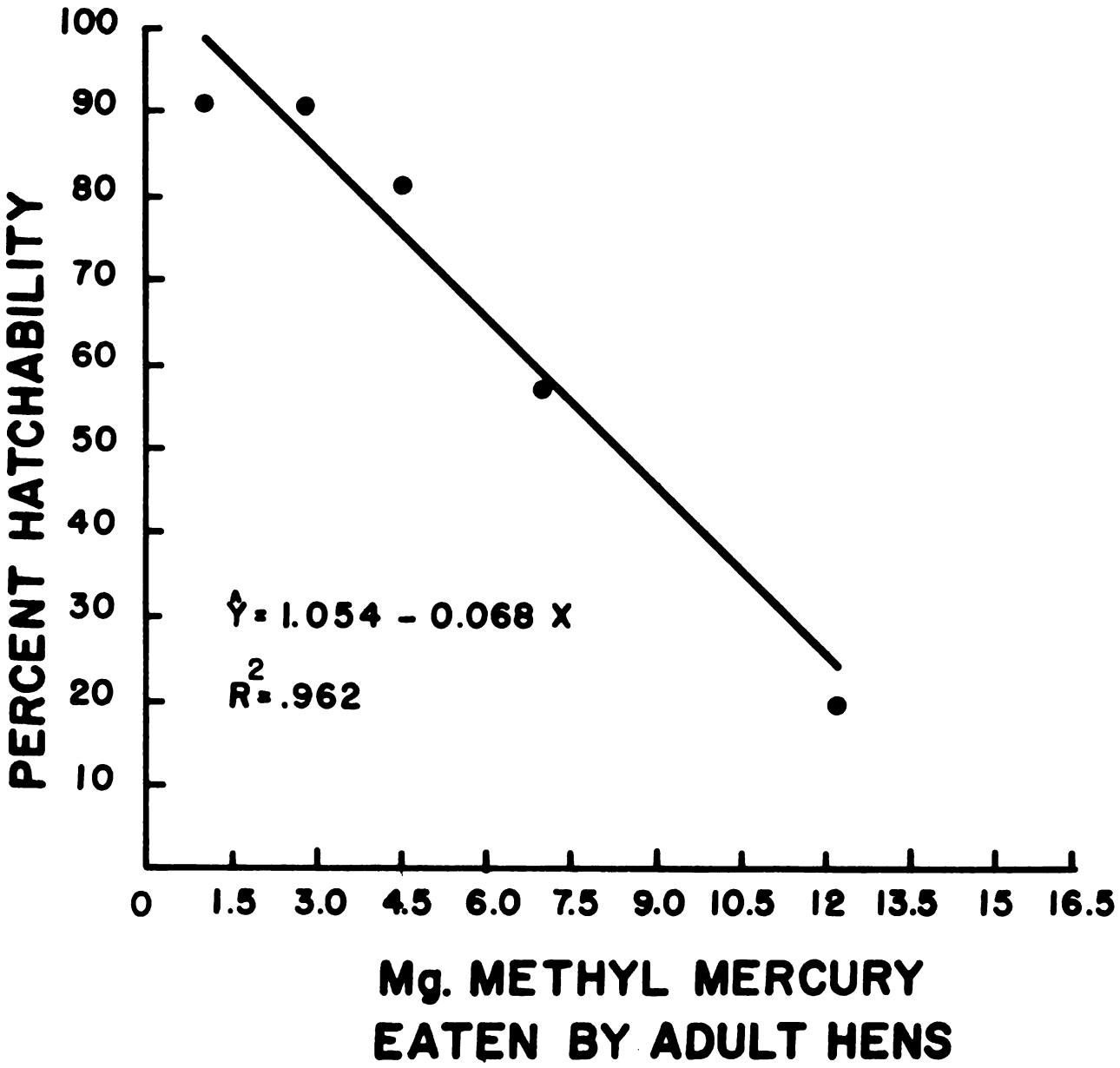

Fig. 7. Hatchability of eggs related to total amount of methyl mercury consumed by hen pheasants.

the incubation period (Fig. 8). There was a shift in the distribution of embryonic mortality from hens fed methyl and phenyl mercury compounds. A significant decrease of mortality in the 20-24 day developmental period occurred for both treatment groups with consistently higher percentages of mortality in the first three developmental periods. The amount of embryonic mortality occurring during the first 14 days of the developmental period was 70, 62, and 48 percent for the methyl mercury, phenyl mercury, and control groups, respectively.

Discussion

Methyl mercury dicyandiamide affected survival and reproduction in pheasants. There was a range of 31 days between the time the first and last hen went out of egg production and a range of 34 days between the first and last death on high and low concentrations, respectively. The average amount of methyl mercury consumed at termination of egg production and at death was 15.8 mg $\pm$ 0.9 mg and 24.7 mg $\pm$ 0.8 mg, respectively. The low variation about the mean over the range of concentrations suggests that methyl mercury accumulates in the body in an additive manner which is a function of concentration and time.

The hatchability of eggs from hens fed methyl mercury dicyandiamide declined at a rate of 6.8 percent per mg consumed. Borg et al. (1969) reported a decline

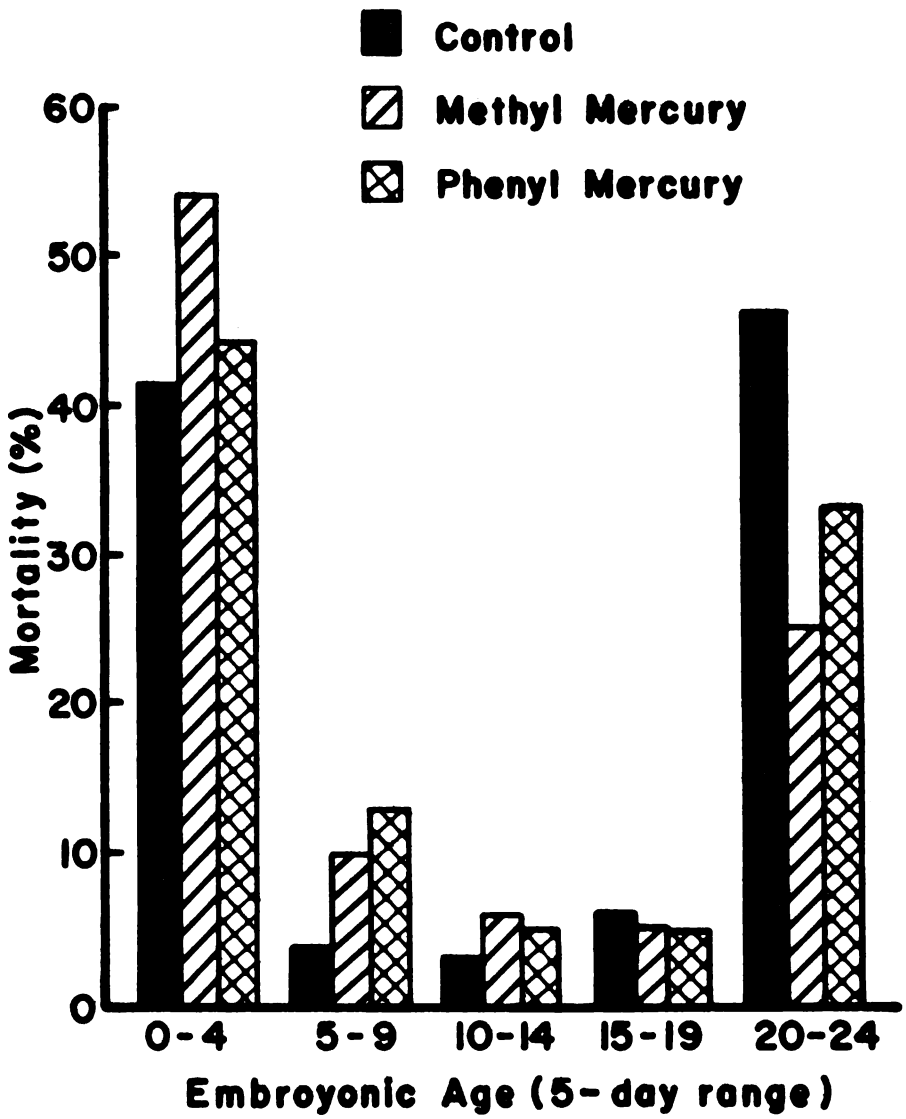

Fig. 8. Distribution of embryonic mortality of eggs from hens in the control, methyl, and phenyl mercury treatment groups.

in hatchability to 55 percent after feeding pheasant hens 15-20 mg/kg methyl mercury dicyandiamide for 9 days. Based on an average daily consumption of 54 grams of food, their birds would have consumed 8.5 mg which according to our regression equation would give 51 percent hatchability. Similar results have been reported for domestic fowl (Gallus gallus) by Tejning (1967b) who found that after 28 days of continuous feeding methyl mercury dicyandiamide at rates of 18.4 mg/kg and 9.2 mg/kg hatchability was reduced to 10 percent and 17 percent, respectively, as compared to a control value of 61 percent.

Successful hatching is related to total accumulation of mercury in the bird and in the egg. Eggs which were laid after a small amount of methyl mercury had been consumed (0-4 mg) had much better success in hatching than those laid after consumption of larger amounts (5-12 mg). This suggests that a certain concentration of methyl mercury, and phenyl mercury to a smaller extent, must be accumulated in the hen's body and incorporated into the eggs before hatchability will be reduced. Tejning (1967b) reported similar results with domestic chickens showing that hatchability depends on the concentration of methyl mercury dicyandiamide in the food, number of days the food has been consumed, and the concentration of the mercury in the egg.

Phenyl mercuric acetate, in comparison with methyl mercury dicyandiamide, is much less toxic. There were no effects on survival, egg production, or food consumption attributed to phenyl mercuric acetate. Hatchability was affected declining at a rate of 0.20 $\pm$ 0.06 percent for each mg of phenyl mercuric acetate consumed and did not decline below the control value until 20 mg had been consumed. Calculations of $L.D._{100}$ by Grolleau and Giban (1966) also show phenyl mercuric acetate to be much less toxic than methyl mercury dicyandiamide.

Although no data are available on the amount of mercury consumed by wild pheasants, Tejning (1967a) has estimated the average spillage of seed grain in Sweden to be 1 percent of the total amount planted with an average mercury concentration of 16 mg/kg. Tejning (1967a) and Ulfvarsson (1965) have shown that the high levels of mercury found in Swedish pheasants can be attributed to the amount of grain left on the ground after spring and fall planting.

In the past, recommended dosages for the use of methyl mercury dicyandiamide in the United States have ranged from 10 mg/kg for cereal grains to 82 mg/kg for corn, peas, soybeans, and navybeans. Based on recommended dosages for wheat and corn and an average daily consumption of 60 grams it would take a wild pheasant hen 6.5 days and 0.8 days, respectively, to consume 4 mg of methyl mercury dicyandiamide. The 4 mg level is the

point at which hatchability declines below the control value. Consumption of more than 4 mg would seriously decrease hatchability and would result in a cessation of egg production if approximately 16 mg were consumed. We believe that methyl mercury dicyandiamide could limit pheasant numbers if treated seed is available.

EXPERIMENT II: LEVELS OF MERCURY IN THE TISSUES OF PHEASANTS CONSUMING TWO MERCURIAL FUNGICIDES

Materials and Methods

Two mercurial fungicides (NOR-AM Agricultural Products, Inc.) were used, Panogen 15, containing 2.2 percent methyl mercury dicyandiamide, and Panomatic, containing 3.4 percent phenyl mercuric acetate (Table 1). Adult hen pheasants were fed Purina game breeder pellets ad libitum treated with varied levels of the two mercurial fungicides. The birds were divided into two groups, 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 birds fed every third day. There was one hen per concentration in all treatments except the three-day treatments which had one cock with each hen.

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 in plastic containers for subsequent analysis. Similar samples were collected from all birds that died during the experiment as soon as possible after death. Fecal material was collected and frozen for subsequent analysis on day 10, 18, and 24 of the experiment from the hens fed methyl and phenyl mercury daily.

All tissues and fecal samples were prepared for analysis by wet acid digestion according to Zabik (unpublished), Dept. Ent. M.S.U., E. Lansing, Mich. (Table 2). Samples were analyzed with a flameless atomic absorption spectrophotometer (Jerrell Ash model 800) for total mercury content with the method provided by the Jerrell Ash Company. All values are reported as mg/kg (ppm).

Results

Distribution of Mercury in the Tissues

Samples were analyzed for total mercury content from 11 control hens, 11 hens and 6 cocks fed methyl mercury dicyandiamide, and 14 hens and 7 cocks fed phenyl mercuric acetate (Table 3). 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 (Fig. 9). There were no significant changes in the mercury concentrations of 4 hens sacrificed at the start and 7 hens at the conclusion of the experiment. The greatest concentration of mercury

TABLE 2. The wet acid digestion procedure used to prepare tissues for analysis of total mercury.

1.	Blend 10 g tissue with 40 ml of 10% trichloroacetic acid for 10 min.
2.	Rinse the blender cups with 5 ml of 10% trichloroacetic acid.
3.	Centrifuge at 2000 x θ for 15 minutes.
4.	Pour supernatant into a flask, cover and save.
5.	Add the precipitate from the centrifuge to 50 ml of concentrated sulphuric acid (cooled to 5° C in an ice bath). Rinse the centrifuge cup with 5 ml sulphuric acid. Break up any lumps in the digestion, and let stand for one hour in the ice bath.
6.	Add 5 ml of 30% hydrogen peroxide to the sulphuric acid digest, cover and let stand overnight (15 hours); this should give a light yellow solution. If the solution is not clear add an additional 5 ml of hydrogen peroxide and let stand for four hours.
7.	Add the supernatant from step 4 to the acid digestion.
8.	Titrate to a light pink color with a saturated solution of potassium permangonate.
9.	Add 20 ml of sodium chloride-hydroxlamine sulfate (60 ml of 25% (w/v) hydroxlamine sulfate and 50 ml of 30% (w/v) sodium chloride diluted to 500 ml with water) to the total digestion. The sample is now ready for reduction to elemental mercury and analysis by flameless atomic absorption.

TABLE 3. Mercury concentrations in the tissues of male and female pheasants fed methyl mercury dicyandiamide and phenyl mercury acetate.

Treatment	Band No.	Sex	Conc. of Hg on the food (mg/kg)	Fate	Consumption of Hg (mg)	Mercury in the Tissue (mg/kg)				
						Breast Muscle	Liver	Kidney	Brain	Gonad
Control	329	♀	0	Sacrificed	0	0.01	0.01	0.01	0.01	0.01
"	331	"	0	"	0	0.01	0.01	0.02	0.01	0.01
"	332	"	0	"	0	0.02	0.01	0.01	0.01	0.01
"	334	"	0	"	0	0.01	0.01	0.01	0.01	0.01
"	337	"	0	"	0	0.05	0.03	0.02	0.02	0.07
"	361	"	0	"	0	0.15	0.04	0.11	0.04	0.07
"	362	"	0	"	0	0.47	0.11	0.32	0.06	0.09
"	363	"	0	"	0	0.01	0.02	0.08	0.03	0.03
"	364	"	0	"	0	0.01	0.03	0.07	0.07	0.02
"	2	"	0	"	0	0.08	0.12	0.05	0.04	0.02
"	17	"	0	"	0	0.17	0.07	0.07	0.02	0.02
Methyl mercury dicyandi-amide	32	"*	5.1	Sacrificed	7.6	4.46	8.21	7.22	2.26	0.24
	3	"	2.6	"	11.2	6.32	10.50	11.89	5.13	1.74
	33	"*	10.2	"	14.3	7.83	12.82	12.01	3.86	0.36
	4	"	5.1	"	22.5	31.36	15.50	19.51	7.35	3.67
	7	"	10.2	Died	25.4	12.72	44.48	46.10	9.43	10.90
	8	"	12.8	"	20.2	19.34	--	57.45	--	13.08
	9	"	15.4	"	21.9	19.06	--	50.16	--	10.64
	10	"	17.9	"	25.7	25.18	--	88.59	--	22.25
	12	"	20.5	"	26.0	20.79	38.32	47.57	18.54	8.24
	14	"	25.6	"	24.8	22.73	31.27	34.46	12.36	6.33
	15	"	30.7	"	26.7	19.75	62.93	51.04	16.14	14.86

Methyl	1452	♂	0	Sacrificed	0	0.27	--	0.05	--	0.03
mercury	1328	"	5.1*	"	3.4	5.09	9.35	3.29	--	1.03
dicyandi-	1536	"	10.2*	"	4.6	8.16	14.35	4.72	--	1.94
amide	1502	"	20.5*	"	11.8	9.08	10.88	10.38	--	2.67
	1	"	0-7.7	"	18.0	11.77	8.15	11.42	0.90	1.23
	11	"	20.5-30.7	Died	45.3	27.25	--	96.83	--	19.11
Phenyl	37	♀*	7.2	Sacrificed	10.0	0.18	0.82	1.47	0.04	0.80
mercuric	18	"	3.6	"	15.3	0.13	0.52	1.19	0.09	0.31
acetate	38	"*	14.3	"	20.6	0.34	1.58	2.78	--	0.68
	19	"	7.2	"	32.3	0.21	1.46	8.10	0.03	0.62
	39	"*	28.7	"	39.6	0.44	2.53	3.03	0.02	1.86
	20	"	10.7	"	45.0	0.14	1.29	7.04	0.06	0.40
	40	"*	43.0	"	60.7	1.87	2.99	5.80	0.08	0.60
	22	"	14.3	"	62.0	0.15	3.28	8.02	0.04	0.22
	23	"	17.9	"	78.1	0.08	6.58	15.80	0.04	1.22
	24	"	21.5	"	95.7	0.12	--	17.38	0.50	--
	25	"	25.1	"	113.5	0.12	2.43	12.67	0.20	0.14
	27	"	28.7	"	122.3	0.50	5.26	12.83	0.07	3.66
	28	"	32.2	"	142.3	1.08	3.46	6.18	0.19	6.37
	30	"	43.0	"	191.2	1.61	6.11	31.82	0.76	6.26
Phenyl	1295	♂	0	Sacrificed	0	0.11	--	0.07	--	0.01
mercuric	1317	"	7.2*	"	4.4	0.79	--	1.23	--	0.28
acetate	1518	"	14.3*	"	8.6	2.97	6.11	4.02	--	0.21
	1443	"	28.6*	"	17.3	1.14	--	5.49	--	0.05
	16	"	0-10.7	"	23.7	2.27	2.51	2.44	0.07	0.31
	21	"	14.3-25.1	"	86.6	0.12	1.13	8.46	0.05	0.21
	26	"	28.7-43.0	"	152.7	2.25	5.72	12.67	0.20	0.19

*Birds fed treated food every third day.

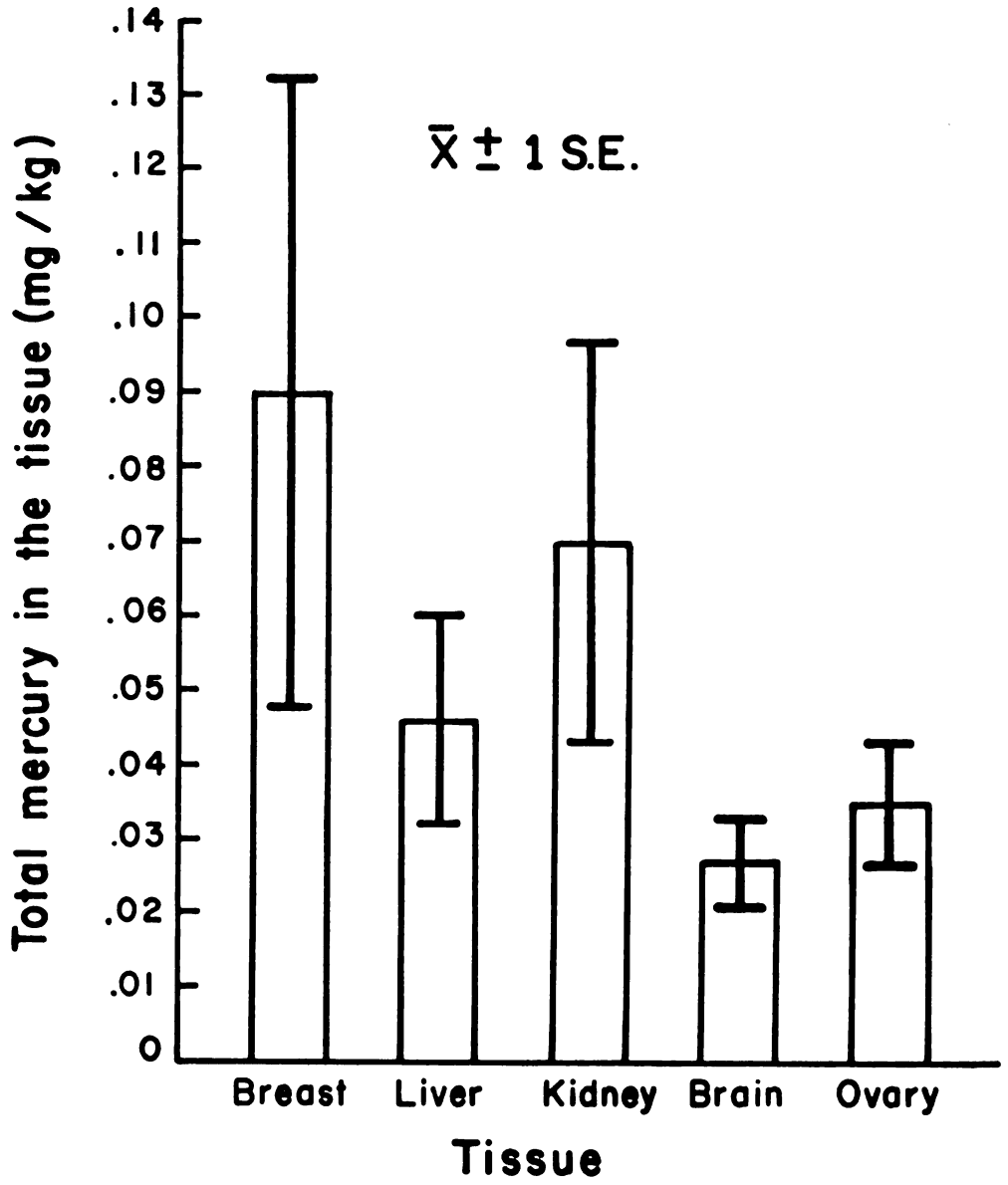

Fig. 9. Distribution of mercury in the tissues of 11 control birds.

occurred in the breast muscle and the kidney with significantly lower quantities in the ovary and brain. The liver values were between the two groups.

The concentration of mercury in the tissues of 7 of 9 hens which had consumed 24.7 ± 0.8 mg of methyl mercury dicyandiamide prior to death showed a significant 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 (Fig. 10). The breast muscle contained significantly less mercury than the kidney and liver, but more than either the ovary or brain. Similar results have been reported by Borg et al. (1969) who fed methyl mercury dicyandiamide to male and female pheasants until death at a rate of 21 mg/kg food and found concentrations of mercury in mixed liver and kidney to range from 30 to 130 mg/kg and breast muscle from 20 to 45 mg/kg. Tejning (1967b) fed methyl mercury dicyandiamide to chickens for an average of 85.5 days at a mean concentration of 18 mg/kg food and found the mean concentration of mercury in the liver, kidney, breast muscle, and brain to be 55.2, 47.8, 15.8, and 13.3 mg/kg, respectively.

The amount of methyl mercury dicyandiamide consumed by 5 cocks ranged from 3.42 to 45.3 mg. The cock which consumed the largest amount died on day 41 of the experiment. There are positive correlations between increasing consumption of methyl mercury dicyandiamide and levels of mercury in the kidney, breast muscle, and gonad

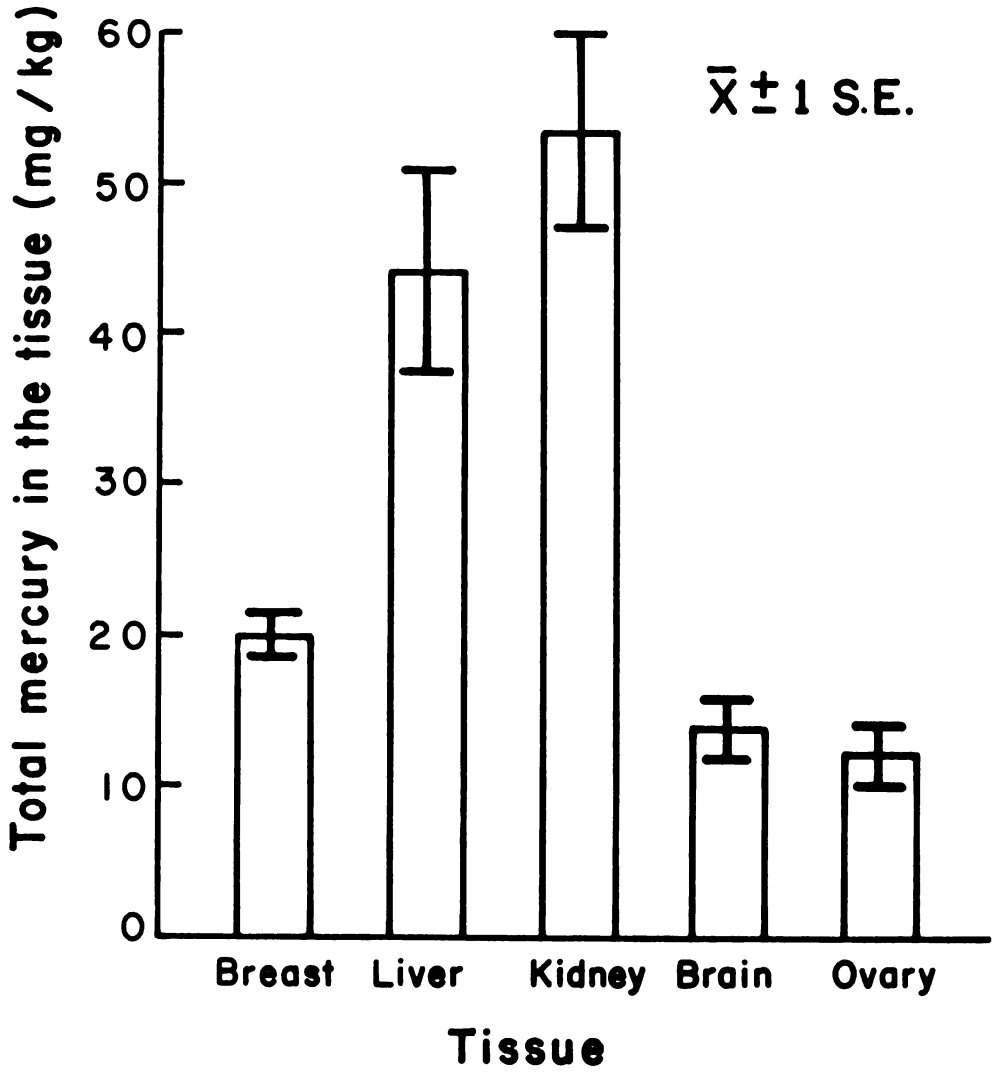

Fig. 10. Distribution of mercury in the tissues of 7 hens that died from consumption of methyl mercury dicyandiamide.

(Fig. 11). The highest rate of increase was in the kidney followed by the breast muscle and gonad.

Consumption of 10 mg of phenyl mercuric 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 phenyl mercuric acetate resulted in mercury levels in the brain that were higher than control birds. The concentration of mercury in hens continued to increase as additional phenyl mercuric acetate was consumed in all tissues except the breast muscle (Fig. 12). The correlation coefficient between the concentration of phenyl mercuric acetate on the food and the concentration of mercury in the breast muscle was not significant ($r = 0.49$). The highest concentration in the breast muscle was 1.61 mg/kg after 191.2 mg of phenyl mercuric acetate had been consumed. All but three of the values were 0.50 mg/kg or smaller.

The greatest accumulation of mercury occurred in the kidney followed by the liver and gonad, breast muscle, and brain. Similar distributions of phenyl mercury compounds have been reported for chickens and quail (Swensson and Ulfvarson 1969; Backstrom 1969). Miller et al. (1960) reported concentrations of ppm phenyl mercuric acetate in the liver and kidney to be about the same, but total mercury concentrations were higher in the kidney than in the liver, similar to this study.

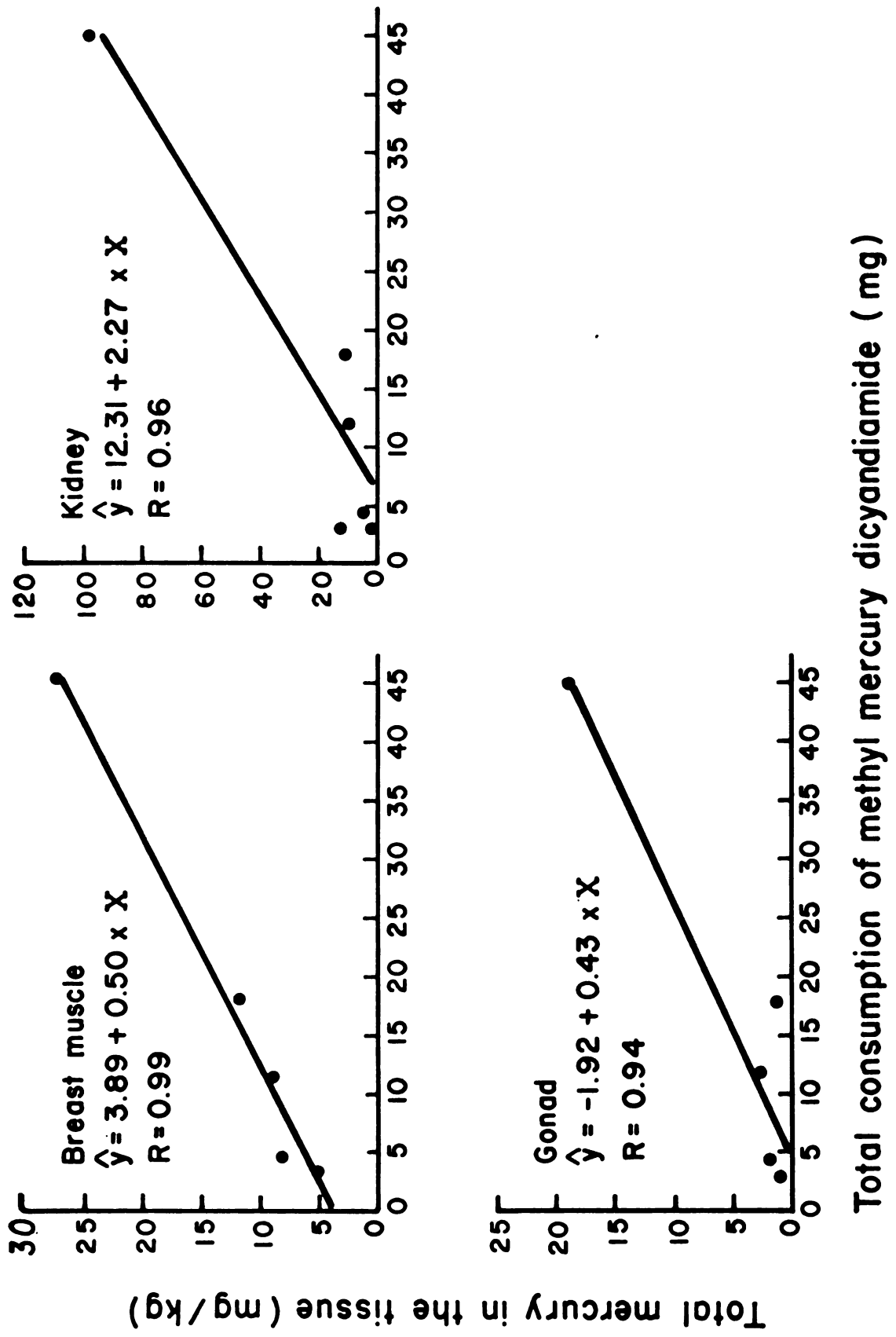

Fig. 11. Distribution of mercury in the tissues of male pheasants as related to the total consumption of methyl mercury dicyandiamide.

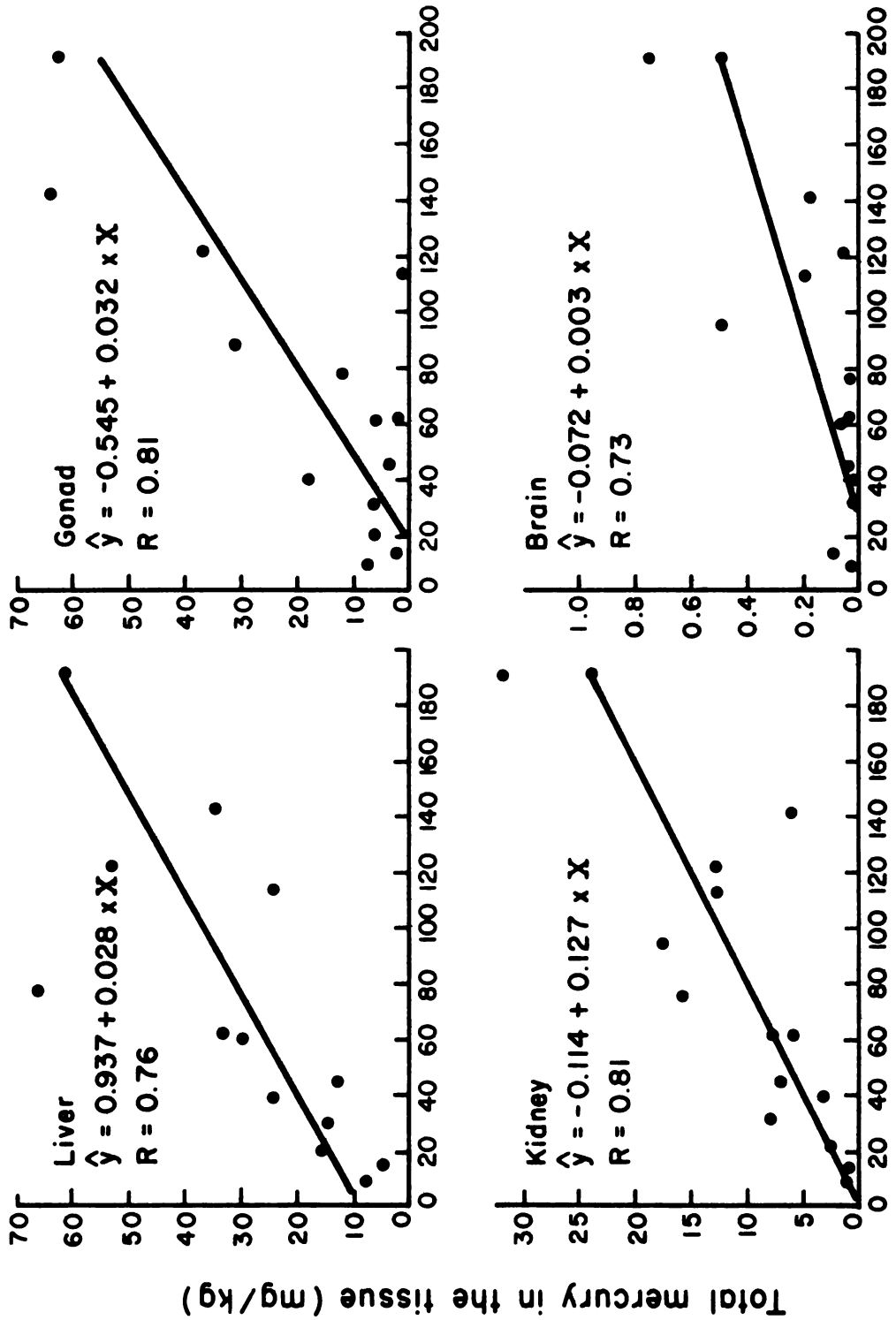

Fig. 12. Distribution of mercury in the tissues of female pheasants as related to the total consumption of phenyl mercuric acetate.

The hens fed phenyl mercuric acetate every third day tended to show higher concentrations of mercury in the breast muscle and liver and lower concentrations in the kidney than the hens fed similar amounts of phenyl mercury acetate daily (Table 3). The concentrations in the brain and gonad showed no differences for the two treatments. The reason for this difference is not clear and should be investigated further.

The amount of phenyl mercuric acetate consumed by 6 cocks, all living at the termination of the experiment, ranged from 4.4 to 152.7 mg. There is a positive correlation ($r = 0.95$) between increasing consumption of phenyl mercuric acetate and levels of mercury in the kidney tissue. This correlation was not significant for any other tissues. The greatest concentration of mercury occurred in the kidney followed by the liver, breast muscle, and brain and gonad.

Mercury Content in the Feces

Mercury concentrations in the feces of birds fed methyl mercury dicyandiamide were lower than the levels in the feces of the birds fed phenyl mercuric acetate and ranged from 0.15 to 3.15 mg/kg and 1.24 to 29.76 mg/kg, respectively, with corresponding concentrations of mercury on the food ranging from 2.6 to 30.7 mg/kg and 3.6 to 43.0 mg/kg, respectively (Table 4). The average

TABLE 4. Relationship of the total mercury (mg/kg) in the feces from hens fed methyl mercury dicyandiamide and phenyl mercuric acetate with mercury concentrations on the food (mg/kg).

Treatment	Band No.	Sex	Conc. of Hg. on the food (mg/kg)	Total Hg (mg/kg) in the Feces		
				Day 10	Day 18	Day 24
Methyl mercury dicyandiamide	2	♀	0	0.01	0.05	0.02
	3	"	2.6	0.31	0.25	0.15
	4	"	5.1	0.18	--	0.95
	5	"	7.7	0.19	0.53	0.23
	7	"	10.2	0.21	0.66	--
	8	"	12.8	0.29	2.07	3.15
	9	"	15.4	0.31	--	0.96
	10	"	17.9	0.28	0.51	1.11
	12	"	20.5	0.42	1.05	--
	13	"	23.0	0.74	0.95	0.90
	14	"	25.6	0.54	--	2.46
	15	"	30.7	--	1.83	1.80
Phenyl mercuric acetate	17	"	0	0.08	0.18	0.06
	18	"	3.6	8.46	1.24	2.31
	19	"	7.2	7.79	--	2.96
	20	"	10.7	9.85	2.17	3.08
	22	"	14.3	--	1.95	--
	23	"	17.9	--	15.08	5.77
	24	"	21.5	3.23	--	5.47
	25	"	25.1	6.73	9.44	7.14
	27	"	28.7	--	29.76	--
	28	"	32.2	13.74	17.16	9.52
	30	"	43.0	5.46	14.49	13.50

concentration of mercury in the feces of the control birds was 0.07 ± 0.02 mg/kg.

Although mercury concentrations from one time period to the next were not significantly different from one another, the mercury levels in feces of hens fed methyl mercury dicyandiamide tended to increase with time. The correlation coefficients for mercury concentrations in the feces and mercury concentrations on the food for hens fed methyl and phenyl mercury were, r = 0.77, 0.57, 0.49, and 0.08, 0.67, and 0.98 for day 10, 18, and 24, respectively. The day 10 methyl mercury and day 18 and 24 phenyl mercury r values are significant. The correlation between the concentrations of methyl mercury dicyandiamide fed and excreted decreased with time while the correlation between the concentrations of phenyl mercuric acetate became more apparent with time and subsequent consumption of additional quantities of the compound.

Discussion

A comparison of methyl mercury dicyandiamide and phenyl mercuric acetate on survival and reproduction of pheasants during a 74-day experiment indicates there are major differences between the effects of these two compounds on the birds. None of the birds consuming phenyl mercuric acetate died during the experiment while an average consumption of 24.9 ± 0.8 mg of methyl mercury

dicyandiamide was lethal to 9 hens. Two cocks that consumed 37.0 and 45.3 mg also died. 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 methyl mercury dicyandiamide. Although the birds consumed greater quantities of phenyl mercuric acetate, up to 191.2 mg, relatively low concentrations of total mercury were present in all tissues with brain and breast 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 shown that alkyl mercury compounds do accumulate in greater quantities than aryl mercury compounds. Other studies (Swensson and Ulfvarson 1968, Miller et al. 1960, and Miller et al. 1961) have shown that some phenyl mercury compounds may accumulate in greater amounts than methyl mercury compounds. There is a great deal of variation in the relative distribution of mercury when different compounds and different methods (intravenously and orally) are used to get the compound into the bird.

Giban (1953) and Miller et al. (1960) have shown the lethal single oral dose of methyl mercury dicyandiamide and phenyl mercuric acetate in chickens to be 12 to 18 and 60 mg/kg body weight, respectively, indicating that methyl mercury dicyandiamide is more toxic than phenyl mercuric acetate. The proportional increase of total

mercury in the tissues as the total quantity of mercury consumed increased was more rapid for methyl mercury dicyandiamide. This was also reflected by smaller concentrations of total mercury in the feces of birds fed methyl mercury dicyandiamide than in the feces of birds fed phenyl mercuric 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 methyl mercury dicyandiamide and by Miller et al. (1960) and Swensson and Ulfvarson (1969) with chickens using phenyl mercuric acetate.

The two cocks that died from methyl mercury dicyandiamide consumed an average of 41.2 mg prior to death as compared to the 24.7 mg consumed by the hens. The mercury concentrations in the tissues of the one cock analyzed were higher than the hens. Borg et al. (1969) has reported that male pheasants fed methyl mercury dicyandiamide have a longer survival time than females. Backstrom (1969) has also shown for quail that males tend to concentrate greater amounts of methyl mercuric nitrate than females in all tissues tested. He also found that males concentrate less phenyl mercuric nitrate than females in all tissues but liver.

The use of methyl mercury dicyandiamide for seed treatment presents a much greater threat to pheasant

populations than phenyl mercuric acetate and it appears that females are more vulnerable to alkyl mercury compounds than males.

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