Accumulation of Mercury in Tissues of Cattle, Sheep, and Chickens Given the Mercurial Fungicide, Panogen 15, Orally

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Yearling cattle and sheep and 6-week-old chickens were given daily oral doses of the mercurial fungicide Panogen 15 (methylmercury dicyandiamide). Cattle and sheep were given 15 mg of the total formulation per kg of body weight daily to 70 days, and chickens were dosed at both 5 and 10 mg/kg daily to 84 days. Two yearling cattle and three yearling sheep were mildly poisoned in the latter stages of the study. Residues of mercury, determined by atomic absorption spectrophotometry, were generally higher in cattle than in sheep except in the liver. The order of increasing residues in both cattle and sheep was brain, muscle, liver, and kidney. With chickens, however, residues in liver were higher than in kidney in both treatment groups. The residues of mercury in the various tissues of the dosed animals could constitute a hazard to future human consumers. Tissues from control cattle, sheep, and chickens contained no detectable amounts of mercury.

Fungicides are used widely for treatment of seed for future planting to reduce losses from plant diseases caused by organisms on the seed or in the soil. A mercurial fungicide that has been used frequently in the past is Panogen 15. The active ingredient in this formulation is methylmercury dicyandiamide (Figure 1) and accounts for 2.2% of the total formulation. The mercury equivalent is 1.5% of the total formulation.

Most known cases of poisoning by mercurial fungicide-treated grain have been with swine, but naturally occurring mercurialism in cattle has been reported by Herberg (1954) and Buck (1969). Palmer (1963) and Palmer *et al.* (1973), at this laboratory, experimentally poisoned livestock with mercurial fungicides.

Since domestic animals might be exposed to some mercurial fungicide-treated grain, residues of mercury could accumulate in their tissues. The objective in this study was to determine the residues of mercury in tissues of cattle, sheep, and chickens given the fungicide, Panogen 15, daily by oral capsule for various lengths of time.

MATERIALS AND METHODS

Reagents. Panogen 15 has 2.2% active ingredient of methylmercury dicyandiamide (1.5% mercury equivalent). It was obtained from Nor-Am Agricultural Products, Inc., Chicago, Ill.

The technical standard was 99% methylmercury dicyandiamide and was obtained from the Research and Development Center of Nor-Am Agricultural Products, Inc., Woodstock, Ill.

Apparatus. A Perkin-Elmer Model 403 Atomic Absorption Spectrophotometer set at 2537 Å resonance line and equipped with a mercury hollow cathode lamp was used in this study. The spectrophotometer and lamp were obtained from Perkin-Elmer Corp., Norwalk, Conn.

Three species of farm animals were chosen for this study. Thirteen yearling cattle and 13 yearling sheep of mixed breed and sex obtained at local auction were used. Twenty-six 6-week-old White Leghorn chickens were also used. Table I shows the number of animals dosed, the dosage of the formulation, active ingredient, and mercury equivalent given the different species daily. The dose given the different species of animals was comparable to that which the animals might consume if accidentally exposed to grain treated with the fungicide in the recommended manner.

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At least one dosed animal was killed weekly up to 10 weeks of treatment to obtain tissue samples. The test with chickens continued 12 weeks. Animals were killed the day after the last dose, and kidney, liver, muscle, and brain were collected from both cattle and sheep; kidney, liver, breast muscle, and thigh muscle were collected from the chickens. Undosed animals that served as controls were killed at the end of the study; then tissue samples were collected for analysis.

The various tissues collected from the test animals and controls were ground in an electric meat grinder. These samples from each tissue were well mixed and were frozen at −20° until analyzed.

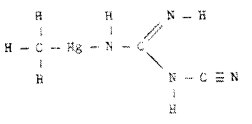
A modification (Dalton, 1969) of the tissue digestion procedure recommended by the Analytical Methods Committee (1960) was used in this study. Duplicate samples (each 5 g) were digested with 30 ml of a nitric–perchloric–sulfuric acid mixture (7:5:5) by refluxing at 135° for 30 min. (Extreme care must be taken in the use of this acid mixture.) The temperature of the digestion mixture was raised slowly to 160° while collecting the distillate. The mixture was refluxed for 15 min at 160° and the distillate was collected while the temperature of the mixture was raised to 178–180°. The mixture was refluxed for 30 min at 180° and, after allowing the apparatus and contents to cool, distilled water was used to quantitatively transfer the distillates and digested sample to a 125-ml flask. The digested sample was raised to pH 3.0 by dropwise addition of 30% sodium hydroxide with cooling.

The procedure of Willis (1962), modified by Dalton (1969), was used to extract the mercury from the digested sample. Ten milliliters of a 1% solution of ammonium pyrrolidine dithiocarbamate (APDTC) was shaken with the digested sample for 1 min. After standing 5 min, the solution was shaken with 10 ml of methyl isobutyl ketone (MIBK) for 1 min. After 5 min, the aqueous layer was discarded and the organic layer was analyzed for mercury by atomic absorption spectrophotometry. The instrument was adjusted according to standard conditions developed by the Perkin-Elmer Corporation (1968). The sensitivity of this method is approximately 0.3 ppm. Recovery of mercury from tissues fortified at 5- and 15-ppm levels of mercury as Panogen 15 averaged 85% of the total mercury present. Residues of mercury reported in the Results section have not been corrected for percent recovery.

RESULTS AND DISCUSSION

Two yearling cattle were mildly poisoned after 56 and 65 daily doses of the fungicide, respectively. Signs of poisoning included muscular incoordination, stiffness in hindquarters, and unsteady gait. All other cattle showed

ACTIVE INGREDIENT OF PANOGEN 15



METHYL MERCURY DICYANDIAMIDE

Figure 1. Chemical structure of the active ingredient of Panogen 15.

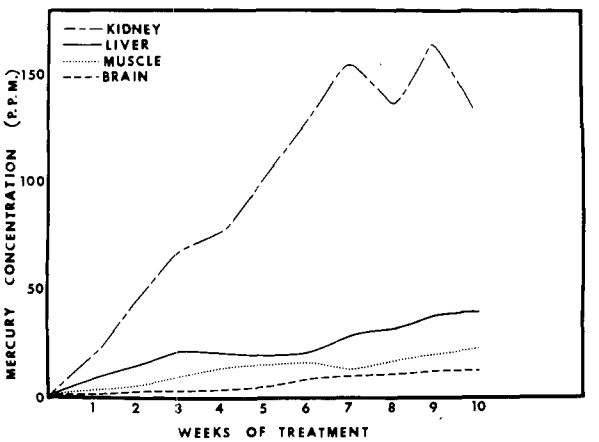


Figure 2. Mercury in tissues of cattle given daily oral doses of Panogen 15 at 15 mg/kg.

no ill effects. Figure 2 shows the residues of mercury found in tissues of cattle dosed daily with the total formulation at 15 mg/kg for various lengths of time. The greatest residues of mercury were in the kidneys (165 ppm at 9 weeks), followed by liver (39.5 ppm at 9 weeks), muscle (23.2 ppm at 10 weeks), and then brain (11.8 ppm at 9 weeks).

Three yearling sheep were mildly poisoned after 42, 56, and 59 daily doses, respectively. Signs of poisoning were muscular incoordination, stiffness, and unsteady gait. Two other sheep received more doses than the affected ones with no adverse effects apparent. Figure 3 shows the residues of mercury found in tissues of sheep given daily oral doses of 15 mg/kg of the total fungicide formulation. Kidney again had the greatest residues of mercury (110 ppm at 6 weeks), followed by liver (54 ppm at 9 weeks), muscle (14 ppm at 8 weeks), and then brain (13 ppm at 8 weeks). Although the animals were dosed at the same rate, residues of mercury in sheep were not as high as those of cattle, except in the liver.

No ill effects were visible in dosed chickens at both dosages to 84 days. Figure 4 shows the mercury found in tissues of chickens given the formulation at 5 mg/kg of body weight. In contrast to cattle and sheep, residues of mercury in chickens were greater in liver (12 ppm at 12 weeks) than in kidney (9.6 ppm at 12 weeks). Residues in

Table I. Number of Animals Treated and Daily Intake (mg/kg) of Panogen 15, Active Ingredient,[a] and Mercury

Number and types of test animals		Daily intake (mg/kg) of Panogen 15	Active ingredient	Mercury
10	Cattle	15	0.33	0.225
12	Sheep	15	0.33	0.225
12	Chickens	5	0.11	0.075
12	Chickens	10	0.22	0.150

[a] Active ingredient is methylmercury dicyandiamide.

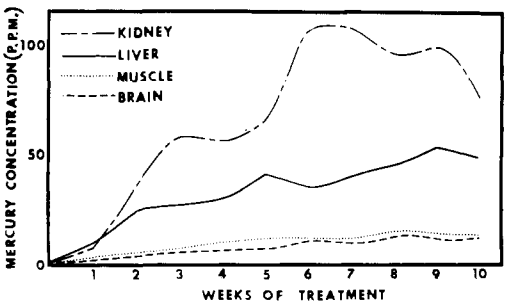


Figure 3. Mercury in tissues of sheep given daily oral doses of Panogen 15 at 15 mg/kg.

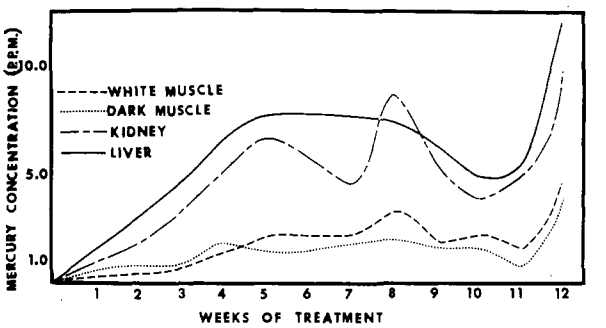


Figure 4. Mercury in tissues of chickens given daily oral doses of Panogen 15 at 5 mg/kg.

breast muscle reached 4.5 ppm at 12 weeks and 4 ppm in thigh muscle at 12 weeks. The results of the determinations in liver and kidney were erratic toward the end of the study, probably because of differences in individual chickens.

Similar results were obtained in the chickens given daily oral doses of the formulation at 10 mg/kg of body weight. As Figure 5 shows, liver again had the greatest residues of mercury (17.4 ppm at 11 weeks), followed by kidney (14 ppm at 11 weeks). Levels were 6 ppm in breast muscle and 5 ppm in thigh muscle at 12 weeks.

Tissues from control cattle, sheep, and chickens contained no detectable amounts of mercury.

Our data compare favorably with those from earlier work by Palmer *et al.* (1973). Because Palmer *et al.* (1973) used Ceresan M and we used Panogen 15 the residues differ, but the deposition patterns are the same in both studies; *i.e.*, greatest residues of mercury were found in kidney, then liver, muscle, and brain of cattle and sheep. Also, in turkeys (Palmer *et al.*, 1973) and chickens (this

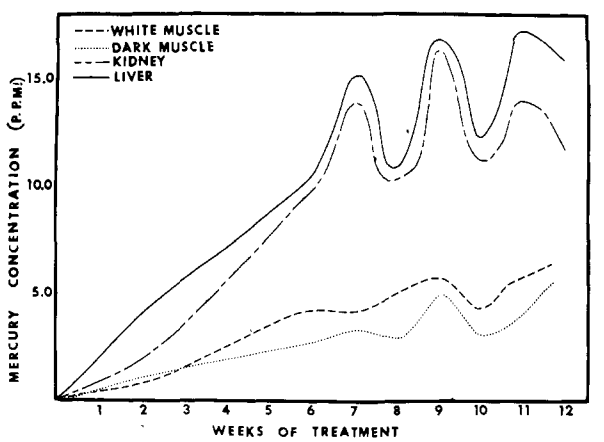


Figure 5. Mercury in tissues of chickens given daily oral doses of Panogen 15 at 10 mg/kg.

study), residues of mercury were greater in liver than in kidney.

In 1970, Panogen 15 was suspended for use as a seed treatment and such treated seeds were suspended from travel in Interstate Commerce. No tolerances are allowed on feed grains. It is still registered for use on cotton as a liquid or granular formulation applied in-furrow and covered at planting time. Registrations are also valid for nongrazed grass areas. The chances of an animal to be exposed to some grain treated with Panogen 15 are small; however, if such an exposure did occur, the consumption of tissues from this animal by humans could be hazardous.

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Metabolism of [^{14}C]Parathion and [^{14}C]Paraoxon with Fractions and Subfractions of Rat Liver Cells

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To obtain information relative to the metabolism of insecticides in mammalian systems, [2,6-ring-^{14}C]parathion and [2,6-ring-^{14}C]paraoxon were incubated with rat liver cell fractions obtained from the same liver homogenate and with highly purified subfractions from mitochondria and microsomes. Different enzymes in the rat liver were responsible for the degradation and detoxication of parathion and paraoxon. It was primarily with the soluble fractions obtained at 105,000 × *g* and 500,000 × *g* forces that parathion was degraded to water-soluble metabolites (20% of the applied radiocarbon) and only to a lesser extent by microsomes (1% of the applied radiocarbon). Based on analyses of the organic solvent extraction phases, enzyme activities in the soluble fraction resulted in a reduction of parathion to aminoparathion, but also in an oxidation of parathion to paraoxon. Microsomes showed some parathion-degrading activity and the amount of *p*-nitrophenol produced was relatively small. Paraoxon, however, was mostly degraded by particulate associated enzymes through hydrolysis, yielding *p*-nitrophenol in the organic solvent phase. The largest amounts of [^{14}C]paraoxon derived water-soluble metabolites (65% of the applied radiocarbon), though, were produced by the soluble fractions which also reduced paraoxon to aminoparaoxon. A biological assay of the water extraction phases from [^{14}C]parathion- or [^{14}C]paraoxon-treated incubation mixtures containing the 105,000 × *g* supernatant did not result in insect mortalities, thus indicating a detoxication of the insecticidal substances.

Detoxification mechanisms of insecticides in biological systems have been studied for years by many research workers. Of particular interest are those investigations that deal with the metabolism of insecticides through enzymatic reactions. In this respect, a number of studies were conducted relative to the degradation of parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate) and paraoxon (*O,O*-diethyl *O*-4-nitrophenyl phosphate) in mammalian systems. Nakatsugawa *et al.* (1969) showed that the arylphosphate cleavage of [^{35}S]parathion was primarily effected by rat liver microsomal oxidases and a minor portion was catalyzed by nonoxidative soluble enzymes requiring reduced glutathione. The authors concluded that "the metabolism of parathion in the rat is mostly initiated by liver microsomal oxidases." Neal (1967), working with microsomes from rat livers, reported that the major metabolites of [^{32}P]parathion were paraoxon, diethyl hydrogen phosphate, diethyl hydrogen phosphorothionate, and *p*-nitrophenol. Utilizing ^{3}H-labeled paraoxon, Kojima and O'Brien (1968) found that this compound was degraded by four distinct enzymatic pathways in soluble and particulate fractions of the liver cells. They reported that the soluble fraction produced *O*-desethylparaoxon. In another investigation, Fukami and Shishido (1966) demonstrated the major role of the soluble cell fraction in degrading methyl parathion. They showed that the supernatant fraction of tissue homogenates from rat liver and insect mid-gut cleaved methylparathion to desmethylparathion. Hollingworth (1970), using methylparaoxon labeled with ^{14}C in one methyl group, demonstrated that the addition of glutathione caused an increase in the degradation of methylparaoxon by the supernatant fraction from mice liver. He further stated that "it seems unlikely that microsomal oxidation plays a general role in detoxifying dimethyl esters in mouse liver." The enzymatic reduction of parathion was demonstrated with soluble, mitochondrial, and microsomal fractions from rat livers (Hitchcock and Murphy, 1967). Gaines *et al.* (1966) showed that parathion, after its "infusion into the hepatic portal system of rats, was more toxic than when infused by way of the femoral vein into the general circulation." The authors state that "parathion is converted to the highly toxic par-

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