Organic Mercury Identified as the Cause of Poisoning in Humans and Hogs

Abstract. *Atomic absorption spectrophotometry and neutron activation analysis showed the presence of mercury in organic extracts of seed grain and in tissues of hogs fed the contaminated grain. Mercury was also found in the urine, serum, and cerebrospinal fluid of humans who ate the contaminated pork. Mass spectral analysis confirmed the presence of organic mercury. This paper reports the first documented episode of indirect mercury poisoning in humans in the United States caused by the ingestion of contaminated meat from animals that had consumed mercury in their food supply.*

In 1961 Uchida *et al.* (*1*) showed that methyl (methylthio) mercury, traced to the ingestion of shellfish, was the cause of an unusual illness in humans in the Minamata Bay area, Japan. In 1966 Ordonez *et al.* (*2*) reported similar unusual illnesses that involved the central nervous system in humans in Guatemala. Because of the nature of the symptoms, this disease was thought to be encephalitis; however, autopsy samples sent to our laboratory were found to contain high concentrations of mercury. The deceased had ingested wheat seed that had been treated with Panogen (*3*) and contained 17 parts per million (ppm) of mercury. Also in 1966 Borg *et al.* observed mercury poisoning in birds in Sweden (*4*), and Takizawza and Kosaka reported methyl mercury poisoning in humans resulting from the ingestion of fish and shellfish in the Niigata Prefecture, Japan (*5*).

In August 1969 a farmer (Mr. H.) and five of his neighbors in the area of Alamagordo, New Mexico, obtained waste seed grain from a local granary. The grain had been treated with an organomercurial fungicide, either Panogen or a formulation of Ceresan (*3*); both fungicides had been used at different times for seed treatment by the manufacturer. The six farmers used this grain in food for hogs. The father of one family (Mr. H.) began feeding his pigs with this grain in late August or early September. After about 2 to 3 weeks, one hog, which had been fed 60 percent more grain than the others, was slaughtered and the family ate the meat during the next 3.5 months. The other pigs were kept on a similar diet but were fed smaller quantities of the grain. By mid-October, 14 of these feeder pigs had developed blindness, lack of coordination, and posterior paralysis. In the next 3 weeks, 12 of the 14 pigs died. Gait disturbances in the surviving pigs improved, but the pigs remained blind and stunted.

In early December one child of Mr. H.'s family became ill. By late December two other members of the family had developed the same illness. Mercury poisoning caused by the ingestion of contaminated pork was suspected. At this time the mother of the children was pregnant. Three months later the baby was born.

Details of the epidemiology, symptomatology and diagnosis, and the clinical history and treatment of the patients for mercury poisoning will be reported by the Center for Disease Control (formerly the National Communicable Disease Center) (*6*). We report here the results of chemical studies associated with the poisoning episode, the first observed case in the United States of indirect mercury poisoning in humans caused by the ingestion of contaminated meat from animals that had consumed mercury in their diet.

Several methods had been developed for the analysis of mercury in either the inorganic or organic form (*7*). We found that modification of the methods of Willis (*8*) for inorganic mercury and of Westöö (*9*) for organic mercury gave satisfactory results.

Samples of hog tissue (brain, liver, kidney, muscle, colon, pancreas, eye, heart, fat, and lymph nodes), human body fluids (serum, urine, cerebrospinal fluid, and amniotic fluid), and seed grain were prepared for analysis by atomic absorption spectrophotometry by modifications of Willis's method (*8*), as follows: 20-g tissue and grain samples in 25 ml of water were refluxed under a 24-inch (61-cm) condenser with 25 ml of concentrated HNO_3-H_2SO_4 (1 : 1, by volume) for at least 2 hours; in some tissues additional amounts of the acid mixture were added until the sample was free of solids. Fuming acids at lower reflux temperatures were used in order to minimize the digestion time of some tissues and mercury losses by volatilization. Perchloric acid (15 ml) was added to complete oxidation, and reflux was continued until the solution was light amber. After cooling, the *p*H of the solution was adjusted to 2.5 to 3.5. Samples were chelated with 5 ml of a 1 percent (by weight) solution of ammonium pyrrolidinedithiocarbamate (APDC) and extracted three times with 50 ml of methyl isobutyl ketone (MIBK). Emulsions were broken up by centrifugation. The combined MIBK extracts were reduced to between 4 and 25 ml for subsequent analysis. Urine (86.0 to 906.5 g), amniotic fluid (24.0 g), and cerebrospinal fluid (0.96 to 2.25 g) were adjusted to *p*H 2.5 to 3.5 with concentrated HNO_3-H_2SO_4 (1 : 1, by volume). These samples were chelated and extracted as before. Serum (5.4 to 36.5 g) was refluxed for 1 hour with 50 ml of 1.0*N* HCl. Then 20 ml of concentrated HNO_3 was added, and the condenser was rinsed with water. Reflux was continued for ½ hour. The sample was cooled, distilled water was added, and the *p*H was adjusted to 2.5 to 3.5 with 40 percent NaOH. The sample was chelated and extracted as before. The combined MIBK extracts were reduced to between 4.0 and 20.0 ml for subsequent analysis. Organic extracts were analyzed by atomic absorption spectrophotometry (*10*).

In order to observe emission effects or the absorbance of other possible interfering species near the absorption line for Hg at 2537 Å, test solutions containing (i) 20 ppm Hg, 1200 ppm Na, and 810 ppm P; (ii) 20 ppm Hg and 900 ppm Fe; (iii) 14 to 24 ppm Na and 156 to 180 ppm P; (iv) 20 ppm K and 155 ppm P; and (v) 1000 ppm Fe were prepared, and their influences on Hg absorption were recorded. These concentrations were chosen to simulate concentrations reported to be present normally in tissues and body fluids. Blank effects (water-saturated MIBK and other reagents) were also examined.

Phosphorus, sodium, potassium, and iron were not observed to interfere in either aqueous solution or organic solvent at the analytical wavelength for mercury. There was no absorption from the air-hydrogen flame. Sample calculations were corrected for the blank effects of MIBK and the signal-to-noise effects of the flame.

Aqueous standard solutions were prepared from analytical grade cyano-(methylmercuri)guanidine (Panogen) with the mercury content equal to 10,

Table 1. Concentration of mercury (in parts per million) in hog tissues and seed grain fed to that particular hog, as determined by atomic absorption spectrophotometry.

Hog or grain owned by	Hog					Grain
	Brain	Liver	Kidney	Muscle	Colon	
Mr. H. (hog slaughtered)				29.4*		
Mr. H. (hog slaughtered)				27.5		32.8†
Mr. H. (blind hog)‡	36.1	25.2	12.0	14.2		32.8
Neighbor 1	13.1	17.3	12.1	9.5	24.2	2.97§
Neighbor 2	15.8	21.0	25.2	23.1	6.5	1.27
Neighbor 3	12.6	3.5	8.4	16.8	14.7	2.76
Neighbor 4	3.5	8.8	20.5	16.8	7.9	
Neighbor 5	21.0	10.5	21.0	12.6	24.2	2.54
Range	< 3.5–21.0	< 3.5–21.0	8.4–25.2	9.5–23.1	6.5–24.2	1.27–2.97
Mean	12.5	12.2	17.4	15.8	15.5	2.39
Standard error	± 3.46	± 3.05	± 3.10	± 2.29	± 3.81	± 0.38

* Pork eaten by members of the family. Animal showed no signs of poisoning. † Mixture of waste grain. ‡ Other tissues analyzed and concentrations (in parts per million): spleen, 7.5; heart, 7.5; fat, 9.8; lymph node, 23.1; cerebrum, 9.8; cerebellum, 42.95; eye, 18.9; heart blood, 31.5; lung, 14.2; pancreas, 15.8; thigh muscle, 11.5; gastrointestinal tract, 17.3; stomach, 8.5. § Possible mixture of waste grain or contaminated feeder chow, or both.

20, 50, 100, and 200 ppm. Standards were also prepared in organic solvent from digestion with the HNO_3-H_2SO_4 acid mixture, chelation with APDC, and extraction with MIBK. These standards were compared with the Fisher atomic absorption spectrophotometric mercury reference standard at concentrations ranging from 2 to 200 ppm.

Some samples (20 g) of tissue and grain and of serum and urine were fortified with Panogen to give mercury contents equal to 12.5, 20, and 50 ppm, and 5, 10, 15, and 50 ppm, respectively. The analytical procedure was evaluated with standards at 20, 50, and 100 ppm.

By means of the analytical procedure 83 to 100 percent of the mercury was recovered from Panogen (mean, 95.8 ± 4.25 percent) at 20, 50, and 100 ppm, and 50 to 60 percent of the mercury (mean, 53.8 ± 1.96 percent) was recovered from tissues and grain fortified at 12.5, 20, and 50 ppm. Losses of mercury from tissue or grain samples may be due to volatilization during prolonged reflux, inefficient condensers, too high a reflux temperature, or combinations of these. Solubility effects during the adjustment of *p*H may also be a factor. Recovery from serum and urine was 98 to 100 percent (mean, 98.7 ± 0.67 percent).

Wet tissue, grain, human blood, and urine were analyzed for mercury by neutron activation analysis at the Nuclear Research Center, Georgia Institute of Technology (*11*). The Reinsch test for mercury (*12*) was positive.

A 600-g sample of waste seed grain was prepared for mass spectral analysis by a modification of the extraction and thin-layer chromatographic procedures of Westöö (*9*). An aliquot of the mercury-containing extract prepared by the method of Westöö was placed in a glass insert, the solvent was evaporated, and the insert was placed into the ion source of a mass spectrometer (LKB model 9000) by direct probe and ionized at 20 and 70 ev (*13*).

A 200-g sample of waste seed grain that had been extracted with methanol–diethyl ether (1 : 1, by volume) was highly colored by the characteristic red dye used to identify organomercury-treated grain. The dye was purified by column chromatography (*14*) for identification by atomic absorption spectrophotometry. Tests for the presence of chlorinated hydrocarbon and organophosphate insecticides were made by gas chromatography (*15*) but the results were negative.

Results of atomic absorption analysis of tissue samples from hogs, waste grain, and feeder chow that might have been contaminated are presented in Table 1. The hog fed large quantities of the waste grain and slaughtered for family consumption contained high concentrations of mercury in the tissues, as did another sick and ultimately blind hog from the same herd; this second hog also had the highest content of mercury (36.1 ppm) in the brain. The waste grain fed to these animals contained 32.8 ppm of mercury. Hogs belonging to the neighbors and fed grain from the same source contained about the same concentrations of mercury in the tissues.

The mixture of grain consisted of floor sweepings and screenings; it contained sorghum, oats, grain, chaff, and rat feces. Some of the grain had been treated with organic mercury compounds as a fungicide. Since there was no homogeneity in any of the grain samples, mercury concentrations varied for each sample. Each hog owner used this mixture in the daily feeding of the animals. The feeder chow might have been contaminated with waste grain. Neighbor farmers transferred feeder chow and waste seed grain by open truck in the rain, which might explain the lower mercury contents (mean, 2.39 ± 0.38 ppm) in the neighbors' grain.

Meat products purchased in area food stores also contained mercury. Atomic absorption spectrophotometric measurements indicated that kidney contained 2.2 ± 1.09 ppm; neutron ac-

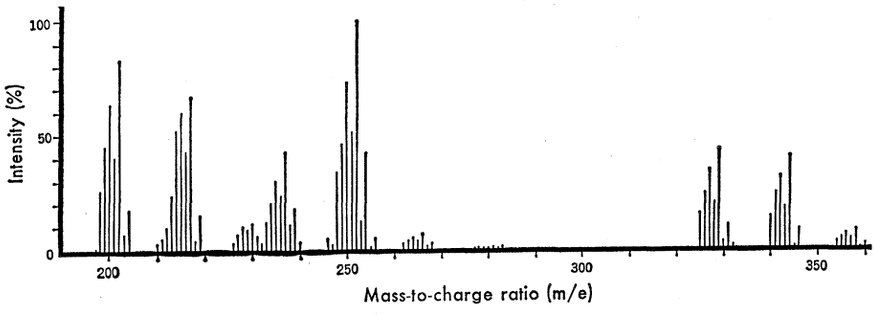

Fig. 1. Mass spectrum of waste grain extract.

tivation analysis (*11*) revealed that liver and sausage contained 0.17 and 0.072 ppm, respectively (the detection limit of the atomic absorption spectrophotometer is 0.3 ppm for a 20-g sample of tissue with a volume reduction of 4.0 to 5.0 ml). The present U.S. Food and Drug Administration tolerance limit for mercury in meat products is 0.5 ppm.

The concentrations of mercury in serum, urine, and cerebrospinal fluid were determined in samples from the human victims. Urine samples obtained from Mr. H., his son (age 13), and his two daughters (ages 8 and 20) on 8 January contained, respectively, 0.16, 0.21, 0.20, and 0.06 ppm of mercury. Concentrations of mercury in the urine samples of the neighbors varied from < 0.05 to 0.18 ppm (33 samples); the content of mercury in the serum samples of the neighbors averaged < 0.2 ppm (38 samples). After treatment with British Anti-Lewisite, the concentrations of mercury in the urine samples of Mr. H.'s son and older daughter (age 20) had increased to 0.50 and 0.49 ppm, respectively [the concentration of mercury in the urine of Mr. H.'s younger daughter (age 8) was < 0.03 ppm]. Concentrations of mercury in the serum samples of these children were approximately 16 times those in the urine. The concentrations of mercury in the serum and cerebrospinal fluid of Mr. H.'s son were about the same (3.0 ppm). The urine of Mrs. H., who was pregnant at onset of the children's illness, contained 0.09 ppm of mercury on 8 January and 0.18 ppm on 11 February; her serum contained 2.91 ppm of mercury on 22 January and 0.47 ppm on 11 February. The amniotic fluid contained < 0.02 ppm of mercury on 11 February. Concentrations of mercury in the newborn baby's urine ranged from 2.70 ppm at delivery to 1.56 ppm several days later. These concentrations of mercury indicate placental transfer to the fetus.

The mercury, identified by atomic absorption spectrophotometry, was confirmed as organic mercury by mass spectrometry (Fig. 1); these results substantiated the clinical diagnosis of organic mercury poisoning. The dye coating on the waste seed grain was isolated by column chromatography; it absorbed at 5440 Å. This dye was identical to that in commercial samples of Panogen and Ceresan. The extracts from the waste seed grain prepared by the method of Westöö contained, according to mass spectral analysis, characteristic Hg^+, methyl Hg^+, methyl $HgCl^+$ (chloride from the analytical procedure), ethyl Hg^+, and probably methoxyethyl Hg^+ isotopic ion clusters at m/e (mass-to-charge ratio) 202, 217, 231, 237, 252, 281, and 296. Other mercury-containing organic ions were observed at m/e 329, 344, and 358.

These data clearly show that mercury accumulated in animal tissues and human body fluids and confirm that compounds containing organic mercury were, in fact, the causative agents in the poisoning incident. The changes in the mercury concentrations in the serum and urine of the mother after delivery and the content of mercury in the urine of the newborn baby indicate placental transfer.

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3. Panogen is the trade name of Morton Chemical Company for a formulation containing methylmercuric dicyandiamide or cyano-(methylmercuri)guanidine as active ingredient. Ceresan is the trade name of E. I. du Pont de Nemours & Co. for a group of formulations consisting of ethyl, methyl, or methoxyethyl mercury compounds as the active ingredient.
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10. Equipment for analysis of the organic extracts comprised a Beckman model 979 atomic absorption spectrophotometer equipped with a total-consumption, turbulent-flow burner assembly for an air-hydrogen flame and a Beckman 10-inch (25.4-cm) potentiometric strip chart recorder, operated under the following conditions: wavelength, 2537 Å; lamp, argon-filled, hollow cathode; lamp current, 10 ma; three burners; elevator position, 7.6 cm; three passes (light beam); support gas, air at 20 to 25 pounds per square inch (1.4 to 1.7 atm); fuel gas, hydrogen at 4 pounds per square inch; lean flame; 0.15-mm slit width.
11. We thank M. E. McLain, Jr., Nuclear Research Center, Georgia Institute of Technology, Atlanta, for neutron activation analyses.
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13. Other parameters were as follows: the temperature of the direct probe varied from ambient temperature to 120°C; temperature of the ion source, 290°C; accelerating voltage, 3.5 kv; filament current, 4 amp; trap current, 60 μa; recording oscillograph, 4 cm/sec; scan speed, 5.
14. Glass chromatographic columns, 8 mm in diameter, were packed with 4.0 cm of silica gel (Woelm), grade 1, below 2.0 cm of anhydrous sodium sulfate. The columns were wet with benzene before introduction of the samples. A 4-ml portion of the supernatant from the extract was eluted with about 14 ml of benzene and 5 ml of acetone and then by an additional 1 ml of acetone. The second acetone fraction containing the dye was collected and the acetone was evaporated. The red dye was dissolved in methanol, and its absorbance was determined on a Cary model 14 recording spectrophotometer.
15. The Micro-Tek model MT 220 gas chromatograph was equipped with tritium electron-capture detectors for chlorinated compounds and dual flame photometric detectors for phosphorus and sulfur.
16. We thank Mrs. E. Gray for her assistance with the statistics and receipt of samples.
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Roadrunners: Energy Conservation by Hypothermia and Absorption of Sunlight

Abstract. *Roadrunners sunning in artificial sunlight consume oxygen at standard (basal) levels at ambient temperatures as low as 9.0°C. Energy savings of sunning roadrunners averaged 551 calories per hour. In the dark, birds may undergo hypothermia. Hypothermic roadrunners can elevate their body temperatures to normal levels by sunning, at reduced metabolic cost.*

One of the most dependable sources of energy in the desert is solar radiation. Small birds exposed to artificial sunlight have been shown to reduce energy expenditures (*1*, *2*). Although the hypothesis (*1*) that sunlight directly affects avian heat budgets has broad implications, the actual ecological role of this phenomenon has yet to be evaluated for any bird. We examined this hypothesis for the roadrunner (*Geococcyx californianus*) because these desert residents frequently sun themselves in the field and in captivity