Bull. Environm. Contam. Toxicol. 24, 65-72 (1980)

Comparative Study of Uptake and Tissue Distribution of Methylmercury in Female Rats by Inhalation and Oral Routes of Administration

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The release of mercury to the environment from coal-fired power plants, mining, and smelting operations is of special concern since these sources are currently uncontrolled and the use of coal is expected to increase significantly. Mercury which is released into the environment becomes available for methylation. Methylation rates in ecosystems are a function of the mercury burden, bacterial population, and other physico-chemical conditions. Analysis of mercury forms in the air around Tampa Bay, Florida revealed that about 21% of mercury in the atmosphere is of methylmercury types (JOHNSON and BRAMAN 1974). No detailed data concerning the uptake and absorption of inhaled vapor of methyl mercury compounds are available. This paper reports a study on the uptake and tissue accumulation of ^{203}Hg-methylmercuric chloride following oral and inhalation administration in female rats.

MATERIALS AND METHODS

Synthesis of ^{203}Hg-methylmercuric chloride (^{203}Hg-MMC). One mmole of pure recrystalized MMC was dissolved in five ml of methanol containing 0.5 ml of 6N HCl. Two mCi of ^{203}Hg nitrate was added to the methanol solution and the mixture was allowed to stand overnight at 50 C. After cooling, 5 ml of water was added to the mixture and the ^{203}Hg-MMC was separated by benzene extraction. The recovery of MMC was generally greater than 75%.

Generation of ^{203}Hg-MMC Vapor. ^{203}Hg-MMC generator consisted of a 30 x 150 mm test tube with a side arm outlet and an inlet tube at the top which extended almost to the bottom of the tube. A known amount of ^{203}Hg-MMC was weighed into a small vial which was then placed in the test tube, and the tube was immersed in a thermoregulated water bath. Air was pumped into the generator from the inlet tube at a constant rate measured by a precision flow meter. Since MMC vaporizes relatively easily at room temperature, the vapor was carried off from the generator and entered into a mixing flask in which air could be added to give a desired concentration. The determination of ^{203}Hg-MMC vapor concentration was achieved by pass-

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Technical Paper No. 5285, Oregon Agricultural Experiment Station, Corvallis, Oregon.

0007-4861/80/0024-0065 $01.60

ing a known volume of air through a hopcalite or charcoal trap, measuring the radioactivity in the trap and calculating the vapor concentration from the known specific activity of ^{203}Hg-MMC. ^{203}Hg-MMC concentration of the atmosphere could be controlled by the temperature of the water bath, the air flow rate into the test tube, and the dilution with MMC-free air.

All measurements were made with a γ-scintillation spectrometer equipped with 3-in NaI well detector (Packard Model 5260) or with a 2-in NaI well detector (Technical Associate Model SM-10). The background was generally less than 20 cpm for Packard Model 5260 and 80 cpm for SM-10.

Adult female Wistar rats (between 2 to 6 months old, averaging 225 ± 27 g) were used in this study. Two rats in an 8-inch all glass metabolism cage were exposed continually to air containing ^{203}Hg-MMC vapor of known initial concentration. Water was available and food was not given during the time of exposure. The flow rate of air was maintained at 500 ml min^{-1} during the inhalation period.

In the first experiment, the rats were exposed to ^{203}Hg-MMC vapor for 6, 12, 18 and 24 hours in order to determine the inhalation uptake and distribution of ^{203}Hg in relation to the time of exposure. In the second experiment, rats were exposed for 24 hours to 50, 100 or 140 nmole of ^{203}Hg-MMC per liter in order to study the uptake in relation to MMC vapor concentration. In the third experiment, rats were dosed orally with either 3 μmole or 9 μmole of ^{203}Hg-MMC in corn oil and sacrificed after 1,2,3 or 4 days in order to compare the tissue distribution of ^{203}Hg between the two routes of administration.

The subcellular fractions of liver, kidney and brain were obtained by differential centrifugation as described previously (ELLIS and FANG 1967) and the ^{203}Hg distribution in these fractions determined. All subcellular fractions were acidified with concentrated HCl and extracted with benzene to determine the amount of organic mercury. The distribution of ^{203}Hg among the proteins of various molecular weights in the soluble fraction of liver and kidney are also determined after separation on a Sephadex G-100 column.

RESULTS

The data obtained from the measurement of ^{203}Hg contents of tissues from rats when exposed to air containing 140 nmole ^{203}Hg-MMC per liter for various periods of time are shown in Table 1. It is apparent that the ^{203}Hg concentration of most organs increased linearly with the time of exposure. Excluding the hair, the highest concentration or the highest rate of accumulation is found in the blood, followed in decreasing order by stomach, kidney, liver, spleen, lung, pancreas, heart, intestine, muscle, and brain. The accumulation of ^{203}Hg in the blood is about two to three times faster than in the liver, kidney, and spleen; four to six times faster than in the lung,

TABLE 1

Pulmonary uptake of ^{203}Hg-MMC by adult female rats in relation to the time of exposure (initial MMC concentration 140 nmole/l, air flow rate 500 ml/min).

Exposure Time, hr	^{203}Hg Concentration, nmole/g Fresh Tissue			
	6(2)	12(7)	18(4)	24(8)*
Blood	1.0	11.9±2.9	25.0	26.4± 6.9
Stomach	0.5	9.1±4.5	14.5	17.9±10.5
Kidney	1.7	7.3±2.0	9.7	17.8± 6.1
Liver	1.6	6.0±1.6	10.4	12.3± 5.1
Spleen	1.0	4.9±1.6	10.2	10.2± 3.3
Lung	0.6	3.9±1.1	8.4	7.0± 1.6
Pancreas	1.0	3.7±0.9	6.9	6.4± 2.6
Heart	0.3	2.8±0.8	4.8	5.5± 1.9
Intestine	0.4	2.9±1.1	5.3	4.9± 1.3
Muscle	0.3	0.8±0.3	1.2	2.2± 1.0
Brain	0.2	0.8±0.3	0.8	1.1± 0.4
Hair	151	948 ±90	856	2030 ±404

*All values are the average of the rats used.

heart, and intestine; and 14 and 33 times greater than in the muscle and brain, respectively.

The accumulation of ^{203}Hg in different tissues of rats exposed for 24 hours to air containing 50, 100, or 140 nmoles of MMC per liter is shown in Table 2. The ^{203}Hg content in various tissues increases with the increased MMC vapor concentration. The relative ^{203}Hg content in each organ or tissue remains fairly constant among the three concentrations used. Since the initial event of pulmonary uptake of any vapor is contact of a gas molecule with the surface of the lung, the frequency of contact is proportional to the pressure or the concentration of the gas and the relation can be expressed by the Freundlich equation as follows:

$$q = aC^{1/n}$$

where q is the quantity of adsorbed substance, C is the concentration, a and n are constants. By plotting the log of the rate of accumulation, a straight line is obtained as shown in Figure 1. At the range of MMC concentration studied here, the Freundlich equation fits the experimental data quite well and can be utilized to estimate empirically the pulmonary uptake of MMC and the deposition of mercury in the tissues.

For comparison of tissue distribution of MMC to the pulmonary uptake, a group of female rats was given a single oral dose of 3 or 9 µmole of MMC in corn oil and sacrificed 1,2,3 or 4 days afterwards. The results are shown in Table 3. In both groups most organs have a maximum ^{203}Hg content at day one and then decline slowly, with the exception of brain in which the ^{203}Hg content increased steadily throughout the four-day period. Since the ^{203}Hg contents were quite different in various tissues

TABLE 2

Pulmonary uptake of ^{203}Hg-MMC by adult female rats in relation to MMC vapor concentration (exposure time - 24 hr; air flow rate = 500 ml/min).

MMC, nmole/l	^{203}Hg Concentration, nmole/g Fresh Tissue		
	50 (5)	100 (11)	140 (8)*
Blood	6.8 (3.3-9.7)	18.4 (9.0-30.7)	26.4 (14.7-49.8)
Kidney	6.1 (3.5-9.4)	11.2 (7.4-21.9)	17.8 (11.5-31.9)
Stomach	5.7 (2.0-9.9)	13.2 (6.7-22.3)	17.9 (8.8-33.4)
Liver	4.0 (2.2-6.7)	9.9 (5.8-16.7)	12.3 (7.2-23.6)
Spleen	3.1 (1.9-4.2)	6.4 (3.3-10.7)	10.2 (6.4-16.3)
Pancreas	2.4 (1.9-2.9)	4.7 (1.7-9.2)	6.4 (4.1-12.3)
Lung	2.7 (2.1-3.3)	6.8 (3.7-12.1)	7.0 (5.5-10.0)
Heart	2.1 (0.6-2.7)	4.9 (2.6-8.9)	5.5 (4.0-9.8)
Intestine	1.7 (1.2-2.4)	3.6 (2.0-5.2)	4.9 (3.8-7.9)
Muscle	1.3 (0.2-2.4)	1.5 (0.8-2.5)	2.2 (1.2-4.1)
Brain	0.5 (0.3-0.7)	0.8 (0.5-1.4)	1.1 (0.7-1.7)
Hair	424 (273-593)	876 (705-1050)	2030 (1263-2590)

*All values are average of all rats used.

following oral administration and pulmonary uptake, the best way to compare these results is using the ratio of blood ^{203}Hg to tissue ^{203}Hg. This comparison is shown in Table 4.

Significant differences were observed in blood/kidney, blood/ brain and blood/muscle ratios between these two routes of administration. Higher ratio values from pulmonary uptake of MMC suggest a greater retention of MMC in the blood. However, this difference could be due to the result of the elapse time after dosing, as the values from oral administration were the average of rats sacrificed from 1 to 4 days after dosing, while the rats from pulmonary uptake were exposed within 24 hours, and sacrificed immediately.

With respect to the subcellular distribution of ^{203}Hg, the majority (42-51%) of ^{203}Hg was in the soluble fraction of both liver and kidney, 31-44% in the crude nuclear fraction, and

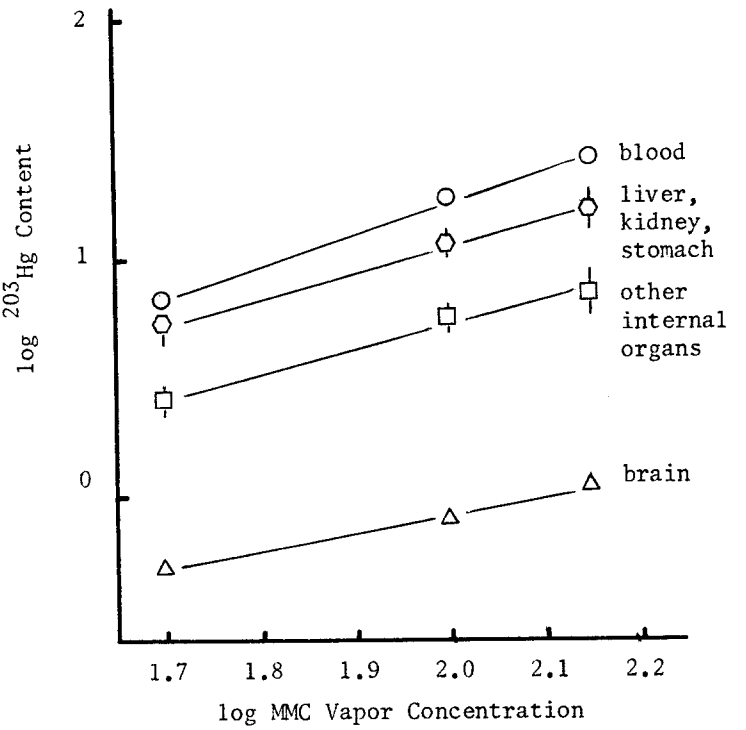

Figure 1 Relationship of ^{203}Hg accumulation in tissues of rats to MMC vapor concentration of the air inhaled by the rats.

4-12% in the mitochondrial and microsomal fractions (Table 5). In contrast, the majority (44-46%) of ^{203}Hg in the brain was found in the crude nuclear fraction and only 29% in the soluble fraction. Extraction of each fraction with benzene showed that from 51-83% of ^{203}Hg was organic mercury. The soluble fraction had the highest benzene extractable ^{203}Hg (81-95%), while the microsomal fraction had the lowest (51-76%), suggesting that microsomes were the major site for MMC transformation.

Chromatography of the liver, kidney, or brain soluble fraction on a Sephadex G-100 gel column resulted into two major ^{203}Hg peaks (Figure 2). The first peak was eluted with the void volume representing a protein of greater than 100,000 daltons. The second peak, with a V_i/V_o value of 1.5 which corresponds to about 70,000 daltons and is probably serum albumin.

DISCUSSION

The results found in this study indicate that ^{203}Hg accumulation in organs and tissues from pulmonary uptake of ^{203}Hg-MMC vapor increases linearly with the time of exposure and, also,

TABLE 3

^{203}Hg accumulation in various tissues of adult female rats receiving an oral dose of 3 μM or 9 μM ^{203}Hg-MMC.

Time After Dosing, Day	^{203}Hg Concentration, nmole/g Fresh Tissue							
	1		2		3		4	
	3 μM	9 μM	3 μM	9 μM	3 μM	9 μM	3 μM	9 μM
Blood	112.0	372.4	95.1	322.8	88.8	209.5	68.2	294.0
Liver	33.7	116.2	25.3	101.9	27.7	119.9	28.5	102.0
Kidney	97.5	314.9	80.4	301.0	94.0	257.1	91.6	297.4
Spleen	32.1	143.2	27.9	131.6	38.0	120.3	25.7	115.4
Pancreas	18.6	74.9	22.7	67.2	17.1	60.5	18.9	60.9
Heart	27.1	60.4	19.8	53.6	18.5	45.8	18.4	50.0
Lung	33.1	107.6	18.6	80.3	26.6	77.2	24.5	71.0
Intestine	9.7		6.2		8.1		8.8	
Brain	4.5	17.5	5.8	19.2	5.3	21.5	6.3	23.0
Muscle	9.1	31.4	10.9	41.0	13.0	43.8	12.6	41.0

TABLE 4

Comparison of ratios of ^{203}Hg concentration in the blood to that in the tissues of female rats received ^{203}Hg-MMC from oral or inhalation route.

	Oral	Inhalation
Blood:liver	3.1 ± 0.4	2.5 ± 0.2
Blood:kidney	1.0 ± 0.2	2.1 ± 0.4
Blood:lung	3.6 ± 0.4	3.7 ± 0.4
Blood:brain	16.8 ± 4.5	25.7 ± 3.7
Blood:heart	4.4 ± 0.4	5.3 ± 0.1
Blood:muscle	9.3 ± 2.2	16.2 ± 2.8

proportionately with the initial vapor concentration. Unfortunately, we measured only the initial MMC vapor concentration at the inlet of metabolism chamber, any uptake by the rat during the passage of air through the chamber will reduce the vapor concentration. Thus, the true vapor concentration in relation to pulmonary uptake is not known.

Although the lung is the first organ to have contact with MMC vapor, the accumulation of ^{203}Hg in this organ is not high in comparison to other tissues throughout the 24-hour period, suggesting that it is quickly transported throughout the whole body. The relative amounts of ^{203}Hg deposition in various tissues and organs are not completely similar between these two routes of uptake as indicated by the ratios of ^{203}Hg in the blood to that in the kidney, brain, heart, or muscle. Most striking differences are in the blood/kidney and blood/muscle

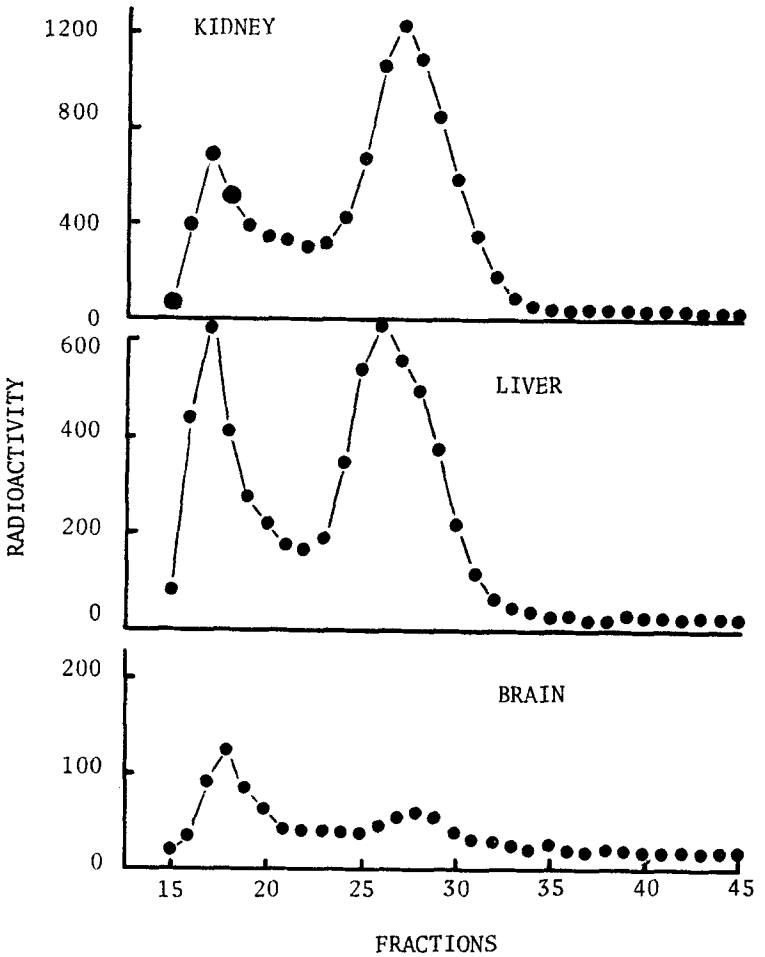

Figure 2 Sephadex G-100 chromatographic separation of soluble fraction of kidney, liver or brain from rats which inhaled ^{203}HG-MMC vapor for 24 hours.

TABLE 5

Subcellular ^{203}Hg distribution in tissues of female rats receiving ^{203}Hg-MMC from oral or inhalation route.

		Total ^{203}Hg Distribution, %				Benzene Extractable ^{203}Hg, %			
Organs	Route	Nu	Mit	Micro	Soluble	Nu	Mit	Micro	Soluble
Liver	0 (6)[a]	32±4	9±2	9±1	50±2	88	80	76	95
Liver	I (6)	43±6	6±1	9±2	42±4	73	66	55	89
Kidney	0 (6)	33±5	7±2	11±1	49±3	81	76	68	89
Kidney	I (6)	35±2	7±2	9±2	50±3	77	81	63	81
Brain	I (6)	27±3	31±11	10±2	29±3	66	63	54	92

[a]Denotes number of rats used. 0 = oral; I = inhalation.

ratios which are about two times higher from pulmonary route than those from oral route. No difference is found in the blood/lung ratio from either route of uptake while a higher value is observed in the blood/liver ratio of oral uptake. The average blood/brain ratio was also shown to be 16 for total mercury in female rats of Sprague-Dawley strain after intravenous injection of MMC at 1 mg/kg (NORSETH and CLARKSON 1970). However, the average blood/brain ratio value was 11.7 when methylmercuric hydroxide was employed (ULFVARSON 1969). All blood/tissues ratios remain quite constant throughout the four-day period with the exception of blood/brain which declines with time in the oral dosed rats.

There is essentially no difference in the subcellular distribution of ^{203}Hg whether the MMC is given orally or by inhalation.

ACKNOWLEDGEMENT

This study was supported in part by a research grant ES-00040 from the U.S. Public Health Service, Institute of Environmental Health Sciences. The technical assistance of E. Fallin and N. Charbeneau are gratefully recognized.

REFERENCES

ELLIS, R.W., and S.C. FANG: Toxic Appl. Pharmacol. 11,104 (1967).

JOHNSON, D.L., and R.S. BRAMAN: Environ. Sci. Technol. 8, 1003 (1974).

NORSETH T., and T.W. CLARKSON: Arch. Environ. Health 21,717 (1970).

ULFVARSON, U.: Toxicol. Appl. Pharmacol. 15,517 (1969).