Bull. Environ. Contam. Toxicol. (1986) 37:890–898

Bulletin of Environmental Contamination and Toxicology

Mercury Distribution Studies Involving Complexes of Low-Molecular Weight Thiols and Methylmercury

James E. Balthrop, Joe L. Wade, and Sylvia Braddon-Galloway

National Marine Fisheries Service, Southeast Fisheries Center, Charleston Laboratory, P.O. Box 12607, Charleston, SC 29412-0607

Most methylmercury (CH_3Hg) circulating through the body is found in the red blood cells (Norseth and Clarkson, 1970). Naganuma and Imura (1979) demonstrated that this CH_3Hg in human erythrocytes was bound to a low molecular weight substance having an elution profile on gel filtration and a Rf value on thin layer chromatography similar to glutathione. Before entering the general circulation, CH_3Hg, like all substances absorbed by the intestine, must pass via the portal circulation to the liver. In fact, the liver is the first major organ with the opportunity to transform or metabolize absorbed nutrients and toxicants. Experiments by Norseth and Clarkson (1970) have shown that the concentration of CH_3Hg in the liver rises very rapidly and reaches a peak within one day after an injection of CH_3Hg. However, the concentration of CH_3Hg in the liver drops rapidly by days 2 and 3. This drop appears to result from the transport of CH_3Hg out of the liver into bile (Refsvik, 1982). A prerequisite for the normal movement of CH_3Hg from the liver into the bile is a high concentration of glutathione in the liver (Refsvik, 1978). Refsvik and Norseth (1975) demonstrated that glutathione bound to CH_3Hg is the predominant mercury compound in rat bile. In addition, a small amount of methylmercuric cysteine is found in the bile of CH_3Hg exposed animals. Thus, it is generally observed that CH_3Hg is associated with thiols in biological materials.

One of the metabolic roles of low molecular weight thiol compounds may be the formation of thiol-methylmercury complexes which can be preferentially translocated across cell membranes. The co-administration of equimolar amounts of the thiol, L-cysteine, with CH_3Hg increased the short-term accumulation of CH_3Hg in liver, kidneys and cerebrum, but reduced the level of CH_3Hg found in plasma (Thomas and Smith, 1982). This modification of the distribution pattern of CH_3Hg by co-administering low molecular weight thiol compounds further suggests that thiol-methylmercury complexes may play a role in the tissue deposition process. In fact,

Send reprint requests to James E. Balthrop at National Marine Fisheries Service, Southeast Fisheries Center, Charleston Laboratory, P.O. Box 12607, Charleston, South Carolina 29412-0607

treatment of rats with CH_3Hg has produced a low molecular weight CH_3Hg complex in cerebral cytosol. This complex accounted for approximately one-third of the soluble CH_3Hg and was identified by column chromatography and electrophoresis to be methylmercury-glutathione (Thomas and Smith, 1979). The object of this study is to determine if CH_3Hg and thiol complexes of CH_3Hg are absorbed and distributed in a manner dependent upon the status of a known ameliorative agent of CH_3Hg toxicity, selenium.

MATERIALS AND METHODS

The CH_3Hg complexes of cysteine, homocysteine and glutathione were prepared by mixing equimolar amounts of the thiol and methylmercury (^{203}Hg) chloride (Amersham)[1] in 0.1 M Tris-HCl, pH 8.0 (Refsvik and Norseth, 1975). The methylmercury complex with cysteine-glycine was prepared by mixing a pH 5.0 solution of cysteine-glycine with a solution of methylmercury chloride (^{203}Hg) in 0.1 M Tris-HCl, pH 8.0 in equimolar proportions (Refsvik and Norseth, 1975).

The thiol-methylmercury complexes were applied to 20 x 20 cm Silica Gel G Redi-Plates (Fisher Scientific). The plates were developed at room temperature in a 70/30 n-propanol/25% ammonia solvent system (Refsvik and Norseth, 1975). Visualization of CH_3Hg was accomplished by spraying with a 0.10% dithizone solution in chloroform (Iwata et al., 1981). To determine the extent of complexation of CH_3Hg by the thiols each plate was divided into 1 cm sections, the silica scraped and counted for ^{203}Hg in a Beckman 8000 gamma counter. The percent CH_3Hg complexed was: glutathione 93%, homocysteine 95%, cysteine 91%, and cysteine-glycine 93%. The Rf values for the CH_3Hg complexes were: methylmercury 0.12, methylmercury-glutathione 0.44, methylmercury-cysteine 0.71, methylmercury-cysteinylglycine 0.63, and methylmercury-homocysteine 0.56.

Female 21 day old ICR mice (Harlan Sprague Dawley) were placed on selenium-deficient (< 0.05 µg/g Se) or selenium-control (0.5 µg/g Se) diets for a period greater than 5 weeks before the start of each experiment. The selenium status (liver selenium and blood GSH-peroxidase) of such animals was reported previously (Balthrop and Braddon, 1985). Animals (n = 4 or 5) were exposed to 5 nmoles CH_3Hg/g body weight by either subcutaneous (SC) or intraperitoneal (IP) injections of thiol-methylmercury complexes diluted in phosphate buffered saline. Animals were injected once daily for 7 days. Twenty-four hours after the final injection the animals were killed. The blood, brain, liver, kidneys, and small intestine of each animal were immediately removed, rinsed in

---

[1] Mention of trade names or products does not imply endorsement by the National Marine Fisheries Service, NOAA.

saline and blotted dry. Tissues were then weighed and counted for ^{203}Hg. Results were calculated as percent of recovered dose and concentration of mercury (nmoles CH_3Hg/g tissue). A Student's t-test was used to compare the means.

RESULTS AND DISCUSSION

The relative distributions of CH_3Hg found in blood, brain, liver, kidney and intestine are shown in Figures 1-4. The results show that there is no difference in the distribution of CH_3Hg due to the selenium status of the animal nor due to the route of CH_3Hg administration (SC or IP). Furthermore, exposure to each of the four complexes of CH_3Hg resulted in the same tissue distribution of CH_3Hg as exposure to uncomplexed CH_3Hg. The liver accumulated approximately 35% of the recovered CH_3Hg while the kidney and intestine each accumulated approximately 25%. The blood accounted for approximately 15% of the recovered CH_3Hg while the brain, the major target tissue for CH_3Hg intoxication, contained approximately 1% of the recovered CH_3Hg.

The concentration of CH_3Hg (nmoles/g tissue) found in the blood, brain, liver, kidney and intestine are shown in Figures 5-8. Once again the results show no differences due to the selenium status of the animals nor due to the route of administration. Furthermore, exposing the animals to complexes of low molecular weight thiols and CH_3Hg resulted in the same tissue CH_3Hg concentration as exposing the animals to uncomplexed CH_3Hg. The kidney contained the highest concentration of CH_3Hg (140-180 nmoles/g), while the liver and intestine contained lower but about equal concentrations of methylmercury (40-60 nmoles/g). The blood had the next to lowest concentration (20 nmoles/g), while the brain had the lowest concentration of the five tissues (10-15 nmoles/g).

Our data provide evidence that CH_3Hg when complexed to thiols is absorbed and distributed throughout the body in a manner similar to uncomplexed CH_3Hg. This may be due to the fact that, following either subcutaneous or intraperitoneal injection, CH_3Hg or thiol-complexes of CH_3Hg are quickly delivered to the liver via the general circulation. The demonstrated conversion in the liver of CH_3Hg to CH_3Hg-glutathione (Refsvik and Norseth, 1975) may be paralleled by liver metabolic interconversions of the injected thiol complexes. Thus the injected CH_3Hg-thiol complexes may lose their distinct identities and all become one similar, though undefined compound.

The relative tissue concentrations of mercury observed in our experiment compares favorably with those found by Fang (1980). He reported the highest level of mercury to be in the kidney and the lowest level in the brain. However, he calculated a 16.8 blood: brain mercury ratio and this is much higher than our ratio of approximately 2. This discrepancy could be due to the fact that the level of CH_3Hg in the brain continues to rise slowly for days

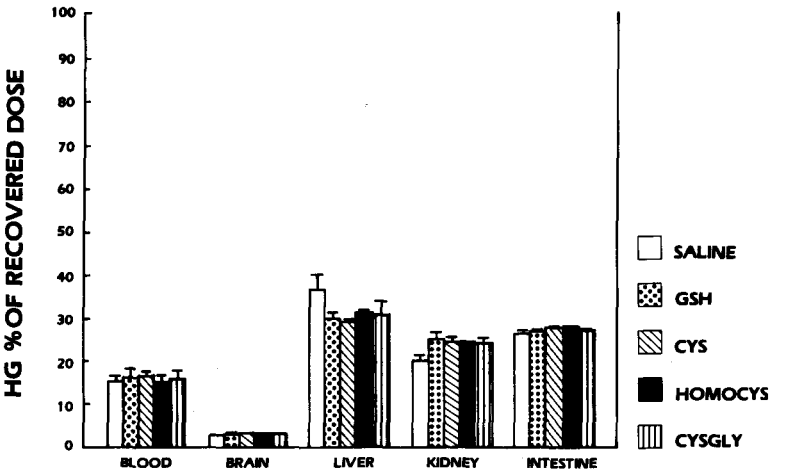

Figure 1. Tissue distribution of methylmercury in selenium deficient mice intraperitoneally exposed to low molecular weight thiol-methylmercury complexes. Selenium deficient mice were injected intraperitoneally daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

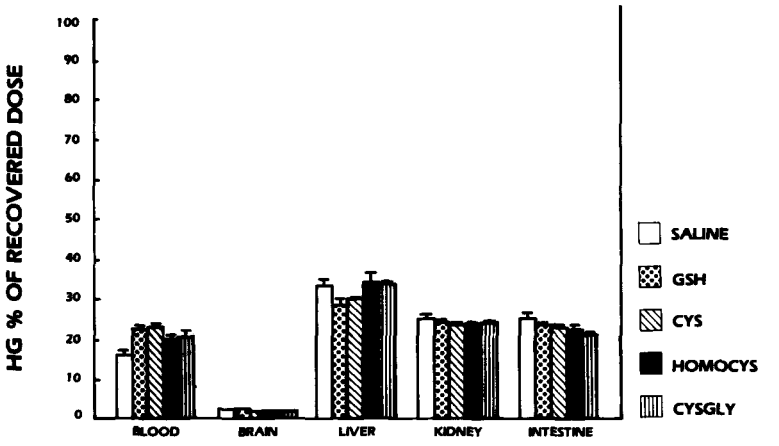

Figure 2. Tissue distribution of methylmercury in selenium control mice intraperitoneally exposed to low molecular weight thiol-methylmercury complexes. Selenium control mice were injected intraperitoneally daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

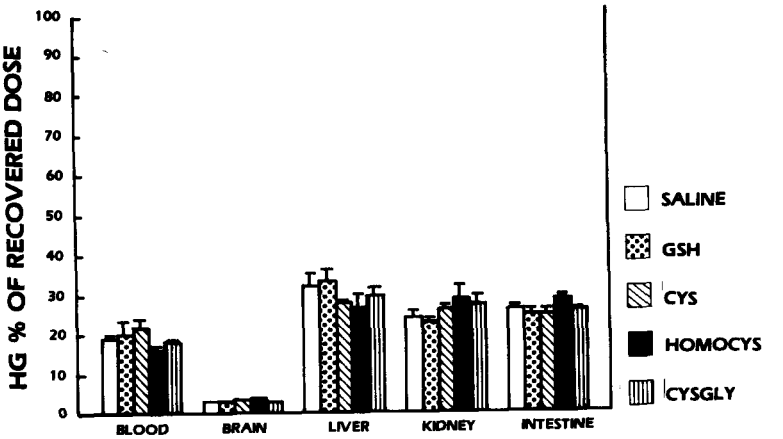

Figure 3. Tissue distribution of methylmercury in selenium deficient mice subcutaneously exposed to low molecular weight thiol-methylmercury complexes. Selenium deficient mice were injected subcutaneously daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

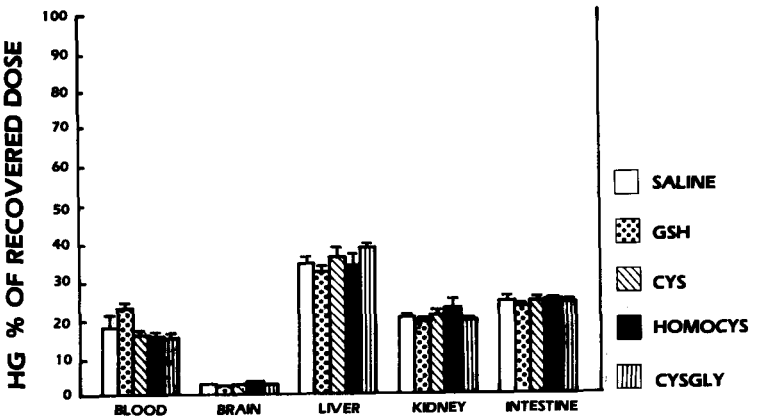

Figure 4. Tissue distribution of methylmercury in selenium control mice subcutaneously exposed to low molecular weight thiol-methylmercury complexes. Selenium control mice were injected subcutaneously daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

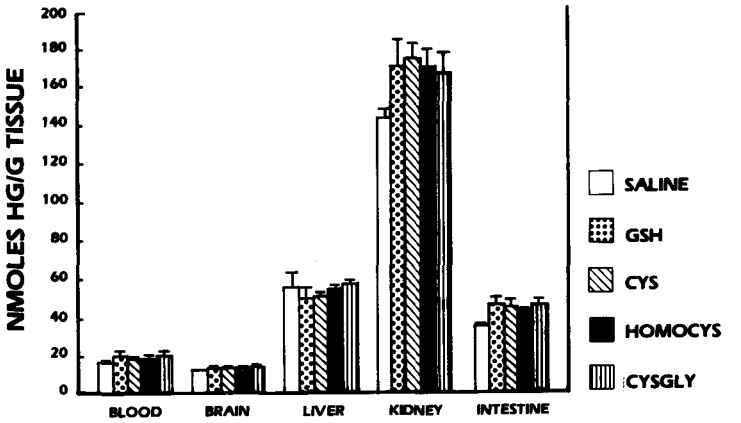

Figure 5. Tissue concentration of methylmercury in selenium deficient mice intraperitoneally exposed to low molecular weight thiol-methylmercury complexes. Selenium deficient mice were injected intraperitoneally daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

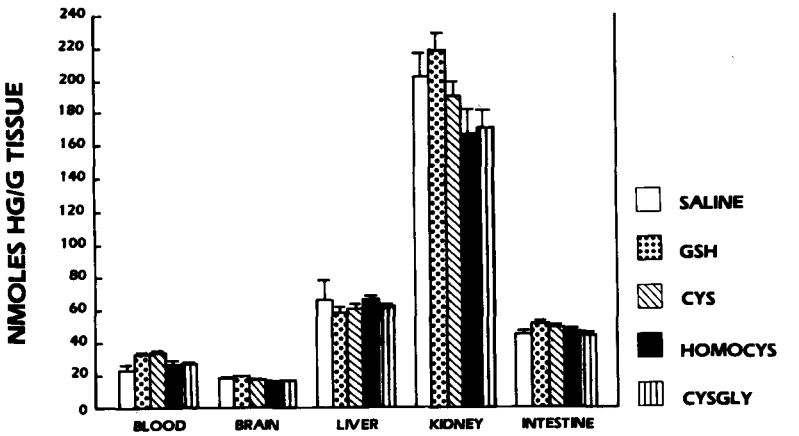

Figure 6. Tissue concentration of methylmercury in selenium control mice intraperitoneally exposed to low molecular weight thiol-methylmercury complexes. Selenium control mice were injected intraperitoneally daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

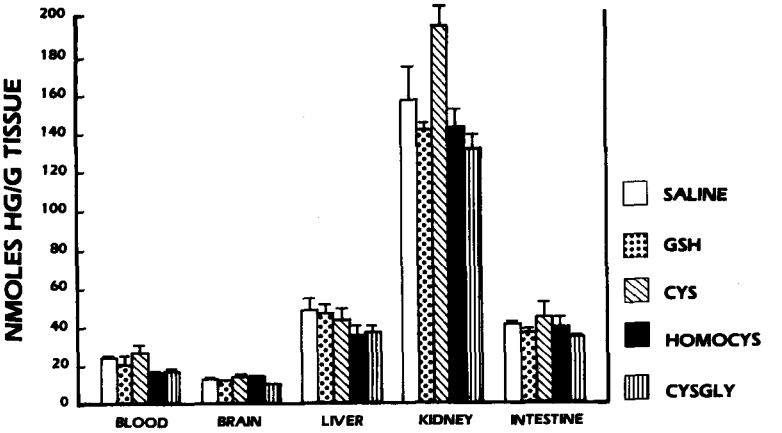

Figure 7. Tissue concentration of methylmercury in selenium deficient mice subcutaneously exposed to low molecular weight thiol-methylmercury complexes. Selenium deficient mice were injected subcutaneously daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

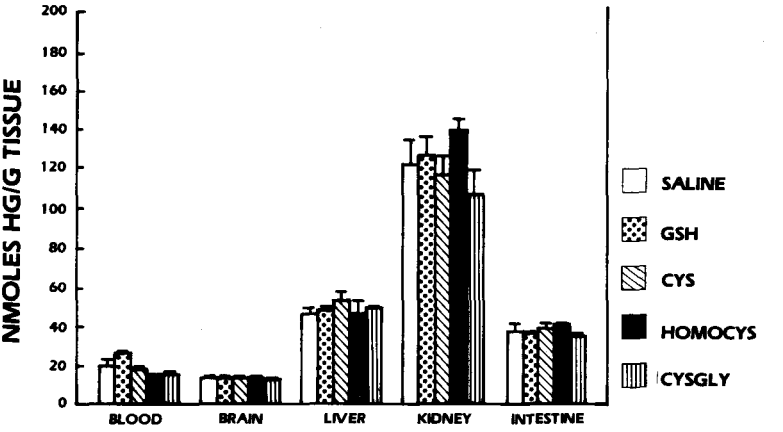

Figure 8. Tissue concentration of methylmercury in selenium control mice subcutaneously exposed to low molecular weight thiol-methylmercury complexes. Selenium control mice were injected subcutaneously daily for 7 days and killed 24 hours after the last injection. The daily dose was 5 nmoles methylmercury/g body weight. The reported values are means ± SE; n=4-5.

(Mehra and Kanwar, 1980). Fang's ratio is calculated after mercury exposure for 2 days, while our data was obtained after exposing animals to CH_3Hg for 7 days.

The selenium status of the animals had no effect on the accumulation and distribution of CH_3Hg following exposure to thiol-methylmercury complexes. This is different from the results of Thomas and Smith (1984) in which they reported that co-administration of sodium selenite with CH_3Hg had an effect on mercury distribution and increased the concentration of methylmercury in the brain.

It is important to note that their study was short term (5-60 minutes) and that, even though the concentration of CH_3Hg in the liver was significantly increased by selenite co-administration at 5 and 20 minutes, it was not increased at 60 minutes after injection. In addition, our study did not involve co-administration of selenite but rather used different selenium dietary regimes.

Our data suggest that the selenium status of the consumer has little if any effect on the distribution and accumulation of injected mercury. Considering our results along with those of Thomas and Smith (1984), it appears that metabolic interactions of selenium with CH_3Hg may occur only when the metals are concurrently administered. Further research is needed to investigate whether or not selenium alters the chemical form of circulating CH_3Hg.

REFERENCES

Balthrop JE, Braddon SA (1985) Effects of selenium and methylmercury upon glutathione and glutathione S-transferase in mice. Arch Environ Contam Toxicol 14:197-202

Fang SC (1980) Comparative study of uptake and tissue distribution of ethylmercury in female rats by inhalation and oral routes of administration. Bull Environ Contam Toxicol 24:65-72

Iwata H, Masukawa T, Kito H, and Hayashi M (1981) Involvement of tissue sulfhydryls in the formation of a complex of methylmercury with selenium. Biochem Pharmacol 30:3159-3163

Mehra M, Kanwar KC (1980) Absorption, distribution, and excretion of methylmercury in mice. Bull Environ Contam Toxicol 24:627-633

Naganuma A, Imura N (1979) Methylmercury binds to a low molecular weight substance in rabbit and human erythrocytes. Toxicol Appl Pharmacol 47:613-616

Norseth T, Clarkson TW (1970) Studies on the biotransformation of ^{203}Hg-labeled methyl mercury chloride in rats. Arch Environ Health 21:717-727

Refsvik T (1978) Excretion of methyl mercury in rat bile: The effect of diethylmaleate, cyclohexene oxide and acrylamide. Acta pharmacol et toxicol 42:135-141

Refsvik T (1982) Excretion of methyl mercury in rat bile: The effect of thioctic acid, thionalide, hexadecyl- and octadecylmercaptoacetate. Acta pharmacol et toxicol 50:196-205
Refsvik T, Norseth T (1975) Methyl mercuric compounds in rat bile. Acta pharmacol et toxicol 36:67-78
Thomas DJ, Smith JC (1979) Partial characterization of a low-molecular-weight methylmercury complex in rat cerebrum. Toxicol Appl Pharmacol 47:547-556
Thomas DJ, Smith JC (1982) Effects of coadministered low-molecular-weight thiol compounds on short-term distribution of methyl mercury in the rat. Toxicol Appl Pharmacol 62:104-110
Thomas DJ, Smith JC (1984) Effects of coadministered sodium selenite on short-term distribution of methyl mercury in the rat. Environ Res 34:287-294
Received March 20, 1986; accepted May 1, 1986.