Bull. Environ. Contam. Toxicol. (1991) 46:255–262

Environmental Contamination and Toxicology

Methylmercury Exposure during Lactation: Milk Concentration and Tissue Uptake of Mercury in the Neonatal Rat

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In recent years toxicological interest in mercury has predominantly been focused on the effects of prenatal exposure to methylmercury on the physical and mental development of children (Marsh et al. 1980; Kjellström et al. 1986; 1989). Thus, there has been a general concern to limit the exposure of pregnant women to methylmercury. Much less attention has been paid to postnatal exposure to mercury. However, there is also a possibility of elevated mercury exposure in the newborn due to exposure via breast milk. Bakir et al. (1973) reported high blood levels of mercury in infants exposed to methylmercury only via breast milk during an outbreak of methylmercury poisoning in Iraq. Mercury levels in the blood and milk of women exposed to methylmercury through fish consumption have been reported by Skerfving (1988). It has been proposed that the recommended restriction of intake of methylmercury-contaminated fish in pregnant women should be extended to include lactating women (Skerfving 1988).

There is a lack of data from both humans and animals on lactational transfer of many metals. However, metabolic evidence suggests that during the neonatal period the infant is sensitive to effects of these compounds (Clarkson et al. 1985). Thus, the gastrointestinal absorption and the retention of metals is higher during this period than adult life (Kostial 1983; Rowland et al. 1983). In the present study the dose-dependent transfer of mercury into milk was studied in lactating rats treated with methylmercury. The uptake of mercury in tissues and blood was followed in the offspring exposed via milk.

MATERIAL AND METHODS

Sprague-Dawley rats, weighing about 300 g, were obtained with litters 5 days after parturition from Möllegaard, Denmark. The rats were kept in individual cages and fed R3 pellets (Astra Ewos, Sweden) and tap water ad libitum. Litters were adjusted to 8 pups each, before being randomly assigned to four dose groups with 4-5 rats with litters in each group.

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On Day 9 of lactation the mothers received a single oral dose of methyl^{203}mercuric chloride, $CH_3{}^{203}HgCl$, specific activity 9.85 mCi/g Hg, purchased from Amersham International, England. The isotope was supplemented with unlabelled CH_3HgCl, dissolved in 5 mM Na_2CO_3 for the highest dose group and dissolved in and diluted with 5 mM Na_2CO_3 for the other dose groups. The rats were administered methylmercury by gavage after 24 h starvation in the following doses; Group I: 0.5 mg Hg/2.48 µCi/kg b wt, group II: 3.3 mg Hg/16.4 µCi/kg b wt, group III: 7.8 mg Hg/46.1 µCi/kg b wt and group IV: 9.4 mg Hg/31.6 µCi /kg b wt.

Milk was collected from all dams at 24 and 72 h after the oral dose. The pups were separated from the mothers during 2 h before milking to allow milk to accumulate in the glands. The dams were anesthetized with an i p injection of 40 mg/kg b wt of sodium pentobarbital (Mebumal vet. ACO, Sweden). A few minutes before milking a s c injection of 6.25 U oxytocin/kg b wt (Syntocinon, 5 IE/ml, Sandoz, Basel, Switzerland) was given to cause milk letdown. The milk was collected using a milking device, previously described by Oskarsson (1987), operated by vacuum and 0.5 - 1 ml milk was obtained from each dam by milking several glands. Whole blood, 0.5 ml, was collected orbitally in heparinized capillary tubes and 35 µl of heparin (25 000 IU/ml, Kabi Vitrum, Sweden, diluted 1:100) was added to each blood sample.

After a nursing period of 72 h, blood and milk was collected from the dams, whereafter all rats were sacrificed and liver, kidney and brain obtained from the mothers and 3 pups from each litter. Two samples of whole blood was collected by heart puncture in heparinized syringes from each litter by pooling blood from 4 pups. After centrifugation of the blood samples at 1800 rpm for 15 min, radioactivity was determined in plasma and erythrocytes as well as in the tissues and milk in a gamma counter (Nuclear Chicago, Model 4230), using the characteristic line of 279 keV photon emission with a counting efficiency of 35%.

RESULTS AND DISCUSSION

A linear dose-related transfer of mercury from plasma into milk was shown in the present study in lactating rats exposed to a single dose of methylmercury. The relationship at 24 h (Fig 1) indicates that the milk level is approximately 10% of the level in plasma. The relationship between the mercury concentration in plasma and breast milk after 72 h was also linear ($y = 0.083x + 0.013$; $r=0.90$, $p<0.001$). The mercury concentration in milk remained at a similar level at 72 h as at 24 h. There was a linear relationship between the mercury concentration in the erythrocytes and breast milk of the dams. As previously shown (WHO 1976) in methylmercury-treated rats the plasma level of mercury is low (less than 5%) compared to the erythrocyte level. Thus, in the present study, the level of mercury in milk was only 0.2% of the level in erythrocytes (Table 1).

There was a close correlation ($r=0.98$, $p<0.001$) between the mercury concentrations in the erythrocytes of the dams and their

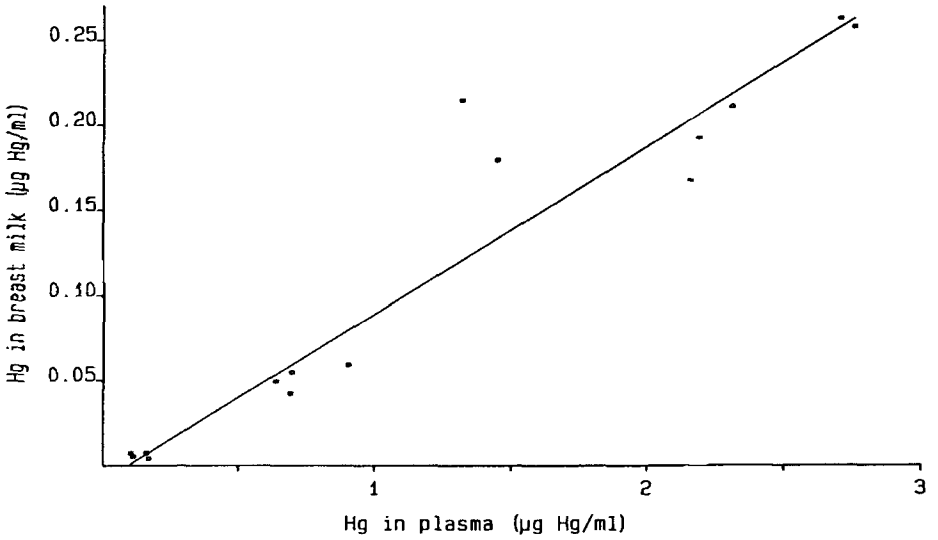

Figure 1. Relationship between mercury concentrations in plasma and breast milk of dams 24 h after a single oral dose of methylmercury, 0.5-9.4 mg Hg/kg b wt, on day 9 of lactation (n = 15). The regression line: $y = 0.097x - 0.004$ shows a highly significant correlation; $r = 0.91$, $p<0.001$.

pups at 72 h ($y = 0.007x + 0.015$). The mercury levels in plasma in the dams and their pups were less strongly correlated ($y = 0.009x + 0.005$; $r = 0.78$, $p<0.001$).

The tissue levels of mercury in kidney, liver and brain at 72 h after administration of methylmercury are shown for dams in Fig 2A and for pups in Fig 2B. There was a good agreement between the dose- dependent tissue uptake in the dams and in the pups exposed via the dams' milk for 3 d. However, in the highest dose group there seemed to be a saturation of the uptake in the kidney and liver of the dams, but not in the pups. The highest tissue levels were reached in the kidneys. There was a linear uptake of mercury in brain, the levels in dams being approximately 110-fold higher than those in pups.

The tissue uptake of mercury in the pups was also plotted against the mercury concentration in milk as a dose indicator of the exposure of mercury to pups (Fig 3A-C). There was a good correlation, especially in brain and kidney. As the milk levels of mercury at 24 and 72 h were similar (Table 1), the milk concentration could roughly indicate the continuously administered dose level to the pups during this period of time.

Table 1. Concentrations of mercury in erythrocytes, plasma and milk in lactating rats given a single oral dose of methylmercury on Day 9 of lactation and in their suckling pups after a 72 h nursing period, expressed as µg/ml (milk and plasma) or µg/g (erythrocytes). Mean ± SD, n = 4-5 dams and 8-10 pups.

	Dose (mg Hg/kg b.wt.)			
	0.5	3.3	7.8	9.4
Dams 24 h				
Erythrocytes	5.52 ± 0.27	37.8 ± 3.9	101 ± 9.8	121 ± 4.6
Plasma	0.14 ± 0.03	0.73 ± 0.12	3.20 ± 1.71	2.24 ± 0.67
Milk	0.006 ± 0.002	0.052 ± 0.007	0.21 ± 0.02	0.23 ± 0.04
Dams 72 h				
Erythrocytes	3.71 ± 0.38	28.1 ± 2.3	80.4 ± 13.9	83.9 ± 13.2
Plasma	0.056 ± 0.012	0.43 ± 0.13	2.53 ± 0.89	2.26 ± 1.33
Milk	0.006 ± 0.002	0.064 ± 0.019	0.16 ± 0.05	0.17 ± 0.04
Pups 72 h				
Erythrocytes	0.030 ± 0.005	0.21 ± 0.05	0.58 ± 0.07	0.66 ± 0.07
Plasma	not detected	0.007 ± 0.003	0.027 ± 0.007	0.024 ± 0.005

A relationship between the concentrations of total mercury in milk and whole blood has been reported from studies carried out in connection with the outbreak of methylmercury poisoning in Iraq in 1971-72 (Bakir et al. 1973; Amin-Zaki et al. 1976). The milk level was approximately 8% of the level in whole blood in the interval from 50 to 1 000 µg total Hg/ml whole blood. The plasma level of total mercury was 18% of the level in whole blood (Bakir et al. 1973) and thus the milk level can be calculated to be about 40% of the level in plasma. The concentration of total mercury in milk in the studied interval was 15-80 µg Hg/ml, of which about 60% was in the form of methylmercury. In women exposed to methylmercury through fish consumption a significant correlation between plasma and milk was found, with milk levels of total mercury approximately 70% of the levels in plasma (Skerfving 1988). The average

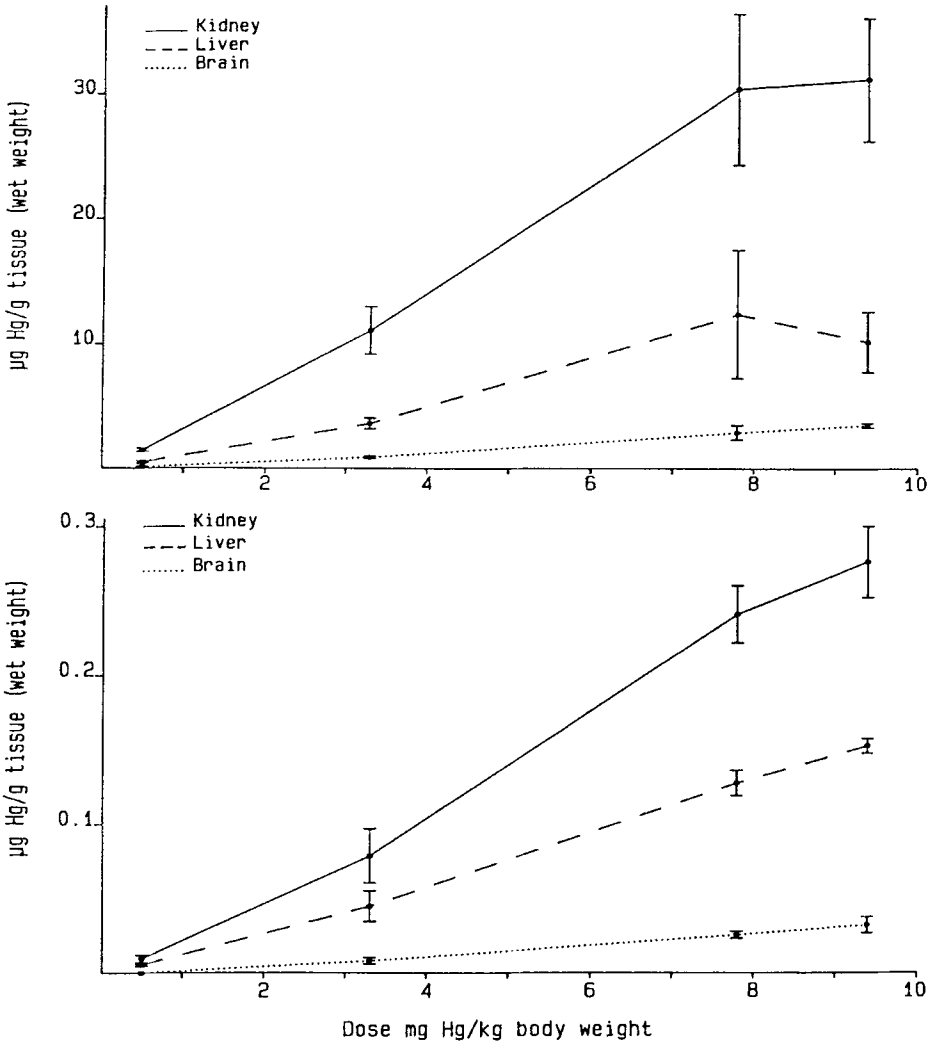

Figure 2. Concentrations of mercury in kidney, liver and brain in dams (A) and pups (B) 72 h after a single oral dose of methylmercury, 0.5-9.4 mg/kg b wt, on Day 9 of lactation. Mean ± SD, n=4-5 dams and 12-15 pups.

concentration of total mercury in milk in that study was 3.1 (range 0.2-6.3) µg/g. However, the proportion of methylmercury in milk was, on average, only 20 % of the total mercury.

Most probably the mercury level in milk reflects the plasma mercury level. There is a difference in distribution in blood between inorganic and methylmercury in that a higher proportion of inorganic mercury is present in the plasma fraction compared to methylmercury, which is predominantly bound to the erythrocytes

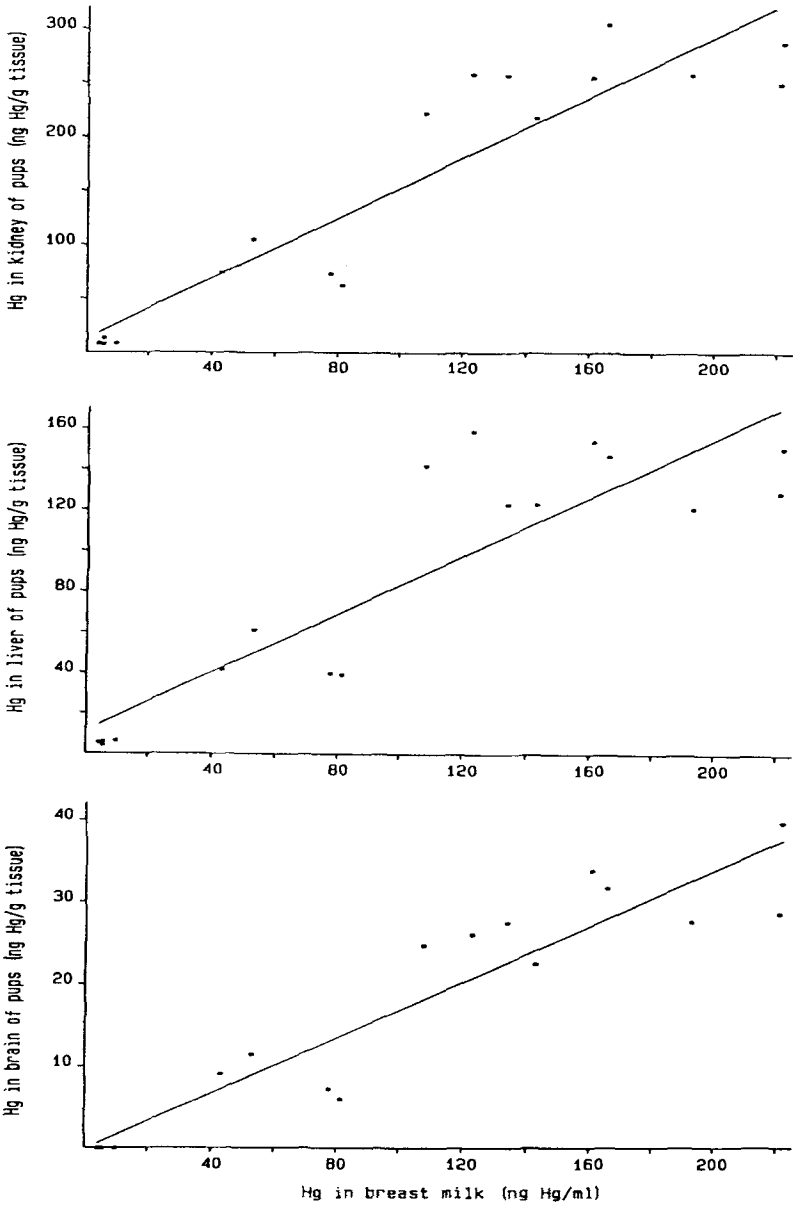

Figure 3. Relationship between mercury concentrations in breast milk and in kidney (A), liver (B) and brain (C) of the pups (mean of 3 pups/litter). For experimental design see fig 2. The regression lines are expressed by the equations (n=17).
(A) $Y = 1.40x + 13.02$; $r = 0.86$, $p<0.001$
(B) $y = 0.72x + 11.14$; $r = 0.79$, $p<0.001$
(C) $y = 0.17x - 0.11$; $r = 0.88$, $p<0.001$.

(Nordberg and Skerfving 1972). Thus, the lactational transfer of inorganic mercury into milk would be favoured. The demethylation of methylmercury that takes place in vivo probably plays an important role for the lactational transfer of mercury. The fraction of total mercury present in blood and tissues as inorganic mercury depends on the duration of exposure to methylmercury and the time after cessation of exposure. Magos and Butler (1972; 1976) showed that the fraction of inorganic mercury in rat tissues tended to approach a constant value, different for each tissue, with long-term daily dosing of methylmercury. In adult rats injected with methylmercury, Norseth and Clarkson (1970) found that approximately 25% of the plasma mercury was in the inorganic form and that this level was constant between 2 and 10 days after injection. It is suggested that the higher lactational transfer shown by Skerfving (1988) after long-term fish consumption is due to a higher proportion of inorganic mercury in plasma compared to plasma in women from the short-term methylmercury exposure in Iraq (Bakir et al. 1973). The low lactational transfer in rats, shown in our study, could be due to the short time after exposure and/or a low degree of demethylation in the lactating rat.

There seems to be a great difference in the transport of methylmercury between the pre- and the postnatal periods. Methylmercury has been shown to cross the placenta easily to the fetus both in humans and in experimental animals (see review by Sager et al. 1986). Mercury levels in the blood of prenatally exposed infants are reported to be higher than maternal concentrations (Amin-Zaki et al. 1974).

It can be suggested that the transfer of mercury into milk is dependent on the exposure situation and that preferentially inorganic mercury is transported into milk. The chemical form of mercury is of great importance for the uptake of mercury in the neonate. Methylmercury is absorbed almost completely from the gastro-intestinal tract, while inorganic mercury is normally reported to be absorbed to only a small extent. However, in suckling rats an absorption of inorganic mercury of approximately 40 % has been reported (Kostial 1978). In the present study it has not been differentiated between different forms of mercury in milk. However, it can be suggested that the major part is in the form of methylmercury, as the tissue distribution of mercury in the suckling rat resembles the distribution in the dam.

Acknowledgments. This study was supported by a grant from the National (Swedish) Environment Protection Board, Project Environmental Health Monitoring System based on Biological Indicators.

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Received May 29, 1990; Accepted July 10, 1990.