

## Blood and Brain Mercury Levels after Chronic Gestational Exposure to Methylmercury in Rats

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Female rats were exposed to 0, 0.5, or 6 ppm Hg (as methylmercuric chloride, 10 rats/group) in drinking water. For half the rats, exposure began 4 weeks before mating and for the others, exposure began 7 weeks before mating. All mating was done with an unexposed male. Maternal exposure continued to post-natal day (PN) 16. Blood and whole-brain mercury concentrations were determined in pups on PN 0 (birth) and PN 21 (weaning). Maternal water consumption was monitored daily during gestation and lactation. Maternal water consumption increased 2- to 3-fold through gestation for all groups. Mercury levels in blood and brain were unrelated to the duration of exposure before mating, although reproductive success appeared to be so related. Mercury levels in both media were closely related to consumption during gestation, but apparently maternal exposure during lactation did not result in exposure to the nursing pups. Brain mercury in offspring decreased between birth and weaning from 0.49 to 0.045 ppm in the low-dose rats and from 9.8 to 0.53 ppm in the high-dose rats. The brain increased in weight only about 5.5-fold during this time, indicating that there was minimal mercury exposure and some net loss from brain during this period. Brain: blood ratios averaged about 0.14 at birth and 0.24 at weaning, suggesting differential loss from neural and non-neural tissue. These ratios are higher than those reported in studies using less chronic exposure conditions or with adult rats. Brain concentrations of mercury in females in the low-dose group were about 10–15% higher than those seen in their male siblings. At the higher dose, the males had slightly higher levels of mercury in the brain than did their female siblings at birth. The relationship between brain concentration (in ppm) and cumulative mercury consumption, also expressed on a ppm basis (cumulative mercury consumed divided by maternal body weight at parturition), was not linear but was well described by a power-function relationship:  $Hg = A * (\text{cum exposure})^b$  where the exponent,  $b$ , was 1.12 and 1.17 for blood and brain, respectively, at birth. This exponent was indistinguishable from 1.0 for both media at weaning, indicating that the relationship between exposure and blood and brain levels became linear.

**Key Words:** methylmercuric chloride; neurotoxicity; brain and blood mercury concentrations; ratios of concentration; chronic exposure; rat.

The concentration of mercury in the brain is the most suitable biomarker of exposure when comparing neurotoxic effects of mercury across studies and even across species (Burbacher *et al.*, 1990; Lapham *et al.*, 1995). This is so even in the face of hundred-fold differences across species in the ratio of methylmercury concentration in the brain to that in other organs or blood (Magos, 1987; Magos *et al.*, 1985). A quantitative relationship between blood and brain concentrations of mercury has been identified for some exposure protocols, but the one currently available for rats is based upon repeated daily administrations of a relatively high dose to adult rats (Magos, 1987; Magos *et al.*, 1985). The applicability of this estimate to low-level exposure or chronic consumption is not known, so extrapolations to such conditions implicitly contain certain assumptions, including linear generalization from repeated acute dosing to chronic oral consumption, and from relatively high to much lower doses (Elsner *et al.*, 1985; Schreiner *et al.*, 1986). The time course of mercury distribution and elimination, however, suggest that data from acute exposure regimens may not apply to chronic exposures (Gray, 1995; Magos and Butler, 1976; Rice *et al.*, 1989; Vahter *et al.*, 1994, 1995). Moreover, extrapolation from a ratio determined at one level of exposure to lower levels implicitly assumes a linear relationship between dose and brain concentration, and such a simple relationship may not hold.

Information about the distribution of mercury, under conditions of chronic, low-level mercury exposure, would be helpful, since this exposure protocol reproduces the most common form of human exposure. If exposure is carried out long enough, then such a protocol establishes some stability in tissue levels. Stability in tissue levels is especially important in studies of the developmental neurotoxicity of methylmercury since estimating tissue levels under the doubly dynamic conditions of equilibrating mercury and fetal development can be very complicated.

Some form of dietary exposure, such as through drinking water, is a convenient way of producing chronic exposure because about 95% of methylmercury can be absorbed from the gastrointestinal (GI) tract (Magos, 1987) while also mimicking human exposure conditions, but such exposures carry with it one distinct disadvantage: the subject exerts some

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TABLE 1  
Exposure, Tissue Levels, and Reproductive Success

Group	Hg <sup>1</sup>	Breeding failures <sup>2</sup>	Litter <sup>3</sup> < 8	Sex	Total births		Blood Hg ( $\mu\text{g/g}$ ) <sup>4</sup>		Brain Hg ( $\mu\text{g/g}$ ) <sup>4</sup>		Brain: blood ratio		Brain weight (g) <sup>3</sup>	
					Births	% male	Birth	PN 21	Birth	PN 21	Birth	PN 21	Birth	PN 21
Control	ND	1/10	0	M	61	55	<0.01	<0.01	<0.01	<0.01			0.273	1.49
				F	49		<0.01	<0.01	<0.01	<0.01			0.268	1.43
Low	0.5	3/10	1	M	34	43	3.5	0.16	0.45	0.046	0.13	0.29	0.261	1.45
				F	45		4.0	0.20	0.52	0.040	0.13	0.23	0.250	1.46
High	6	1/10	1	M	57	54	66.8	2.90	10.30	0.54	0.17	0.21	0.249	1.51
				F	49		74.5	2.50	9.10	0.52	0.14	0.22	0.246	1.48

<sup>1</sup> ppm mercury (as methylmercury) in drinking water.

<sup>2</sup> A breeding attempt was scored a failure if a sperm plug was noted but the rat did not give birth.

<sup>3</sup> Excludes breeding failures.

<sup>4</sup> Based on one male and one female (where possible) from 8 control, 7 low-dose, and 8 high-dose litters.

<sup>5</sup> Variability in blood and brain concentrations are visible in figures presented elsewhere. The standard error in brain weight range from 2% to 10% of the mean.

control over exposure since the dose is determined jointly by the concentration in the medium and the amount consumed. Accordingly, daily and cumulative exposure are influenced not only by the concentration of mercury in water, which can be controlled experimentally, but also by individual differences in fluid consumption and differences in fluid intake through the course of pregnancy and lactation.

The present study was aimed at quantifying blood and brain concentrations of mercury, and their ratios, in neonatal and weanling rats after chronic exposure to methylmercury in drinking water. The information gained can contribute to better estimates of brain levels of mercury after this exposure protocol. By comparing blood and brain levels of mercury at parturition and weaning, it will be possible to determine the extent of lactational exposure in rats. Individual differences in fluid intake and mercury levels at both birth and weaning were all examined in order to identify how much variability in these factors contribute to mercury in blood and brain.

## MATERIALS AND METHODS

**Subjects.** The subjects were 88 Long-Evans rat pups, some of which were exposed to methylmercury chloride ( $\text{CH}_3\text{HgCl}$ , Alfa Chemicals) as described below. They were the offspring of unrelated male and nulliparous females purchased from Harlan Sprague-Dawley.

**Exposure.** Exposure of females to methylmercuric chloride began 28 or 49 days prior to mating. Two exposure groups, comprising 10 females each, received 0.5 or 6.4  $\mu\text{g/ml}$  (hereafter the 6-ppm or high-dose group) of mercury (as methylmercuric chloride) in tap water, a solution that served as their sole source of drinking water. Mercury concentrations were confirmed by atomic absorption spectrophotometry. Within each group, 5 rats began exposure 28 days prior to mating and the remainder began 49 days prior to mating. Ten control females were treated in the same manner as exposed rats, except that mercury was not added to their drinking water.

Measurement of daily water consumption began 2 weeks before exposure and continued until the pups were 16 days old, when they could drink from the water spout. Two water bottles were kept on empty cages and weighed daily

to determine fluid loss through spillage or leakage. Exposure ended when the pups were able to reach the water bottles themselves, at 16 days-of-age; the only source of mercury was through the mother during gestation or lactation.

**Breeding.** Rats were bred at about 4.5 months of age. The water bottle was removed from each female's home cage and one male was placed in the cage with her overnight. Removing the water bottle prevented exposure to the male and prevented the female from consuming unadulterated water during the active cycle (night) and thereby reducing exposure. The same pairings were arranged nightly until a sperm plug was observed. The date of conception was entered as the morning that the sperm plug was observed. Table 1 shows reproductive information.

**Offspring.** Mating resulted in 25 (9 controls, 7 low-dose, and 9 high-dose) litters of 295 pups, some of which were paternally related. Twenty-four litters produced pups for this study; one high-dose litter comprised only 2 pups and was not represented here. All pups were tattooed on PN 1 and surviving pups were kept with their biological mothers until weaning at PN 21. All surviving pups were weighed daily until weaning and inspected for the occurrence of developmental landmarks: surface righting, pinna separation, incisor eruption, eye opening, and air righting.

One male and one female were chosen at birth from each of 7 control, 7 low-dose, and 9 high-dose litters. One male and one female were chosen at weaning from each of 8 control, 4 low-dose, and 5 high-dose litters. Complete representation (one male, one female at both birth and weaning) was available for 7 control, 4 low-dose, and 5 high-dose litters. For other litters (2 control, 3 low-dose, and 3 high-dose) only partial representation of the conditions was possible. The maximum litter size before weaning was 12 (3 control, 1 low-dose, 1 high-dose litters). Pups not used in the present study were used in behavioral studies.

**Tissue analysis.** Rats were decapitated on post-natal day 1 (PN-1: 23 m, 23 f) or 21 (PN-21: 19 m, 23 f). The 21-day-old rats were killed with carbon dioxide gas prior to decapitation. Blood was collected from the carotid arteries of the neck with pipettes, added to a scintillation vial containing 2 ml of 30-unit/ml heparin solution, and weighed. Care was taken to avoid collecting stomach contents. Whole brains (including cerebellum) were immediately removed, placed in a dry scintillation vial, and weighed. The frozen blood and brains were stored for up to 40 days at  $-28^\circ\text{C}$  until they were shipped on dry ice to the analytic laboratory at the Department of Environmental Medicine, University of Rochester. Total mercury in these samples and in samples of the drinking water was determined using atomic absorption spectrophotometry (Cernichiari *et al.*, 1995; Magos and Clarkson, 1972).

**Statistical analyses.** To identify differences among sex, age, and exposure on blood and brain concentrations and brain: blood ratios, 3-way repeated-measures analyses of variance were conducted on the dependent measures ( $\log_{10}$ [brain Hg],  $\log_{10}$ [blood Hg], brain: blood ratio,  $\log_{10}$ (brain weight)). The log transform served to stabilize variance across groups. Any mercury present in control pups was below the limits of detection, so only data from low- and high-dose pups were used in analyses involving mercury concentrations.

For these analyses, the litter was the statistical unit. Dose was entered as a between-groups effect but sex and age were analyzed as repeated measures, which is justified even though the data came from different pups. The litter is considered a statistical unit, so pups within a litter can be viewed as a repeated measure grouped according to age and sex.

This approach permits a more powerful comparison of pups against litter-mates, but can reduce the degrees of freedom if a representative pup is not available from each condition. The repeated-measures algorithm (SPSS) drops the entire litter from the analysis, lowering the degrees of freedom in the error term and potentially reducing power. Accordingly, the analyses were re-conducted with sex as a repeated measure separately at PN 1 and PN 21, with age as a repeated measure separately for males and females, and as a between-groups 3-way ANOVA (dose by age by sex, with each litter was represented multiple times in the analysis). To assess the validity of the between-groups ANOVA, the effect of dam-within-dose (a maternal effect) was examined. In many cases there was a significant effect of dam so the repeated measures approach was appropriate. Regression analyses relating maternal consumption to tissue levels, described elsewhere, show that one cause of an effect of dam is that exposure within dose groups varied according to how much fluid was consumed by individual dams. Nevertheless, the conclusions from the between-groups analyses were not substantially different from the repeated measures analysis with the full design, and are not otherwise described.

The role of duration-of-exposure prior to breeding was examined using *t*-tests. There was no effect on blood or brain concentrations of mercury in the offspring. Therefore, this variable was not used as a factor in the analysis of tissue levels. Litter size was examined using analysis of variance and Chi square (number of litters < 8). Visual inspection revealed no differences across groups in the day at which the developmental landmarks appeared, so these data were not examined any further.

The relationship between maternal exposure and brain levels was examined using linear and nonlinear regression. The details are described in the relevant part of the Results section.

## RESULTS

Table 1 summarizes exposure, breeding success, average blood and brain levels, and brain weight at PN 1 and PN 21. Breeding failures included 1 in the control group, 3 in the 0.5- $\mu\text{g}/\text{g}$  and 1 in the 6- $\mu\text{g}/\text{g}$  exposure groups. The single failure in the control group occurred with a male that had sired a litter of 14 pups with another dam. The 3 failures in the 0.5- $\mu\text{g}/\text{g}$  groups had been mated with male rats that had sired offspring with other females so they could not be attributed to male reproductive problems. All 3 failures occurred in females exposed for 49 days prior to mating. The single failure in the 6- $\mu\text{g}/\text{g}$  group occurred with a female exposed for 49 days prior to mating, but the male had sired a litter of only two pups with another female so this plausibly could be related to the male. A Chi square test applied to these data yields  $\chi^2 = 9.12$  (4 df),  $p = 0.058$ , but this should be interpreted cautiously, because the small sample size makes data on prevalence unstable.

Control rats gave birth to litters ranging in size from 10 to 14 pups. In the 0.5-ppm group, five litters were between 12 and 14

pups, one litter had 9 pups, and one had 5 pups. The litters with 9 and 5 pups came from a dam exposed for 28 and 49 days, respectively, prior to mating. In the 6-ppm group, 7 litters were between 10 and 15 pups, and one had 2 pups. The litter with 2 pups came from a dam exposed for 28 days prior to mating, but the male had experienced a breeding failure with another dam. There were no statistically significant effects of exposure on litter size or weight at birth or at weaning. Pups averaged 6.6 g at birth and 46.2 g at weaning.

### Brain Levels at Birth and Weaning

Figure 1 shows levels of mercury in blood and brain in individual pups and brain: blood ratios, all taken within 24 h of birth and at weaning (post-natal day 21). The concentration of mercury in blood increased with drinking water concentration ( $F_{1,7} = 6379$ ,  $p < 0.001$ ) and was higher in the neonates than in the weanlings ( $F_{1,7} = 3614$ ,  $p < 0.001$ ). No interactions (day by dose, sex by dose) were significant on this measure; all *p*'s were greater than 0.10. Figure 1 and Table 1 show that blood levels increased about 20-fold as water concentration increased from 0.5 to 6 ppm. Blood concentrations decreased about 20- to 30-fold between birth and weaning.

The concentration of mercury in the brain increased with drinking water concentration ( $F_{1,7} = 6379$ ,  $p < 0.001$ ) and decreased with age ( $F_{1,7} = 3262$ ,  $p < 0.001$ ). Figure 1 and Table 1 show that mercury brain concentrations in the 6-ppm group were about 20-fold higher than in the 0.5 ppm group. There was a significant interaction between dose and age in brain concentrations ( $F_{1,7} = 23$ ,  $p < 0.002$ ). Brain concentrations at weaning were about 10-fold lower than at birth in the 0.5-ppm group but 20-fold lower in the 6-ppm group.

A marginal interaction between sex and dose ( $F_{1,11} = 4.8$ ,  $p = 0.063$ ) was detected in methylmercury concentration in the brain when a repeated-measures ANOVA was conducted by comparing a male against a female litter-mate using the full model (sex and age both entered as a repeated measure). When this interaction was re-examined using repeated measures on PN-1 and PN-21 separately, the effect appeared at birth ( $F_{1,13} = 8.1$ ,  $p = 0.014$ ), but not at weaning. In the rats exposed to 0.5 ppm, concentrations in the female brains were higher than those found in the male litter-mate, but at the higher-dose concentrations, brain mercury concentrations were higher in the males than in female litter-mates. The difference was about 10-15% in the low-dose group and smaller in the high-dose group. In one litter a 2-fold difference appeared. The magnitude of the effect was small compared to other effects reported here. When the ANOVA was conducted using sex as a between-group variable (not reported here), that is, when a male was not compared with its sister but instead all males were compared, as a group, with all females, there was neither a main effect of sex nor an interaction between sex and dose (*p*'s greater than 0.1). This explains why the interaction is not visible in Figure 1, where brain levels are presented only as a

## Brain and Blood Mercury Levels

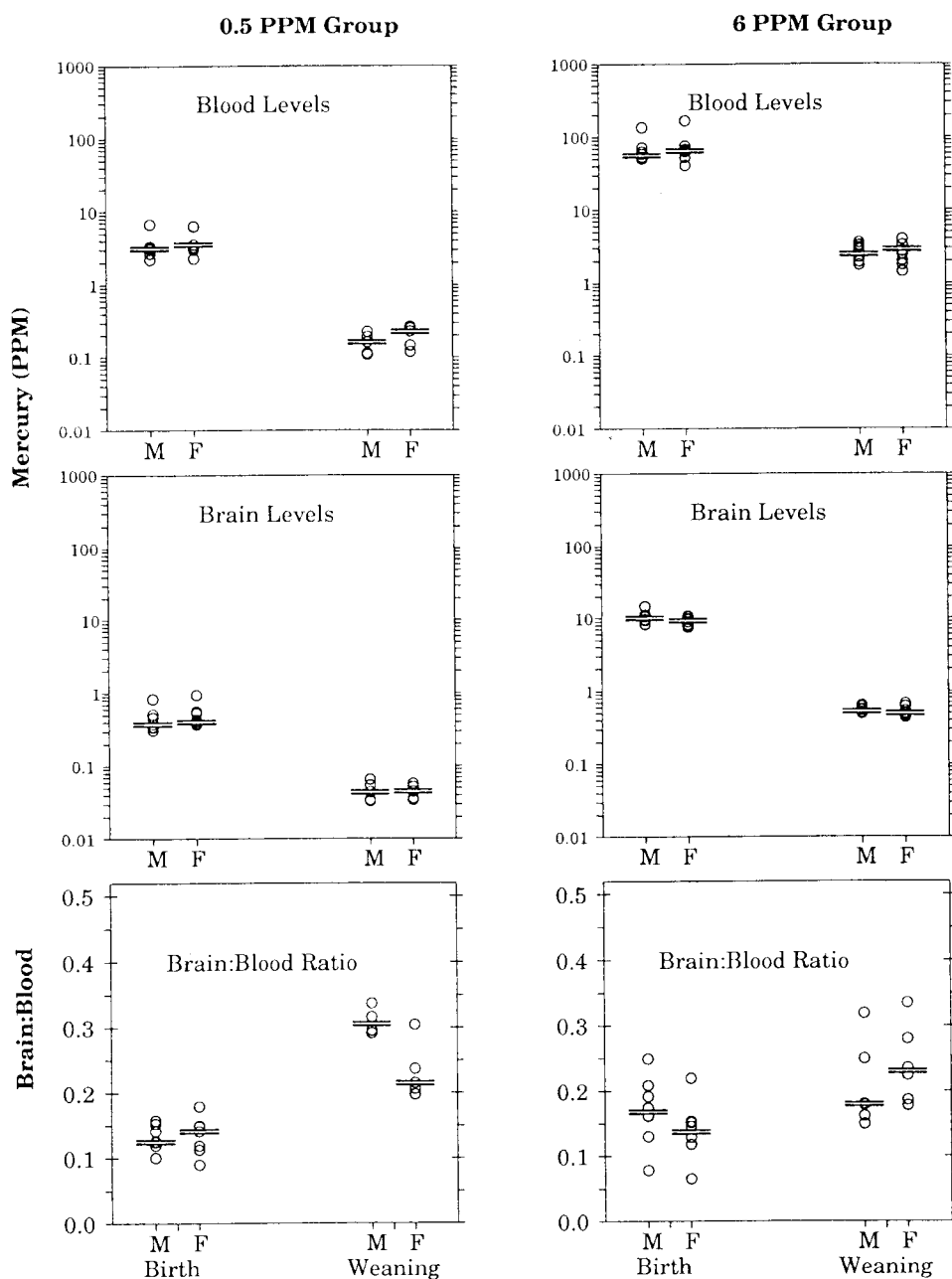


FIG. 1. Concentration of mercury in blood (top row) and brain (middle) and their ratio (bottom) for the 0.5-ppm (left) and 6-ppm (right) groups. Data are shown separately for males and females at birth and weaning. Horizontal lines show group medians. Note the logarithmically scaled axes. Mercury concentrations are expressed as parts per million.

function of group, but can be seen in Figure 3, discussed below, where information about litters is detectable.

The ratio between brain and blood concentration of mercury was higher in the weanling rats than in the neonates ( $F_{1,7} = 63$ ,  $p < 0.001$ ), but how much higher it was depended upon the exposure group; the interaction between dose and brain: blood ratio was statistically significant ( $F_{1,7} = 7.6$ ,  $p = 0.029$ ). Between birth and weaning, the brain: blood ratio increased about 2-fold, from 0.13 to 0.26 (mean of both sexes) in the 0.5-ppm group and from about 0.15 to 0.22 in the 6-ppm group.

No other interactions were significant involving brain: blood ratio as the dependent measure (all  $p$ 's  $> 0.10$ ). Inspection of Figure 1 suggests that the males in the low-exposure group had a particular high brain: blood ratio on PN 21, but this three-way interaction could not be assessed unambiguously here. A relationship to litter size was sought, but none was detected.

Brain weight increased between birth and weaning ( $F_{1,14} = 5020$ ,  $p < 0.0001$ ) about 5.5-fold. No main effect of sex or exposure and no interactions among any of the variables on brain weight were identified on this variable. ANOVAs were

### Daily Water and Mercury Consumption

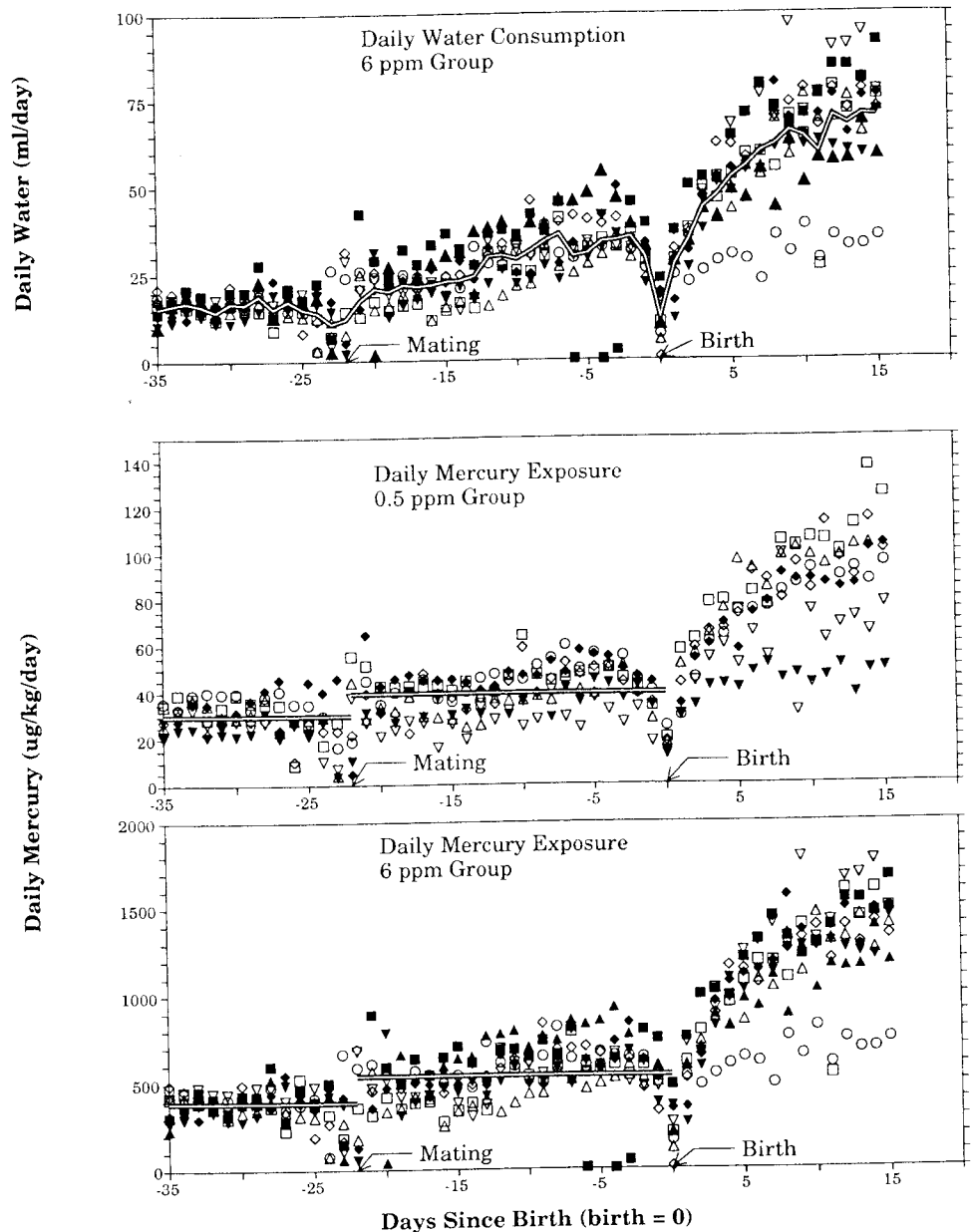


FIG. 2. The top panel shows water consumption before mating, during gestation, and lactation for the rats exposed to 6 ppm of mercury (as methylmercury) in drinking water. Each symbol represents an individual rat. The figures for the control and 0.5-ppm exposure groups were similar. The bottom two panels show calculated mercury exposure ( $\mu\text{g}/\text{kg}/\text{day}$ ) determined by multiplying concentration by daily water consumption and dividing by maternal body weight. The horizontal lines show average group consumption prior to mating and during gestation.

conducted as repeated measures (sex and age as repeated measures, dose as a between-group measure) on the  $\log_{10}$  (brain weight).

#### Individual Differences in Mercury Intake during Gestation

The amount of fluid consumed was examined as being a source of individual variability in brain and blood levels of mercury. The top panel of Figure 2 shows fluid consumption for rats exposed to 6 ppm of methylmercury through gestation and lactation. Consumption in the other groups was similar.

Fluid consumption was not affected by adding mercury to the water in either group; consumption for the 6-ppm group was indistinguishable from that for the control or 0.5 ppm groups (data not shown). Fluid consumption was stable prior to mating, so data from 15 days before mating are shown, and it increased gradually over the course of gestation, from an average of about 15 ml/day prior to mating to about 35 ml/day by delivery. Consumption at mating (-22 days) was low because water bottles were removed when males were introduced in order to avoid exposing them. Consumption declined pre-

cipitously at parturition, although fluid was available, and then continued to increase through lactation. By post-natal day 16, when pups could reach the water bottle and monitoring of fluid consumption had been stopped, the median fluid consumption for rats nursing a full litter had increased to 75–80 ml/day.

Individual variability in fluid consumption increased through gestation and lactation. Litter size was related to these individual differences: those rats consuming little fluid, e.g., those depicted in inverted triangles in the middle panel or open circles in the bottom panel of Figure 2, also had small litters. Pre-mating fluid consumption was a weak predictor, at best, of consumption through gestation, but gestational fluid consumption was a fair predictor of litter size.

Mercury exposure through gestation was estimated by multiplying daily fluid consumption by concentration (0.5 ppm or 6.4 ppm) and, to accommodate the individual variability in fluid consumption, dividing by maternal body weight; these data are shown in the bottom two panels of Figure 2. Normalizing by body weight shows that, despite a large increase in fluid consumption during gestation, there was only a small increase in the level of exposure as measured by concentration. The reason is that fluid intake was related to body weight and to litter size, so increased fluid intake did not necessarily expose the fetus to higher concentrations of mercury. Nevertheless, mercury intake was not constant through this study. After successful mating, overall maternal intake and exposure increased over pre-mating levels, as indicated by horizontal lines showing average intake before mating and during gestation. Mercury exposure increased immediately after successful mating. Through gestation, there are slight deviations from the overall gestational average such that exposure was slightly lower early and slightly elevated later in gestation. An average lactational exposure is not shown because it would be unrepresentative and because milk appeared to be an insignificant route of exposure (discussed below).

To relate blood and brain mercury concentration at birth and weaning to exposure through maternal fluid consumption, mercury concentrations at birth were regressed against the estimate of cumulative exposure through drinking water. Cumulative exposure was estimated by accumulating the daily estimates of mercury consumption (not normalized for maternal weight) from mating to parturition, and then dividing the result by maternal body weight just prior to parturition. This permits an estimate of mercury exposure in units of concentration ( $\mu\text{g}/\text{g}$ ). The analysis was accomplished first using linear regression. The intercept in this analysis was indistinguishable from zero, so the analysis was repeated with the intercept forced to be zero. The resulting equations were  $\text{Hg}_{\text{brain}} = 1.01(\text{cum exposure})$  and  $\text{Hg}_{\text{blood}} = 7.5(\text{cum exposure})$ ; the standard error of the slope estimates were, respectively, 0.03 and 0.6. Inspection of the residuals, however, revealed systematic deviations for the lower exposure group such that mercury concentrations in both blood and brain would be overestimated by 150 to 200%.

A regression was subsequently conducted on the logarithm

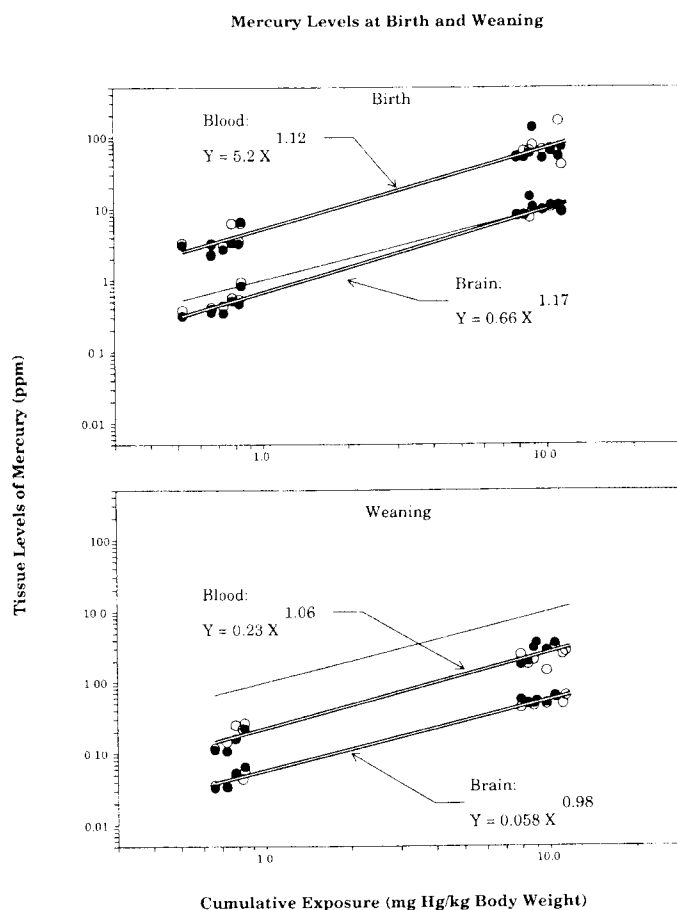


FIG. 3. Concentration of blood and brain mercury at birth (top) and weaning (bottom) expressed as a function of cumulative mercury exposure (also expressed as a concentration) through gestation. To express exposure as a concentration, cumulative exposure during gestation was divided by maternal body weight just prior to parturition. Note the logarithmically-scaled axes. The double lines shows the relationships indicated by equations on the figure. A single, thin line shows the linear relationship  $Y = X$  for reference. A power function was required to describe this relationship at birth, but the relationship at weaning was linear.

(base 10) of brain or blood levels at birth vs. log of exposure, which corresponds to the scaling of the axes in Figure 3. The linear regression, then, took the form  $\log Y = \log a + b \log X$ , where  $X$  is the cumulative exposure in ppm and  $\log a$  and  $b$  are, respectively, the intercept and slope of the linear regression conducted on the log-transformed data. When converted back to the original units, this yields the equation  $Y = 10^a X^b$ , a power function. This analysis applied to brain mercury resulted in the equation (converted back to units of concentration)  $\text{Hg}_{\text{brain}} = 10^{-0.18} * (\text{cum exposure})^{1.17} = 0.66 * (\text{cum exposure})^{1.17}$ , an equation that accounted for 98% of the variability. The standard errors of the intercept ( $\log a = -0.18$ ) and slope ( $b = 1.17$ ) terms were  $\pm 0.09$  and  $\pm 0.03$  respectively. When applied to blood mercury, equation was  $\text{Hg}_{\text{blood}} = 10^{0.72} (\text{cum exposure})^{1.12} = 5.2 (\text{cum exposure})^{1.12}$ . The standard errors of the intercept (0.72) and slope (1.12) were, respectively, 0.034

and 0.047, and the function accounted for 95% of the variance. Thus, the intercepts for blood and brain differed from one another by about 8-fold, corresponding to the brain: blood ratios reported in Figure 1. The slope terms, which appear as the power to which cumulative exposure is raised, were indistinguishable from one another.

A similar regression analysis was conducted on the blood and brain concentrations taken at weaning. In this case, the slope terms were indistinguishable from 1.0, indicating a linear relationship between cumulative exposure during gestation and blood and brain concentrations at weaning. The ratio of the intercepts was 0.25, again corresponding to the brain to blood ratios reported in Figure 1. The actual equations were  $Hg_{\text{blood}} = 0.023 (\text{cum exposure})^{1.06}$ , where the standard error of the intercept ( $\log a = -0.64$ ) and slope (1.07) were 0.035 and 0.05 respectively, and  $Hg_{\text{brain}} = .058 (\text{cum exposure})^{0.98}$ , where the standard error of the intercept ( $\log a = -1.24$ ) and slope (0.98) were 0.019 and 0.025 respectively.

There was no relationship between brain-to-blood ratios and cumulative exposure. At birth and weaning the slopes describing this relationship were 0.0000058 and 0.000014, respectively, and were not significantly different from 0. This analysis yielded intercepts of 0.13 and 0.27 at birth and weaning, respectively. This result is consistent with the analysis reported above.

## DISCUSSION

The present study aimed to link *in-utero* methylmercury exposure via maternal consumption to blood and brain concentrations of methylmercury at 2 critical stages of development. The role of two contributors to dose, methylmercury concentration in drinking water and individual variability in daily water consumption during gestation, was also examined. The results indicate that: (1) the ratio of brain to blood mercury, an index used to estimate brain levels from measurements of blood levels, is higher in the developing rat when exposure is chronic and continuous than has been reported when exposure is via multiple injections to adults; (2) during exposure, both blood and brain concentrations are not linearly related to exposure but a linear relationship appears after exposure terminates; and (3) with rats exposure through lactation is minimal. These conclusions will be discussed in order.

### *Brain:Blood Ratios of Mercury*

Estimates of mercury levels in the rat brain have been made by using the average fetal-brain:fetal-blood ratio of 0.06, a value obtained after repeated administration of methylmercury to adult rats (Magos and Butler, 1976). The ratio for the neonatal or weanling rat obtained in the present report, seen under conditions of chronic exposure to the dam, is about 0.13–0.17 at birth and 0.21–0.29 at weaning, so applying the ratio of 0.06 underestimates actual brain mercury at both ages.

The ratio was larger in the weanling rats, an observation that may correspond to a report of an increased ratio in brain: blood ratio in nonhuman primates after exposure has terminated (Vahter *et al.*, 1994). There was also an interaction between age and exposure. The increase in brain: blood ratio between birth and weaning was smaller for the higher level of exposure, reflecting a dose-related difference in the change in blood and brain mercury between birth and weaning. The brain: blood ratio obtained at birth corresponds to a value of 0.12 to 0.15 that can be calculated from a study examining blood and brain mercury in the rat fetus after a single administration the day before tissues were taken (Wannag, 1976). A ratio of 0.06 has been used to estimate brain concentrations of mercury from blood levels at which motor function was impaired in rodents (Elsner *et al.*, 1988). Since that study used a dosing regimen similar to the one used here, any estimates of brain concentrations of methylmercury should be revised upward.

### *Relationship between Blood and Brain Mercury and Fluid Consumption*

The duration of mercury exposure prior to mating had no discernable effect on mercury levels in the brain or blood of offspring. If it is assumed that the half-life of elimination in adult female rats is 14 days (Magos and Butler, 1976), then the rats exposed for 28 days before mating would have mercury levels of 75% of steady-state at mating and 91% at birth (assuming that pregnancy does not change the kinetics). The rats exposed for 49 days prior to mating would have levels of 91% and 97% of steady-state at mating and birth respectively. No effect of duration of exposure was found on mercury levels in the pups at either birth or weaning. It appears that 28 days, about 2 half-lives, provides time for mercury levels to stabilize. A statistically-significant effect of exposure duration on breeding success was identified, but this conclusion must be treated with caution because of the small sample relative to that required for firm estimates of dichotomous data and the lack of a clear dose-effect relationship.

If mercury exposure had been estimated based upon drinking patterns prior to mating, then the daily dose would have been underestimated 2-fold or more because of the sharp increase in consumption beginning after conception. Fluid consumption increased throughout gestation, so no stable level, and therefore no single average, could describe accurate exposure at any point of gestation. When body weight was considered, daily exposure (expressed as mg/kg or parts-per-million (ppm) as in Fig. 2) was less variable than raw fluid consumption but nonetheless still increased some through gestation. Fluid consumption also varied across individual subjects. Both body weight and fluid consumption were related to litter size, but even when this was considered, there was about a 2-fold range in exposure across individuals within a single dose group.

Brain concentrations of mercury at birth increased with the concentration of mercury in drinking water but this increase

was not quite linear within or across exposure groups. A 12-fold increase in mercury concentration in drinking water, from 0.5 to 6.4 ppm, resulted in a 20-fold increase in average brain concentration of mercury at birth. The nonlinearity between exposure and mercury concentrations at birth was confirmed by regression analyses of consumption, using all animals.

Regression analyses to relate brain and blood mercury to consumption were conducted after expressing exposure and tissue concentration in similar units, parts per million. Cumulative exposure throughout gestation was used, because mercury readily passes the placenta and enters fetal circulation (Berlin and Ulberg, 1963; Eccles and Annau, 1987; Wannag, 1976), where it may accumulate, since mercury leaves the fetal circulation more slowly than it enters (Reynolds and Pitkin, 1975). The blood-brain barrier is not established prior to birth, so mercury in fetal circulation would readily appear throughout brain development. Dividing cumulative exposure by body weight at parturition permits a linear regression to be conducted in consistent units, since the concentration of mercury in the neonatal brain can be related to exposure in units of concentration. Normalization by body weight also reduces the inter-subject variability in fluid consumption since maternal weight was influenced by litter size. Dams that gave birth to large litters consumed more mercury-containing water than those did that had exceptionally small litters, but they also gained more weight. An exposure estimate based upon raw mercury consumed without accounting for body weight increases would overestimate exposure to members of large litters and underestimate exposure to members of small litters.

Although a simple linear relationship, brain mercury in ppm = cumulative consumption in ppm (the obtained regression was  $Hg_{\text{brain}} = 1.01(\text{cum exposure})$ ) provided a good estimate and accounted for 94% of the variability, inspection of the scatter plot indicated that such an approach consistently overestimated brain concentrations obtained from low levels of exposure. A better description was provided by a power function relationship,  $Hg_{\text{brain}} = 0.66(\text{cum exposure})^{1.17}$ , which accounted for 98% of the variability. A power function relationship with a smaller exponent and different intercept described the relationship between exposure  $Hg_{\text{blood}}$  at birth. Inspection of Figure 3 reveals that the regression line accounts for trends both between and within exposure groups.

To determine the replicability of a power-function relationship between exposure and blood or brain levels of mercury, data were examined from 2 studies in a broad range of exposures, in which adequate time was given for maternal levels to stabilize. Suter and Schon (1986) exposed pregnant rats to 1.5, 5, and 15 mg/l methylmercury, beginning 13 days before mating, and reported that blood levels in offspring on the day of birth were, respectively, 10, 47, and 127 ppm; a regression applied to these means yields an exponent of  $1.11 \pm 0.1$ , consistent with our exponent of 1.12. Since the intercept is a scaling parameter, its value is not relevant here. Brain levels

were not reported in that study. Rice *et al.*, (1989) exposed female monkeys (*Macaca fascicularis*) to 10, 25, or 50  $\mu\text{g}/\text{kg}/\text{day}$  of methylmercury and bred them when mercury levels reached steady-state. The relationship between blood levels at birth (from their Table 1) and exposure levels can be described as a power-function relationship, with an exponent of  $1.16 \pm 0.13$ , again, consistent with our slope for brain levels of 1.17. Berlin *et al.* (1975) also reported that blood and brain levels in squirrel monkeys exposed to methylmercury during gestation increased more rapidly than cumulative exposure but a regression analysis could not be performed on their data.

The form of the relationships between exposure and blood or brain levels of mercury at birth and weaning carries implications important to the development of physiologically based pharmacokinetic models of mercury levels, and to extrapolation to low level exposures if the forms can be generalized (Gray, 1995; Farris *et al.*, 1993). The multiplicative form indicates that with zero exposure there are zero levels of mercury in the brain, rather than a residual amount. The multiplicative constant, 0.66 ( $= 10^{-0.18}$ ) for brain mercury, is related to the units of measure. The "origin" (0,0) in log-log space converts back to linear coordinates of  $(X^0, Y^0) = (1,1)$ . The "intercept" of  $10^{-0.18} = 0.66$  describes brain concentrations at an exposure level of 1.0 ppm.

The greater-than-one exponent indicates that brain levels increase faster than dietary levels. If this nonlinearity also applies to lower levels of exposure, then its generalization also suggests that brain levels decrease faster than dietary levels. Applying the power function to extrapolation from experiments using high-exposure levels to lower exposures will yield an estimate of brain mercury lower than what would obtain from a linear estimate. Doing this would carry with it the assumption that the mechanisms by which mercury enters and leaves the brain apply across the range of extrapolation, an assumption that may become untenable as characterizations of low-level exposures become more comprehensive. The relationship between brain and blood mercury is described as a power function because of its utility in describing nonlinearities and because such a form corresponds to the log-log scaling on the axes in the relevant figures. The true functional form may be different, but still nonlinear.

The reason for the nonlinearity between exposure and tissue levels cannot be ascertained at present, but possibilities can be raised that can be tested quantitatively (see also Berlin *et al.*, 1975). One set of explanations flows from the binding of mercury in red blood cells or increased plasma volume in maternal and fetal circulation. The transport of methylmercury across the placenta and other barriers is closely related to mercury concentration in plasma (Greener and Kochen, 1983; Kajiwara *et al.*, 1996; Skerfving, 1988). However, in rats only about 5% of mercury in blood is found in this compartment under normal conditions (Berlin *et al.*, 1975; Clarkson *et al.*, 1988). With a 12-fold increase in mercury intake such as produced here, the number of available binding sites in blood

might fall enough to leave a relatively larger amount of unbound mercury available for transport across the placenta at the higher concentrations. The binding capacity of adult rat blood is probably high enough, under normal conditions, to accommodate most of the elevated mercury concentrations experienced in this experiment (White and Rothstein, 1973), but pregnancy could alter this.

Changes in body water or plasma content of blood could also alter the equilibrium dynamics of mercury in blood. Both absolute and relative plasma volume increase in maternal circulation during pregnancy, sometimes enough to produce anemia, and this too could raise the amount of methylmercury available to fetal circulation (Council, 1989; Mattison *et al.*, 1991; Young *et al.*, 1997). On the fetal side, the percent of body water is relatively high early in development (Council, 1989; Kleinman, 1975). Methylmercury accumulates preferentially to body water, (Vahter *et al.*, 1994) and biotransformation of methylmercury may not take place in the fetus (Wanng, 1976). The high level of body water could shift the equilibrium toward higher unbound levels. In sum, diminished or looser binding, or elevated plasma or body water could result in more mercury being available to the fetal circulation, and therefore to the fetal brain in a fashion that is not linearly related to dose during exposure.

The power-function between exposure and blood and brain levels disappeared by weaning, and was replaced by a linear relationship with a slope indistinguishable from 1.0. This observation lends some credibility to the speculation that the power-function relationship between cumulative exposure and brain and blood levels at birth is related to the dynamics of the partitioning of bound and unbound mercury in the blood during exposure. While there was persistent exposure prenatally, there appeared to be little post-natal exposure (see below), so the mercury would have an opportunity to equilibrate and the number of available binding sites in blood would be less likely to be a consideration after equilibration. A new equilibrium linearly related to exposure could be achieved within a few days (Berlin *et al.*, 1975).

#### *Decline in Mercury between Birth and Weaning*

Brain concentrations of methylmercury fell 10- to 20-fold between birth and weaning, despite continued maternal consumption throughout most of this period. During this same period, brain weight increased only about 5.5-fold. The fact that brain concentration decreased faster than brain growth suggests that total levels of mercury in the brain were lower than they were at birth, i.e., there was some loss of mercury from the brain and minimal replenishment from milk. The decreased concentration in brain and blood mercury at weaning, as compared with birth, reported here (see also Elsner *et al.*, 1988; Suter and Schon, 1986 for similar results) is consistent with reports that mercury levels in rat milk are considerably lower than in blood (Oskarsson *et al.*,

1995; Sundburg *et al.*, 1991) and is in a form, mercuric mercury, that does not readily enter the brain (Bakir *et al.*, 1973; Skerfving, 1988, 1991). The elimination of mercury from routes, such as pelt or fur, that can accumulate mercury and which grow rapidly during this stage, may also have contributed to this decline in brain mercury levels (Magos, 1987). Maternal drinking water may not be an appropriate vehicle to use when studying methylmercury's effects on rat neural development between birth and weaning, a period during which cerebellar, hippocampal, and cortical structures and neuronal interconnections are developing in the rat (Bayer *et al.*, 1993).

Another interpretation of the decline in brain and blood mercury levels at weaning might be that exposure terminated on PN 16, while brain levels were determined on PN 21, but this interpretation is unlikely to explain the data. Dams were not exposed to mercury from days 16 to 21 of lactation because the pups could reach the water bottles at this stage and we wanted to avoid direct exposure to the pups. If mercury levels declined with the half-time of 14 days, as reported in adults, then levels at weaning would be only about 78% of those at 16 days, and the half-time of elimination of mercury is longer in the neonatal rat than in the adult, because hepatic clearance is slower in the neonate (Ballatori and Clarkson, 1982). A 10- to 20-fold loss of mercury between days 16 and 21 of life cannot be attributed to clearance in these young rats unless the half-life of elimination is much shorter.

Brain to blood ratios were higher at weaning than they were at parturition. Several factors could contribute to this. The brain grew at a slower relative rate than the whole pup between birth and weaning; brain weight increased 5.5 fold but pup weight increased about 7-fold during this period (see also Goldey *et al.*, 1994). This growth apparently was not accompanied by a significant degree of continued exposure to the pups. Another potential contributor, consistent with observations in adult primates, is that the brain converts methylmercury to a form that does not exit the brain as readily as methylmercury enters it (Aschner and Aschner, 1990; Vahter *et al.*, 1994, 1995). This explanation requires that the metabolic processes responsible for this conversion be in place prior to weaning. Other possible mechanisms for this increasing ratio, such as differential binding to tissues, differentiation of brain tissue during this period of rapid growth, or slower elimination of methylmercury from the brain than from blood cannot be ruled out with the present data.

A sex by dose interaction was observed in the concentration of methylmercury in the brain, but interpretation of this result should remain guarded at present. Female offspring exposed to 0.5 ppm of mercury during gestation had about 10–15% more mercury in their brain than did their male littermates. At the higher concentration, males showed a statistically significant increase in brain mercury over their female littermates, but the magnitude of the effect was even smaller than the effect noted at lower exposure

levels. The direction of the effect was different at the 2 doses, so the interaction may have been spurious. Nevertheless, increases in mercury concentrations in the female fetus have been noted at low levels of exposure (Inouye *et al.*, 1986). A complex interaction between sex and behavior of nonhuman primates under fixed-interval schedules of reinforcement has also been reported (Gilbert *et al.*, 1996). In adult animals, sex differences have been attributed to hepatic involvement in the uptake and distribution of mercury (Nielsen *et al.*, 1994) and to hormonal influences (Tanaka *et al.*, 1992).

### Summary

In summary, under conditions of chronic oral exposure, brain: blood ratios of methylmercury are much higher than those reported after less chronic exposure regimens. With oral exposure through maternal drinking water, mercury exposure is not constant through development: it increases somewhat through gestation and declines considerably postnatally. Blood and brain concentrations were nonlinearly related to cumulative mercury exposure at birth, although the reason for this cannot be determined at present.

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