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Lactational exposure to mercury in experimental models

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The extent to which there is methylmercury (MeHg) exposure via breast milk is an important issue for at least two sets of reasons. First, the degree to which the form and concentration of mercury in the milk in the animal model resembles that in the human bears directly on our ability to model human exposures in the laboratory. Second, laboratory studies are designed to model human nervous system development, but the models are imperfect since rodents are born at an earlier stage of nervous system development than are humans (Rice and Barone, 2000). In both rodents and humans, formation of much of the hippocampus, cortex, and ventral striatum occurs during gestation (Bayer et al., 1993). Golgi, stellate, and granule cells in the cerebellum and granule cells in the dentate gyrus, however, develop during the first 2 weeks of post-natal life in the rodent but during the last trimester of human fetal development. Rodents still provide excellent models of human MeHg neurotoxicity, as our review sought to show (Newland et al., 2008), but the laboratory evidence must be understood in the context of species differences in CNS development. In rodent models the first two trimesters of fetal development are represented adequately but the extent to which the last trimester of human development (about birth to post-natal day 10 of rodent development) is modeled depends upon the presence of mercury in the nervous system. An important determinant of this is the presence of methylmercury (MeHg) in breast milk.

MeHg in a nursing animal can come from three sources: maternal exposure during gestation, breast milk during nursing, or by direct consumption of food or water containing MeHg. The determinants of exposure include, respectively, the levels of MeHg that cross the placenta, the levels in milk, and the ability of a pup to reach food or water that contains MeHg. If there is no significant exposure via breast milk, then mercury concentrations should begin to fall at birth. This is so even if all of the mercury present at birth remains present at the age that blood or organs are collected, since the animal grows and concentration is influenced by both the amount of a compound and the volume in which it is diluted. If there is direct consumption by the pup then tissue levels should begin to increase at about the age that a pup can begin drinking on its own. The experimental work reviewed below shows that mercury levels do indeed fall at birth and that mercury levels increase if there is an opportunity to consume MeHg directly.

Gestational exposure to MeHg is significant. Determinants of gestational exposure include maternal uptake of MeHg from the gut, its partitioning to different compartments in blood, its transport across the placenta, and its persistence in the fetus. These conditions all conspire to provide substantial fetal exposure. About 90% or more of the MeHg that is consumed in food or water is absorbed from the gut and appears in the blood (Clarkson, 1989; Magos et al., 1984). Once in the blood of a pregnant animal, MeHg crosses the placenta readily to reach the fetal blood supply where it accumulates in the pup to concentrations that often exceed those in maternal tissue (Clarkson, 1989; Magos, 1987, 1997). As a result, the exposed neonate is born with mercury levels matching or exceeding that of maternal levels.

The situation with lactational exposure is more complex, but the general issues surrounding bioavailability still apply. In comparison with blood, breast milk contains relatively low levels of total mercury (organic + inorganic). This has been reported in rats (Sundburg et al., 1991), mice (Manfroi et al., 2004; Sundberg et al., 1999b), hamsters (Nordenhall et al., 1995), guinea pigs (Yoshida et al., 1994), humans (Bjornberg et al., 2005; Sandborgh-Englund et al., 2001; Skerfving, 1988), and killer whales (Endo et al., 2007). The reason is that breast milk derives from plasma. The concentration of organic (e.g., MeHg) and inorganic (e.g., $HgCl_2$) mercury in plasma is key to lactational exposure (Oskarsson et al., 1995; Sundberg et al., 1998, 1999a). The distribution of MeHg between erythrocytes and plasma varies considerably across species, but for all species, MeHg highly prefers erythrocytes. In mouse, human, and rat, the ratio of MeHg in erythrocytes to that in plasma is, respectively, 7:1, 21:1, and 145:1 (Magos, 1987). This means that for these species, the percentage of blood MeHg that is in plasma is 12.5, 4.5, and 0.68%, respectively.

Mercuric mercury, which is usually very low in blood, distributes about evenly between blood and plasma. The net result is that plasma contains a relatively small amount of the total mercury found in blood and much of it is inorganic mercury. In mice, for example, about 45% of plasma mercury is inorganic (Sundberg et al., 1999a,b).

To determine empirically the distribution of MeHg to breast milk, Sundberg and colleagues administered radiolabeled MeHg by oral gavage to lactating dams (Sundburg et al., 1991). Milk was collected 24 and 72 h after dosing and blood was collected at 72 h and the radioactivity in plasma and erythrocytes was analyzed. This radioactivity reflected MeHg content in these compartments, exclusive of a small amount that may have been demethylated to inorganic mercury. The concentration of MeHg in milk was 10% of that seen in plasma and less than 0.2% that seen in erythrocytes. The concentration of MeHg in pups' erythrocytes was about 0.8% of

that in dams' erythrocytes. Accordingly, the MeHg in the brains of pups was only 17% of the low level seen in breast milk, and 0.9% that seen in the brains of the dams after 72 h.

Overall, then, milk contains a fraction of the organic and inorganic mercury found in plasma, and much of this mercury is inorganic mercuric chloride ($HgCl_2$). The form is important because only about 10% of mercuric chloride is absorbed in the adult gut (Chang, 1997; Clarkson, 1989, 2002) and absorption in the infant is also low (Bjornberg et al., 2005). Thus, much of the mercury in breast milk is in a form that is poorly absorbed.

With chronic exposures, even to MeHg, inorganic mercury could still be a factor since about 1% of MeHg is converted to divalent mercury per day (Clarkson, 2002). To determine whether this inorganic mercury can be neurotoxic, Franco et al. (2007) injected 0.5 mg/kg of $HgCl_2$ directly into the peritoneum of a nursing dam, bypassing the low uptake from the gut. This dose did not affect pup weight, locomotor activity, rotorod performance, or markers of oxidative stress in the offspring. A higher dose did affect some of those parameters. The dose of 0.5 mg/kg is many times higher than would be experienced by a nursing animal, so the small amount of $HgCl_2$ received by the pup, whose dam is exposed to MeHg, is unlikely to be neurotoxic.

These theoretical considerations can be validated by examination of mercury levels in the tissue of nursing animals experiencing chronic exposure. In animal colonies, the nursing pups usually remain with the dam until at least 21 days of age, and sometimes longer. At birth the pups are virtually helpless and entirely dependent on the dam for sustenance, but by weaning they are very active and quite capable of drinking or eating if water or food can be reached.

Whether MeHg in weanling mice derives from direct consumption of food or water, rather than lactational exposure, can be examined indirectly by determining blood and brain MeHg in weanling rats under two conditions, one in which maternal exposure continues throughout lactation and a second in which it stops at about the age at which pups can reach the drinking spout. A recent study with mice did this. MeHg concentrations in blood and brains of mice were evaluated on Post Natal Day (PND) 4 and PND 21 (weaning) (Stern et al., 2001) under different exposure regimens. For some dams, the MeHg remained in the drinking water throughout the entire preweaning period and for others it was removed on PND 13. When MeHg was removed at PND 13, brain mercury concentrations declined 8.6–12-fold between PND 4 and weaning and blood concentration declined 9–12-fold. (Body weight increased about 3.8-fold during this same period.) When MeHg remained in water bottles during this same period, however, brain and blood levels declined by only 3.4–6.6-fold and 1.5–2.6-fold, respectively. Mercury levels were higher at weaning when pups could reach the water bottle, during the last week of lactation, than when they could not reach it.

In an earlier study with rats, MeHg was removed from water bottles at PND 16 and weaning occurred on PND 21 (Newland and Reile, 1999). Brain mercury concentrations were 10–20 times lower at weaning than at birth and blood mercury concentrations were 20–30 times lower. Brain weights increased 5.5–6-fold during this period and pup weights increased about 7-fold. It was concluded that the decline in brain and blood MeHg between birth and weaning was not due to elimination between PND 16 and PND 21, which would have had to be very rapid. Instead, it was concluded that the large fall in concentration was due to minimal replenishment of mercury by breast milk, growth of the pup and therefore the volume of distribution, and elimination of mercury during the nursing period. In another study, MeHg in food, rather than in water, remained in the cage containing the mother and offspring until weaning at 30 days of age (Sakamoto et al., 2002). MeHg in the neonatal brain and blood at birth was 50% higher than in the maternal brain but by PN 10 these levels dropped about 5–7-fold. Subsequently, by PN 20, there was an increase in brain and blood levels and an even greater increase by PN 30 when the pups were weaned.

If MeHg exposure via breast milk is minimal then how is it possible that some studies show effects of lactational exposure (Franco et al., 2006; Manfroi et al., 2004) or that there can be large variations across studies in the estimates of mercury content in weanling pups? One possibility is that of direct exposure to near-weanling animals when there is MeHg in the drinking water available in the cage containing the dam and nursing litter. Direct contact is not an unreasonable assumption for a weanling mouse. The difference in body length between a weanling mouse and an adult mouse is only a couple of centimeters. Water bottles are rarely positioned above the floor with sufficient precision that a dam can reach it but a weanling mouse or rat cannot. We have observed a 20-day old rat and mouse not only drinking vigorously from the water supply but also climbing on it, rubbing against it, and playing on it. Since the weanling rodent is only days away from drinking on its own, it seems unreasonable to argue that it cannot reach the bottle at 19 days but can do so at 22 days.

In a recent review (Newland et al., 2008), it was speculated that weanling mice (mistakenly referred to as rats in the review) might have been able to reach the water spout (Manfroi et al., 2004), and this was the basis for a recent letter (Farina, 2008). The speculation was based on an understanding of: (1) the low levels of bioavailable mercury in breast milk, (2) the levels of mercury in blood and brain of the neonatal pup when mercury was explicitly removed from the water bottle, as against levels when mercury was available, and (3) our observations of weanling rodents. It was also based on the relative values in the Manfroi study, which were only 6.5 times greater than in the dams. While this value may sound high, it is closer to the dam:weaning ratio of 8.5 when pups had access to the source than to the ratio of 13.6 when pups did not have access (Stern et al., 2001). (It should be noted that the dams were 75 days old at the time that mercury was collected.) We have no direct knowledge of whether the pups in the Manfroi et al. study actually did drink the water, and this is why the statement was qualified ("the pups may have been able to drink contaminated water"). In light of the very low exposure levels via breast milk and the ambiguity of whether a weanling pup can be exposed directly, it is incumbent on the investigator to design experiments so that there are no ambiguities; mercury should be removed from contaminated water or chow before the late preweaning period, if not at birth.

In that review it was noted that "functionally, MeHg exposure ended at birth." In light of the very low levels of bioavailable mercury in milk, this statement is reasonable for experimental models. This does not mean that milk is an irrelevant route of elimination of MeHg. About 5% of an administered dose is eliminated in breast milk. Nor does it mean that the low levels associated with lactation are irrelevant to risk assessment. For a nursing child, who is not sharing that milk with seven littermates, this might represent sufficient exposure to warn nursing mothers about avoiding fish containing high mercury levels (Bjornberg et al., 2005). That, however, is a risk assessment issue separate from the one at hand, which is whether the small amount of mercury present in the milk of nursing rats and mice substantially increases the body burden of mercury in the offspring. Experiments with reasonable sample sizes simply cannot discriminate between two exposure regimens, one with gestational exposure only and a separate one with additional exposure via breast milk since the latter would increase exposure by only a small fraction. Therefore, functionally, in the studies conducted by Newland and

colleagues (as reviewed in Newland et al., 2008), exposure to the pups ended at birth.

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