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SYMPOSIUM OVERVIEW

Toxicity Assessment of Mercury Vapor from Dental Amalgams[1]

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Introduction (W. Don Galloway and Peter L. Goering)

The toxicity of the various chemical species of mercury and its organic and inorganic compounds is well known. In the past few decades, increased concern about toxicity, often heightened by tragic mass outbreaks of mercury-induced human disease, has led to the virtual elimination of mercurials in drugs and cosmetics, increased monitoring of mercury contamination of seafood, and reduced industrial exposure.

In terms of the number of exposed individuals, the most prevalent remaining source of deliberate mercury exposure is almost certainly dental amalgam. The World Health Organization (WHO, 1991) has estimated that in industrial countries about 3% of the total consumption of mercury is used for dental amalgam, and in the US each dentist uses an average of more than 1 kg of amalgam per year. Recent studies have suggested that dental amalgam releases mercury vapor, which is absorbed by the lung and distributed throughout the body, concentrating in brain, kidney and fetal tissue (for a review, see Clarkson *et al.*, 1988). Implications of this very low-level mercury exposure are unclear. In 1991, a Food and Drug Administration dental advisory panel (FDA, 1991) and an NIH-sponsored conference (NIH, 1991) concluded that no scientific studies definitively link amalgam mercury to human disease states. However, these panels emphasized that more research is required to characterize the risk of amalgam mercury exposure.

Because of the relative dearth of published studies of toxic effects resulting from very low-level mercury vapor exposure and because of the recent public interest in exposure from dental amalgam, the Society of Toxicology conducted a symposium on mercury vapor toxicity, with emphasis on effects of chronic, low-level exposure. Since there has been no recent conference on the effects of low-level exposures to any mercury vapor source, and since inhalation exposure can be expected to produce identical effects, whatever the mercury vapor source, consideration was not limited to dental amalgam exposure alone. The presentations considered both laboratory animal and epidemiologic studies of low-level mercury vapor exposure in general.

The goals of the symposium were to review mercury vapor toxicokinetics and target organ toxicity, to determine what appropriate toxicity endpoints might be, and to consider future research needs. To this end, Dr. Tom Clarkson reviewed the toxicology of inhaled mercury, with special emphasis on human studies. Dr. Fritz Lorscheider summarized work from several laboratories, including his own, involving the effects of mercury vapor on cell function. Dr. Maths Berlin reported reproductive and neurotoxicologic data from exposure of monkeys and rats to mercury vapor in two Swedish laboratories. Dr. Andrew Rowland reviewed the epidemiologic data on mercury vapor reproductive toxicity and reported on his own investigations of fertility among dental assistants exposed to mercury vapor.

In a general discussion following the formal presentations, the speakers indicated that much remains to be learned about

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the effects of chronic, low-level mercury vapor exposure. Since blood and urine levels of mercury are of questionable reliability for predicting toxic effects, new and sensitive biomarkers of exposure and toxicity, such as urinary porphyrin patterns (Bowers *et al.*, 1992), need to be developed. Sensitive populations must be identified and studies initiated to evaluate potential effects of amalgam exposure. Since current information suggests that neurologic, reproductive, developmental, and renal effects may predominate, additional research is required to identify appropriate endpoints, especially functional endpoints, in these areas. Research is also needed to elucidate the mechanisms underlying these observations. A specific potential mechanism which needs to be investigated is the autoimmune-mediated renal injury induced by mercury.

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Overview of Mercury Vapor Toxicity, Toxicokinetics, and Critical Target Organs (Tom Clarkson, University of Rochester, New York)

Living cells have been exposed to mercury, at least in the inorganic form, during most of the period of evolution of life on this planet. Even methyl mercury compounds probably appeared early in evolution as some of the oldest known microorganisms are capable of methylating mercury. Human exposure to inorganic mercury probably became significant only after mercury found applications in human society. The mining of mercury ore was probably the first major cause of exposure. Historical accounts date back 2000 years to the Roman use of the Almaden mines in Spain, but mining and use of mercury must have occurred earlier. Thus cinnabar, the commonest ore of mercury, is credited with use in red ink in China about 2000 BC, and therefore, perhaps, with the invention of bureaucracy! The liquid form of metallic mercury was named quicksilver by Aristotle in about 400 BC (Goldwater, 1972).

Exposures to mercury vapor followed its use in the treatment of syphilis and its numerous applications in the industrial revolution. The use of mercury in amalgam fillings dates back to the first half of the nineteenth century and became widespread in the United States toward the end of the last century (Horsted-Bindslev *et al.*, 1991).

Disposition in the body. Mercury vapor (the monatomic gas, Hg^0, released by metallic, liquid mercury) is probably the most important form of mercury that determines human exposure from dental amalgam fillings (for a review, see Horsted-Bindslev, 1991). It vaporizes from the surface of amalgam fillings, particularly during and shortly after chewing. Thus air, inhaled through the mouth, carries mercury vapor to the lungs where it is efficiently absorbed into the blood stream. In theory, mercury vapor may be absorbed across the mucous membranes of the oral cavity. Also, vapor dissolved in the saliva may undergo oxidation to ionic mercury and be swallowed and absorbed in the GI tract. However, based on our knowledge of the disposition of mercury vapor, it seems most likely that inhalation is the major route of exposure from amalgams.

On entering the blood stream, the dissolved mercury vapor almost instantaneously distributes between plasma and red blood cells (for more detailed reviews, see Magos, 1991; WHO, 1991a). The high lipid solubility of this form of mercury allows it to cross cell membranes without hindrance. On entering the red cell, the atoms of mercury vapor are able to penetrate to the active site of the catalase–hydrogen peroxide complex (catalase compound one) where it is oxidized in a two electron shift to divalent ionic mercury, Hg^{2+}. This reactive species combines with nearby SH groups in hemoglobin and possibly reduced glutathione.

However, despite the rapid intracellular oxidation, some mercury vapor remains dissolved in plasma for a sufficient time to reach the blood–brain barrier, to cross it, and to enter the brain. In this tissue, as in the case of the red cells, mercury vapor is oxidized to Hg^{2+}. Mercury vapor is probably carried by plasma to all cells in the body where it is oxidized. As in the case of the blood–brain barrier, it readily crosses the placenta. Hg^{2+} is believed to be the proximate toxic species responsible for the effects of inhaled mercury vapor.

Hg^{2+} slowly leaves the cells, perhaps in the form of a glutathione complex and is redistributed throughout the body. Eventually the kidney becomes the major site of accumulation of mercury after exposure to mercury vapor.

The urine and feces are the major media of excretion. Although small amounts of dissolved mercury vapor may be found in urine shortly after exposure, compounds of Hg^{2+} are the predominant forms in urine and feces. The half time in the whole body and kidneys after short exposure to nontoxic levels of mercury vapor is about 2 months. The brain has a surprisingly shorter half time, about 21 days, and the blood has two compartments of half times 3 and 30 days.

The level of mercury in urine is commonly used as the biological indicator of the body burden of mercury after occupational exposure to mercury vapor. Urine levels probably

reflect kidney levels where most of the inhaled mercury resides. However, once an individual has attained a steady-state balance, expected after about 1 year of exposure, levels in urine and kidneys should, at least in theory, be proportional to levels in other tissues, including the brain and fetal tissues.

Effects and dose–response relationships. Given the long history of human exposure to mercury vapor, the clinical effects are well known (Suzuki *et al.*, 1991). The classical signs of poisoning in severe cases are gingivitis, intention tremor, and erethism. The latter is a bizarre psychological phenomena typified by the behavior of the "Mad Hatter" in the novel "Alice in Wonderland." The underlying dysfunction in the brain is not understood even to this day.

Recent studies have identified a number of more subtle preclinical effects on the nervous and neuromuscular system, including changes in verbal intelligence and memory, psychomotor disturbances, and abnormal nerve conduction tests (for an extensive review, see WHO, 1991b).

Preclinical changes in kidney function have also been reported such as increased urinary excretion of albumin, of other high-molecular-weight proteins and of certain enzyme markers of kidney damage. These kidney effects are believed to be mediated, at least in part, by the action of mercury on the immune system (Skerfving, 1991). Major differences in susceptibility of the immune system to inorganic mercury have been seen in different strains of the same animal species (Druet, 1991). Thus individual differences in susceptibility in humans is a possibility.

These preclinical effects are most commonly observed above urine levels of 50 μg Hg/g creatinine. Normal or reference urine levels in "nonexposed" adults are in the range of 1 μg Hg/g creatinine. Persons with large numbers of amalgam fillings may be expected to have urine values as high as 4 μg Hg/g creatinine (Magos, 1991).

The margin of safety between urine levels associated with preclinical effects and those associated with amalgam tooth fillings is a question of great current interest. The urine levels quoted above suggest a safety factor of slightly more than 10. However, it is difficult, if not impossible, to identify the lowest threshold for preclinical effects. It is also difficult to predict what fraction of the population might exceed 4 μg Hg/g creatinine due to release of vapor from amalgams. For example, individuals suffering from bruxism may develop much higher tissue levels from amalgams due to prolonged grinding of their teeth.

Conclusions

(1) Mercury vapor, dissolved in plasma and other biological fluids, is the highly mobile species of mercury after inhalation of the vapor. It crosses the blood–brain and placental barriers without hindrance.

(2) On entering the cells, the dissolved mercury vapor is oxidized to divalent inorganic mercury by the hydrogen peroxide–catalase pathway; the latter is believed to be the proximate toxic species.

(3) The half time in the whole body is about 60 days following a brief (about 20 min) exposure to a nontoxic dose of mercury vapor. Two half times are found in the blood compartment: one of 3 days, accounting for about 90% of the mercury in whole blood, and the other of 30 days, accounting for the remainder.

(4) Urine is the most commonly used medium for biological monitoring.

(5) The severe signs and symptoms consist of a triad–erethism, intention tremor, and gingivitis. The more subtle effects found at lower doses include preclinical neurological and cognitive disturbances and increase excretion of albumin in urine.

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Mercury from Amalgam Tooth Fillings: Its Tissue Distribution and Effects on Cell Function

(F. L. Lorscheider, A. O. Summers, P. O. Magner, N. D. Boyd, and M. J. Vimy, University of Calgary Faculty of Medicine, Calgary, Alberta, and *University of Georgia, Department of Microbiology, Athens, Georgia)*

Within the past decade several laboratories, including our own, have established in humans that mercury (Hg) vapor is continuously released from dental "silver" amalgam tooth fillings which contain approximately 50% Hg by weight (Svare *et al.*, 1981; Vimy and Lorscheider, 1985a; Patterson *et al.*, 1985; Aronsson *et al.*, 1989). The release rate of this

Hg is enhanced within 10 min after the occlusal (biting) surfaces of these amalgams are stimulated by chewing (Svare *et al.*, 1981; Vimy and Lorscheider, 1985a,b; Aronsson *et al.*, 1989) or tooth brushing (Patterson *et al.*, 1985). Levels of Hg vapor in intraoral air, both before and after chewing, correlate significantly with the number and type of amalgam fillings (Vimy and Lorscheider, 1985a,b). With continuous chewing over a prolonged period (30 min) the intraoral air Hg vapor levels remain elevated, and then slowly decline to basal levels 90 min after chewing ceases (Vimy and Lorscheider, 1985b). Plasma Hg levels also correlate highly with the number and total surface area of amalgam fillings (Molin *et al.*, 1990).

Based upon *in vivo* electrochemical measurements, it is estimated that a single amalgam filling with a surface area of only 0.4 cm^2 will release 15 μg Hg/day through mechanical wear, evaporation, and dissolution (Gross and Harrison, 1989). This would mean that for an average subject with 8 occlusal amalgam fillings (Vimy and Lorscheider, 1985b), approximately 120 μg Hg would be released daily into the human mouth. This latter amount is consistent with recent fecal excretion estimations of 60 μg Hg/day in subjects with an average number of amalgams (Skare and Engqvist, 1992).

Our original estimate of the absorbed Hg dose from dental amalgams, in a randomly selected group of human subjects, was 20 μg Hg/day (Vimy and Lorscheider, 1985b), and a compartmental model for Hg body burden accumulation from 1 to 10,000 days was proposed (Vimy *et al.*, 1986). Subsequent estimations of amalgam Hg dose by other laboratories have varied from 1.2 to 27 μg Hg/day, with a current consensus of approximately 10 (range 3–17) μg absorbed/day (Vimy and Lorscheider, 1990; WHO, 1991). This is in contrast to a total of only 2.6 μg Hg (inorganic and organic forms) being absorbed daily from food, water, and air (Clarkson *et al.*, 1988a; WHO, 1991). It is now believed that dental amalgams constitute the major source of Hg exposure (see Table 1) in the general population (Clarkson *et al.*, 1988a,b; WHO, 1991). This belief is supported by human autopsy studies demonstrating significantly higher Hg levels in brain and kidneys of subjects with dental amalgams than in control subjects with no amalgams (Nylander *et al.*, 1987).

These clinical studies raise several important questions; such as, what is the metabolic fate of amalgam Hg vapor and are there potential pathophysiological consequences? To address these questions, we have recently employed an experimental animal model in which sheep received dental amalgam tooth fillings containing a radioactive Hg tracer. Whole-body image scan and tissue analysis revealed several possible uptake sites: oral tissues, jaw bone, lung, and gastrointestinal tract. Once absorbed, the Hg is rapidly localized in kidney and liver (Hahn *et al.*, 1989). Similar studies in pregnant sheep indicate that both maternal and fetal tissues begin to accumulate Hg within several days following amalgam placement and this accumulation is progressive with time (Vimy *et al.*, 1990). Another study in the monkey (whose dentition, diet, feeding regimen, and chewing pattern closely resemble those of humans), likewise demonstrates high levels of Hg concentration in kidney, intestinal tract, and other tissues 4 weeks after placement of amalgams (Hahn *et al.*, 1990). The primate kidney continues to accumulate amalgam Hg for as long as 1 year after placement of such fillings (Danscher *et al.*, 1990) at levels comparable to those seen in sheep (Letters, 1991). After treatment with a chelating agent (DMPS), urinary excretion of Hg is significantly greater in human subjects with amalgams than in similarly treated subjects without amalgams. At least two-thirds of excretable Hg may be derived from dental amalgam, and amounts are correlated with total amalgam surface areas (Aposhian *et al.*, 1992). Current investigations in sheep and primates are designed to resolve the possible pathophysiological significance of dental amalgam Hg concentration in body tissues and to evaluate parallel issues in humans.

TABLE 1
Daily Mercury Retention in the General Population (WHO, 1991)

Exposure source	μg Hg/day absorbed	
Dental amalgam	3.0–17.0	
Fish or seafood	2.34	2.6
Other food	0.25	
Water	0.0035	
Air	0.001	

Amalgam Hg alters sheep kidney function by reducing the filtration rate of inulin clearance, increasing urinary excretion of sodium and decreasing urinary albumin levels (Boyd *et al.*, 1991). In 10 human subjects, Molin *et al.* (1990) demonstrated a significant increase in urinary albumin levels 12 months after all amalgam fillings were removed. This latter finding is the reciprocal of our demonstration that urine albumin levels fell in sheep after amalgam placement, suggesting a Hg-induced reduction in blood flow to the glomerulus (Boyd *et al.*, 1991). In rabbit and rat, the kidney proximal tubule (the primary site of sodium reabsorption) is the site of inorganic Hg accumulation (Zalups and Barfuss, 1990; Zalups, 1991). Our findings in sheep also suggest that kidney sequestration of dental amalgam Hg may reduce the renal tubular capacity to selectively conserve sodium (Boyd *et al.*, 1991).

Many human intestine bacterial species are known to carry plasmids encoding resistance both to Hg and to antibiotics (Gilbert and Summers, 1988). A preliminary report now demonstrates that amalgam Hg markedly increases the proportion of Hg-resistant bacteria in the primate mouth and intestine within 2 weeks after amalgam installation (Summers *et al.*, 1990). The significance of this phenomenon has been examined in additional monkeys with similar results. This

has medical importance, because 80% of these proliferating Hg-resistant bacterial strains also demonstrate increased resistance to one or more commonly used antibiotics (Summers *et al.*, 1991).

Reports from other laboratories implicate inorganic Hg in the pathogenesis of human brain dysfunction. Postmortem metal analyses of brain tissue from subjects with Alzheimer's disease demonstrate particularly high Hg concentrations in brain regions involved in memory function compared to tissue from age-matched control subjects, and dental amalgams are suggested as a possible Hg source (Thompson *et al.*, 1988; Wenstrup *et al.*, 1990). Abnormal microtubule formation in brains of subjects with Alzheimer's disease has been related to a defect in tubulin depolymerization that is associated with increased neurofibrillary tangle density (Khatoon *et al.*, 1989). Because Hg-treated rats display an irreversible tubulin defect similar to that seen in Alzheimer's disease (a defect not evident in aluminium-treated rats), others have concluded that inorganic Hg is involved in the etiology of this disease (Duhr *et al.*, 1991). The implications of such a conclusion, if confirmed by further studies, would attach an additional dimension to the view that dental amalgams provide the major source of exposure to Hg body burden (WHO, 1991).

In conclusion, the evidence to date indicates that a substantial amount of dental amalgam Hg vaporizes into mouth air, the Hg vapor is inhaled and swallowed, and significant concentrations of this Hg are distributed in body tissues resulting in alteration of cell function. Blood and urine Hg levels remain relatively low during amalgam Hg exposure, and therefore may be poor diagnostic indicators of the very high Hg levels that accumulate in some body tissues as a consequence of such exposure. The foregoing experimental findings are in marked contrast to opinions recently pronounced by spokesmen for the dental profession (Letters, 1990) and the American Dental Association (1990). Based on experimental evidence, the potential pathophysiological effects of chronic low-dose Hg exposure from dental amalgam in humans warrant continued investigation.

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Prenatal Exposure to Mercury Vapor: Effects on Brain Development (M. Berlin, J. Hua, B. Lögdberg, and K. Warfvinge, University of Lund, Sweden)

The lipophilic properties of mercury vapor explain why 10 times more mercury accumulates in brain tissue of adults after exposure to vapor than after injections of mercuric salts (Berlin and Johansson, 1964; Berlin *et al.*, 1969). Similarly, the fetal brain accumulates mercury when exposed to vapor in contrast to mercuric salts, for which the placenta constitutes a barrier (Clarkson *et al.*, 1972). However, mercury accumulation in the fetal brain is less than in the maternal brain, perhaps because mercury vapor is oxidized in the liver before reaching the brain. In a pilot study on two pregnant squirrel monkeys acutely exposed to mercury vapor during late pregnancy, Weiss and Clarkson (1987) observed that the mercury concentration in the fetal brain was one-third of that in the maternal brain.

It is conceivable that exposure to mercury vapor during pregnancy may interfere with brain development in view of the neurotoxic effects of mercury. A project is ongoing in Sweden with the aim to answer this question in an attempt to create a scientific basis for assessing the health risk of low-level mercury exposure from dental amalgam fillings. Two research groups, Dencker and co-workers in Uppsala and our own group in Lund, are involved.

In Lund we have developed an animal model based on a nonhuman primate, the squirrel monkey, for studies of chemical neuroteratogenic agents (Lögdberg *et al.*, 1987). The work with this model has included development of techniques for breeding, timed pregnancies, and morphological diagnoses (Lögdberg, 1992; Lögdberg and Berlin, 1992). Reference material from fetal brains in different stages of development and from human child and fetal brains with pathological brain development have been collected (Lögdberg and Brun, 1992). We have characterized normal fetal and postnatal growth in our colony, obtaining statistics on pregnancy outcome, abortion rate, prenatal mortality, and growth parameters for 15 years. These data are used as historical controls for our current studies.

Pregnancy outcome. Ten pregnant monkeys were exposed to mercury vapor at a concentration of 1 mg/m^3, beginning at Week 3 to Week 7 of gestation and continuing to the termination of pregnancy (approximately Week 22). One monkey was exposed for 24 hr a day, four monkeys were exposed for 7 hr a day, 5 days a week, and five monkeys were exposed for 4 hr a day, 5 days a week. Ten unexposed monkeys were assigned as controls in addition to historical controls. Exposure was monitored by recording mercury concentration in chamber air and by monitoring monthly the mercury concentration in monkey blood. Blood concentrations in the exposed monkeys ranged from 0.11 to 0.92 μg/g.

There was 60% incidence of abnormal pregnancy outcome among the 10 exposed pregnant squirrel monkeys compared to 5% seen in our breeding colony. The incidence of abortion and neonatal mortality showed a dose-related increase. A decrease in birth weight was also observed. The brains of two live offspring were perfused with a glutaraldehyde–formaldehyde mixture and brains from three deceased offspring were immersion-fixed in formaldehyde. The mercury concentration in four of the offspring brains ranged between 0.20 and 0.30 μg/g and was about eight times less than that found in the maternal brains. The fifth offspring deviated from the rest with total retention of mercury more than 10 times greater than the others, which was a brain mercury concentration of the same order seen in the maternal brains.

Mercury distribution in the neonatal brain. A coronal slice was cut just anterior to the central fissure of the offspring brains. The slice was dehydrated, embedded in paraffin, and sectioned at 5 μm. The sections were processed by a chemographic histochemical method to visualize mercury and stained with hematoxylin–eosin or just stained with hematoxylin–eosin.

Silver grains, due to presence of mercury, were found in both neurons and glial cells. In all sections, the walls of the blood vessels, the epithelium covering the brain, and the ependyma lining the ventricles and plexus choroideus contained considerable amounts of mercury. The density of stained cell bodies in the section, both glial cells and neurons, was generally higher in the white matter than in gray matter, with the exception of cortex piriformis, nucleus basalis Meynert, and nucleus fascicularis diagonalis Broccae, where the density of strongly stained neurons was high. In the cortical plexiform layer the density of stained cells, mostly glial cells, was higher than in other layers (Warfvinge *et al.*, 1992).

Histopathological changes in fetal and neonatal brains. Brain weights for all mercury-exposed offspring were low for

the fetal ages. The pattern of cerebral sulci of three cases was more irregular than normal. Some of the sulci seemed immature, particularly in the frontal part of the cerebrum. In one offspring, collections of heterotopic neurons and preferential localization of tissue mercury to these cells were seen. There were also indications of increased frequency of disoriented pyramidal neurons in some parts of cerebral cortex for this offspring. Other cases also showed indications of an increased number of heterotopic neurons in the subcortical white matter but further morphometric analyses are necessary to confirm this (Lögdberg *et al.*, 1992a). Thus, indications were found that mercury vapor induces growth retardation, sulci abnormalities, and increases in the number of heterotopic neurons in the cerebrum. The subpially localized heterotopic neurons with their disoriented apical dendrites are of special interest as they may indicate arrested cell migration from the subpial granular layer. The subpial granular layer normally disappears by migration of its cells across the margin of the zone to the cortical plate.

The disturbances seen in these brains have not been reported in previous experimental studies of mercury vapor exposure. However, similar changes with a persistence of subpially localized cells have been discovered following prenatal methyl mercury exposure in both humans (Choi *et al.*, 1978) and squirrel monkeys (Lögdberg *et al.*, 1992b).

Functional consequences of pre- and neonatal exposure. Dencker and the group in Uppsala have studied the functional consequences of prenatal (Danielsson *et al.*, 1992) and neonatal exposure (Fredriksson *et al.*, 1992) to mercury vapor in rat pups. Prenatal exposure to mercury vapor at a concentration of 1.8 mg/m^3 occurred during Days 11–14 and 17–20 of gestation. Two exposure durations were used, 1 hr/day (low dose) and 4 hr/day (high dose). Maturation variables studied, such as surface righting, negative geotaxis, pinna unfolding, and tooth eruption of the mercury-treated pups, were normal. Tests of spontaneous motor activity showed that the animals were hypoactive at 3 months of age and hyperactive at 14 months. In the spatial learning tasks, the radial arm maze and circular swim maze, the prenatally exposed pups showed a retarded acquisition of the former but no differences in the latter. A simple test of learning, habituation to a novel environment, indicated a reduced ability to adapt. These data suggest that prenatal mercury vapor exposure results in behavioral changes similar to those reported in offspring exposed to methyl mercury.

Neonatal exposure of rat pups to a mercury vapor concentration of 0.05 mg/m^3 was studied with two exposure durations, 1 hr/day (low dose) and 4 hr/day (high dose). Exposure occurred on Days 11–17, the period of rapid brain growth. Spontaneous motor activity was assessed at the ages of 2 and 4 months. Rats exposed to the high dose showed a marked increase in locomotion and total motor activity, but a decrease in rearing at 2 months. At 4 months these rats showed marked hypoactivity with respect to all three variables. Rats exposed to the low dose showed no significant difference at 2 months compared to controls. However, at 4 months the same pattern, i.e., an increase in locomotion and total motor activity but a decrease in rearing, seen in the high-dose group at 2 months was observed. In the spatial learning tasks, the radial arm maze and circular swim maze, exposed rats showed a retarded acquisition in the former but there was no difference compared to control in the latter. These behavioral changes are similar to those seen in prenatally exposed rats.

Conclusions. The above-described observations in nonhuman primates and in rats strongly indicate that mercury vapor is a potent neuroteratogenic agent.

The fetuses of female dentists, dental staff, and female workers exposed to mercury vapor are likely to be at risk. However, available evidence does not allow any conclusions concerning low mercury vapor exposure such as that resulting from release from dental amalgam fillings. These results, however, underline the necessity for further information concerning those dose levels for the assessment of the health risks attributable to mercury vapor exposure from amalgam in dental fillings. The WHO (1991) task group concluded in the criteria document for inorganic mercury, that the available evidence concerning exposure to low levels of mercury vapor was not sufficient to allow any conclusions concerning the risks or safety of use of amalgam in dental fillings and this is still true. Amalgam in dental fillings should be looked upon as a pharmaceutical drug used to prevent illness. All drugs have side effects and the acceptable risk levels depend on the benefits connected with use. In the case of amalgam in dental fillings the larger part of the population is exposed in industrial countries. Even a low-risk level has significance from the point of view of population health and cost for society. The main task in risk assessment would be to identify sensitive groups and the most critical effects. A fairly deep understanding of the mechanisms involved in the toxic action of mercury will be required to accomplish this task with any precision for low levels of risk. This understanding will enable the use of specific indicators of adverse effects in animal and epidemiological studies.

There are two main areas which have to be investigated.

(i) Effects of mercury vapor on the growing nervous system. There is at present no indication that such effects may occur at the level of vapor released from dental amalgam fillings. However, dentists and dental staff may be at risk from occupational exposure.

(ii) Effects of mercury vapor exposure on the immune system. If low levels of mercury exposure can trigger adverse reactions in the immune system, such as autoimmune disease, such effects are likely to be dependent on the genotype. Also, other components of amalgam in addition to mercury may induce an immune response.

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Reproductive Effects of Mercury Vapor (Andrew S. Rowland, NIEHS, Research Triangle Park, North Carolina)

The question addressed by this symposium is what, if any, are the long-term health consequences resulting from exposure to mercury, which makes up about 50% of dental amalgam. Although amalgam has been used in dentistry for over a century, we do not yet know the answer to this question. Experimental work on animals will be very important in developing a better understanding but because it is so difficult to extrapolate between species, human studies will also be important.

In recent years, researchers have found a direct relationship between the number of amalgam surfaces in a person's mouth and their urinary mercury levels (Olstad *et al.*, 1987). This suggests that people with dental amalgams have continuous low-level exposure to mercury. To date, there have not been any thorough epidemiologic studies of the health effects of dental amalgam but there is literature on the health effects of mercury vapor. This paper summarizes the epidemiologic data on the reproductive effects of mercury vapor and the gaps in that literature.

In laboratory animals, inorganic mercury impairs reproduction. Chronic exposure disrupts the estrous cycle, increases preimplantation loss, increases postimplantation loss, and decreases live litter size (Lach and Serebro, 1972; Lamperti and Prince, 1973, 1974; Watanabe *et al.*, 1982; Baranski and Szymczyk, 1973). The lowest exposure levels at which these effects occur have not been established and the mechanisms involved are not well understood. Mercury vapor is able to cross the "blood–brain barrier" and the placenta and accumulates in fetal tissue (Clarkson *et al.*, 1972; Clarkson *et al.*, 1985). Mercury is excreted in breast milk, and the neonatal period seems to be one of particular vulnerability because mercury is efficiently absorbed through the gastrointestinal tract of suckling animals but not in more mature animals (Clarkson *et al.*, 1985). Laboratory animal studies also suggest that mercury may impair spermatogenesis and interfere with testosterone production (Lee and Dixon, 1975; Berlin *et al.*, 1982; Ng and Liu, 1990).

Data on the effects of mercury vapor on human reproduction are scarce. Six studies, mostly conducted in Eastern Europe, have reported menstrual cycle abnormalities among mercury-exposed workers (Goncharuk, 1977; Panova and Dimitrov, 1974; Mikhailova *et al.*, 1971; Marinova and Chakarova, 1973; DeRosis *et al.*, 1985; Sikorski *et al.*, 1987; Barlow and Sullivan, 1982). Two of these studies involved dental workers (Marinova and Chakarova, 1973; Sikorski *et al.*, 1987). Changes in menstrual cycle length was the abnormality most frequently reported in these studies but because menstrual abnormalities were described so differently in each study, it is difficult to get a consistent picture of how mercury disrupts the menstrual cycle and at what exposure levels this might occur.

In the mid 1970s, an autopsy study conducted on mercury miners in Yugoslavia found extremely high levels of mercury in the pituitary glands, thyroid glands, kidneys, and brains of exposed workers compared to two control populations (Kosta *et al.*, 1975). More recently, Nylander and his colleagues at the Karolinska Institute have reported similar findings in occupationally exposed dental workers who had much lower mercury exposures (Nylander, 1986; Nylander and Weiner, 1991). In both the mercury miners and the dental workers, researchers observed that mercury can remain sequestered in these tissues for many years and selenium levels tended to be correlated with mercury. This raises the question about whether the mercury is bound to selenium or some other endogenous substance and therefore somehow rendered "inert" or whether it is biologically active and able

to interfere with neurologic or reproductive function (Cavanaugh, 1988). Two small studies have looked for changes in pituitary gland or thyroid gland function among dental workers; these studies were largely negative but did report differences in serum prolactin levels, the clinical significance of which is not clear (Langworth *et al.*, 1990; Erfurth *et al.*, 1990). More research on this issue is needed, particularly in light of recent studies demonstrating a relationship between the number of amalgam surfaces in a patient's mouth and the amount of mercury found in the occipital lobe of their brains and pituitary glands upon autopsy (Nylander *et al.*, 1987, 1989).

Mercury has been suspected of being an abortifacient agent since the era when syphilis was treated with mercury compounds and it was observed that many pregnant women who were treated for this disease miscarried (Alfonso and Alvarez, 1960). Since that time, there have been six studies of spontaneous abortion among female workers exposed to mercury. Unfortunately, data from these studies are conflicting and difficult to evaluate. Two of the six studies, one conducted among Polish dental workers (Sikorski *et al.*, 1987) and the other among female mercury smelter workers in the former Soviet Union (Goncharuk, 1977) reported a relationship between mercury exposure and spontaneous abortion. The other four studies were negative (DeRosis *et al.*, 1985; Heidam, 1984; Erickson and Kallen, 1989; Brodsky *et al.*, 1985) although one, a study of Danish dental workers (Heidam, 1984) reported increased rates of spontaneous abortion in a subgroup of women working with mercury in public clinics.

Taken together, the four negative studies do not rule out an effect. Exposure data were crude in all four studies which means that subtle effects may have been missed. Two of the negative studies were conducted among dental workers in Scandinavia where exposure levels are extremely low. Exposure data in a third study of American dental assistants (Brodsky *et al.*, 1985) were estimated at the time the questionnaire was completed even though the pregnancies being studied had occurred as many as 10 years before. Another study conducted in a lamp factory in Italy was small and exposure was described as intermittent, so a possible effect could not be ruled out here either (DeRosis *et al.*, 1985).

In preliminary analyses of our own unpublished data on spontaneous abortion in female dental assistants, we have found that women who prepared 50 or more amalgams per week were at increased risk for spontaneous abortion compared to dental assistants with either no exposure or low mercury exposure, but this difference was not statistically significant. We interpret this preliminary finding as suggestive of an effect but inconclusive because our measure of mercury exposure was crude.

There have been two studies of the effects of paternal exposure to mercury and risk of spontaneous abortion that are interesting because they used urinary mercury levels to estimate exposure. Workers at a Department of Energy plant that used large quantities of mercury during the 1950s (Alcer *et al.*, 1989) showed dose-related increases in the risk of spontaneous abortion ($p < 0.02$). This was reported as a negative study because the effect became weaker when number of previous spontaneous abortions was included in the statistical model. However, to the extent that any of the previous spontaneous abortions were caused by mercury exposure, controlling for previous spontaneous abortions would represent over-control and a weakening of the relationship would be expected. A more recent study at a chloralkali plant in France also reported a dose–response relationship between level of paternal mercury exposure and risk of spontaneous abortion (Cordier *et al.*, 1991). Although suggestive of a paternal effect, both studies commented that they were not adequately able to rule out the effect of maternal exposure to mercury or other occupational exposures and point out the need for more research along these lines.

It is difficult to evaluate the teratogenicity of inorganic mercury from the existing data. Although some studies have demonstrated increased chromosomal aberrations and micronuclei among mercury-exposed workers (Barregard *et al.*, 1991; Anwar and Salah Gabal, 1991; Popescu *et al.*, 1979), others have not (Mabille *et al.*, 1984; Verschaeve *et al.*, 1979). The positive spontaneous abortion study conducted in Poland (Sikorski *et al.*, 1987) also reported six congenital malformations of 117 pregnancies in their exposed group, and five of these were cases of spina bifida. This is an extremely high rate of spina bifida but similar findings have not been reported elsewhere. A registry study conducted in Sweden where occupational exposure to mercury is low, found no evidence of increased malformations, including spina bifida, among the children of dental workers (Erickson and Kallen, 1989). Large epidemiologic studies among populations with higher exposures have not been conducted.

For the last few years, our research group at the National Institute of Environmental Health Sciences has been conducting studies on the fertility of female dental assistants. Our primary outcome for these studies was fecundability, the probability of conception during each menstrual cycle. We estimated fecundability using data on time to pregnancy, the number of menstrual cycles it takes a woman to become pregnant after discontinuing use of birth control, adjusted for frequency of unprotected sexual intercourse (Baird *et al.*, 1986). In preliminary analyses we have found that female dental assistants who prepared 30 or more amalgams per week and who worked in offices using poor mercury hygiene showed evidence of reduced fecundability. We also found that dental assistants who used good mercury hygiene or who prepared less than 30 amalgams actually had better fecundability than unexposed dental assistants. One explanation for the improved fecundability of the low mercury-exposed group might be that the "unexposed" controls had other occupational exposures that were impairing their fertility. We interpret these preliminary data as suggestive of a

fertility effect among women with relatively high occupational exposure to mercury but again, inconclusive, because we were not able to isolate the reason for increased fertility among the low exposed groups.

The only investigation of the fertility of male workers exposed to mercury vapor reported small but statistically nonsignificant decrements in the expected number of births for all three age groups studied (Lauwerys *et al.*, 1985). This study had limited statistical power and their control population, while free of mercury, experienced other toxic exposures.

There are many gaps in the literature on reproductive effects of mercury vapor. Because the experimental animal data and the menstrual cycle data suggest an adverse effect on fertility, this needs to be followed up. The types of menstrual cycle abnormalities caused by mercury exposure and the underlying mechanisms need to be clarified. We need to determine whether the mercury that accumulates in the brain, thyroid gland, and pituitary gland is biologically active and if it is disrupting neurological or reproductive function. We need better studies of spontaneous abortion that account for both paternal and maternal factors and there need to be more studies on the possible teratogenic effects of mercury. Studies on the exposed fetus and infant are important: we need to know whether occupationally exposed women can safely breast feed their children and what the possible long-term developmental consequences of low-level mercury exposure might be. It is also reasonable to ask whether there might be any negative consequences from having elective dental work performed during pregnancy because brief peaks in mercury exposure to the fetus are likely to occur during these procedures. Because we are concerned about the consequences of low-level exposures, attention should also be paid to mechanisms the body may have for detoxifying mercury (for example, the possible role that selenium or induction of metallothionein might play) and at what exposure levels these protective pathways might become overwhelmed.

Public concern about the toxicity of mercury in dental amalgam is reasonable given the uncertainties involved and the lack of adequate data to address the issue. There is a need for sensitive markers of subclinical reproductive, neurologic, immunologic, and renal disease. The most reassuring data will be generated when, and if, it can be shown that markers of subclinical disease which are sensitive to mercury are within the normal range in people with large amounts of amalgam in their mouths.

Finally, it is important to recognize that the amalgam question does not exist in isolation. There is a danger that we jump out of the frying pan and into the fire by condemning the use of mercury in dental amalgam before the toxicity of the alternatives has been determined. The toxicity of methyl methacrylate and some of the other components of composite resins has not been adequately evaluated and needs to be, if we are to make rational scientific decisions about this issue.

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