Mercury Induced Autism

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Introduction

Mercury (Hg), a heavy metal, is widespread and persistent in the environment. Among other effects, exposure to hazardous Hg levels can cause permanent neurological and renal impairment. Elemental Hg, or inorganic Hg, is released into the air or water from sources such as coal-burning power plants or manufacturing facilities, and it becomes methylated in the environment where it accumulates in animal tissues and increases in concentration through the food chain. The US population is primarily exposed to methyl-Hg by eating fish. This exposure to methyl-Hg is of great concern because fetuses are highly susceptible to Hg's adverse effects. In addition, numerous prescription and over-the-counter drugs have contained, or continue to contain, inorganic or organic forms of Hg. As a result, Hg is a ubiquitous source of danger in drugs for the eye, ear, nose, throat, and skin; in bleaching creams; as a preservative in cosmetics, tooth paste, lens solutions, vaccines, allergy test, and immunotherapy solutions; in antiseptics, disinfectants, and contraceptives; in fungicides and herbicides; in dental fillings and thermometers; and many other products (Geier et al. 2008a, 2010).

Environmental studies and human studies warn of increasing Hg levels. According to the 2002 United States (US) Department of Interior/US Geological

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Survey, the USA has a growing Hg problem. This problem is not confined to the USA, as demonstrated by a 2009 study of mercury levels in the oceans led by scientists from Harvard University and the US Geological Survey. It found that the ocean's mercury levels have risen about 30 % over the last 20 years, predominately from industrial emissions. The authors stated that mercury is circulated over vast distances and that Asia's burning of coal is the primary source of mercury emissions worldwide (Sunderland et al. 2009).

These increases in the environment are reflected in human studies. Laks (2009), for example, recently reported on time trends for blood inorganic mercury (I-Hg) levels in 6,174 women, ages 18–49, in the National Health and Nutrition Examination Survey (NHANES), 1999–2006 data sets. Laks found that, in the US population, I-Hg detection rose sharply from 2 % in 1999–2000 to 30 % in 2005–2006. In addition, the population average mean I-Hg concentration rose significantly over that same period, from 0.33 to 0.39 μg/L. I-Hg was significantly associated with age suggesting bioaccumulation. Laks stated that the study provided evidence that I-Hg deposition within the human body is a cumulative process, having increased with age and in the population over time since 1999, as a result of chronic Hg exposure. If blood I-Hg levels in women are increasing, as suggested by the NHANES data, then it is safe to assume that fetal exposure to I-Hg is also increasing. Worrisomely, studies have consistently shown that fetal cord blood Hg levels are higher than the maternal blood levels.

The most recent data by Lederman et al. (2008) from the US Centers for Disease Control and Prevention (CDC) evaluated a cohort of several hundred individuals in New York. It was observed that about 6 % of women of child bearing age had blood Hg levels above the safety limit (≥5.8 micrograms (μg)/liter (L)) established by the US Environmental Protection Agency (EPA). Far worse, when evaluating the blood Hg levels present in newborn babies, about one in three infants tested had blood Hg levels above the US EPA safety limit, and a number of infants had blood Hg levels above the level defined by the US CDC as the threshold level for Hg poisoning (≥10 μg/L).

Furthermore, most infants in the USA have received and continue to receive additional doses of Hg following birth as part of the routine childhood vaccination schedule. The Hg compound added to vaccines to help prevent bacterial and fungal contamination in multidose vials is called thimerosal. Thimerosal, a trade name for sodium ethylmercurithiosalicylate, a drug component that has never been adequately tested for its safety in humans, contains organic Hg moiety (ethyl-Hg). It is about half Hg by weight (49.55 %) (Geier et al. 2007).

During the mid-1980s, infants received a cumulative dose of 100 μg of organic Hg during the first 18 months of life from the 25 μg Hg in each diphtheria–tetanus–pertussis (DTP) vaccine routinely administered at 2, 4, 6, and 18 months of age. Additionally, during this time, infants may have incurred additional Hg exposure through breast milk if they were born to mothers with Hg amalgam fillings and/or Rh-negative mothers, since many Rho(D)-immune globulin formulations,

used to prevent isoimmunization in the Rho(D)-negative individual exposed to Rho (D)-positive fetal blood, contained thimerosal (10.5 to >50 μg Hg/dose). Rho(D)-immune globulins were routinely recommended for administration to these mothers within 72 h of birth (Geier et al. 2008a, 2010).

Starting in the late 1980s/early 1990s, the cumulative dose of Hg children received from thimerosal-containing childhood vaccines/biologics more than doubled. Specifically, a thimerosal-containing *Haemophilus influenzae* type b (Hib) vaccine (25 μg Hg/dose) was recommended for routine administration at 2, 4, 6, and 18 months of age. Furthermore, a thimerosal-containing hepatitis B vaccine (12.5 μg Hg/dose) was recommended for routine administration at birth, 2 and 6 months. As a result, an infant could have potentially received a cumulative dose of 237.5 μg Hg during the first 18 months of life. Furthermore, since many formulations of Rho(D)-immune globulins were thimerosal containing (10.5 to >50 μg Hg/dose) and were then recommended for routine administration to all Rh-negative pregnant women at 28 weeks of gestation starting in the late 1980s/early 1990s (in addition to the recommendation for its routine administration within 72 h of birth), the cumulative dose of Hg received from thimerosal-containing vaccines/biologics was certainly even higher for many US infants (Geier et al. 2008a, 2010). As a result, the cumulative estimated Hg doses some children received during fetal and infant periods from thimerosal-containing vaccines/biologics exceeded 300 μg Hg.

By the summer of 1999, the realization that the Hg exposure to American infants was incurring through the immunization schedule exceeded some, if not all, safety limits, caused alarm among both private health organizations and public agencies. On July 7, 1999, the US Public Health Service (USPHS) and the American Academy of Pediatrics (AAP) issued a joint statement that urged "all government agencies to work rapidly toward reducing children's exposure to Hg from all sources." The statement recommended that thimerosal be removed from vaccines as soon as possible as part of this overall process. Between 1999 and 2001, many of the thimerosal-preserved vaccines recommended for children less than 6 years of age began to be made available in reduced-thimerosal ("preservative-free") formulations in the USA (Geier et al. 2008a, 2010).

Despite this call, issued by the USPHS and the AAP, to reduce children's exposure to Hg from all sources, thimerosal was reintroduced into the US routinely recommended childhood vaccine schedule in 2002 with recommendations to administer two doses of influenza vaccine in the first year of life and to vaccinate all children who were 6 months to 23 months of age (Geier et al. 2008a). In addition, starting in 2002, the federal recommendations, initially issued in 1997, emphasized the need to vaccinate all pregnant women who would be in their second or third trimester of pregnancy during the US "flu" season (December to March) as well as those who have medical conditions that might increase their risk for complications from influenza, regardless of the stage of pregnancy. It is important to note that the vast majority of influenza vaccines have contained and

continue to contain a preservative level of thimerosal (25 μg Hg/0.5 milliliter (mL) dose). The US Department of Health and Human Services recently estimated more than 90 million doses of the 2010–2011 influenza vaccine contained preservative levels of thimerosal. Moreover, the 2002 recommendation for the influenza vaccine has been continually expanded to the point that, in 2008, the US CDC recommended that all pregnant women should receive an influenza vaccine (without regard to the trimester of pregnancy) and that all infants should receive two doses of influenza vaccine in the first year of life, with an additional influenza vaccine administered on a yearly basis thereafter until the child is 18 years old (Geier et al. 2008a, 2010). As a result, the cumulative estimated Hg doses some children may receive from thimerosal-containing influenza vaccines exceed 400 μg Hg.

The administration of thimerosal-containing vaccines to infants in the USA was found to result in increased blood Hg levels with some infants having blood Hg levels in excess of the blood safety limit from the US EPA, as well as the blood Hg level defined by the US CDC as the threshold level for Hg poisoning. In addition, administration of thimerosal-containing vaccines to infants in the USA was found to induce hair Hg levels in excess of the hair Hg safety limit established by the US EPA for significant periods of time during the first several years of life (Geier et al. 2008a, 2010).

All told, researchers have reported that Hg exposures in early childhood from both potential environmental and vaccine sources resulted in some infants receiving in excess of 350 μg Hg during the first 6 months of life. It was estimated that about 50 % of the total Hg doses to which some infants were exposed came from routinely recommended thimerosal-containing childhood vaccines. The cumulative exposure resulted in infants receiving doses of Hg in excess of Hg exposure limits established by the US EPA, US CDC, US Food and Drug Administration (FDA), and Health Canada during key developmental periods during the first year of life (Bigham and Copes 2005).

The relationship between Hg body burden and exposure in children appears to be measurable. For example, a study by Kern et al. (2011a) examined urinary porphyrin levels (a biomarker of Hg body burden) in American children (in Texas) as compared to French children (in eastern France). These two groups were chosen because of Hg exposure differences based on two factors: (1) exposure from thimerosal in vaccines and (2) Hg from coal-burning plants. Comparing the relative presence of thimerosal in vaccines administered in the USA and France in 1999, thimerosal was present in approximately 30 different childhood vaccines used in the USA, whereas there were only two in France that contained thimerosal. In the USA, thimerosal is still in most of these doses of the influenza vaccines recommended to be given every year to pregnant women, infants, and children as well as to most adults, and it was also in the A/H1N12009 pandemic inactivated influenza vaccine – both of which are reportedly seldom administered in France. In regard to coal burning, energy produced from coal in France in 2003 was 6 % versus 51 % in the USA and

36.5 % in Texas. Additionally, the children in the Texas group lived in an area that has one plant alone producing about 1,500 lbs of Hg per year which, because of the prevailing winds, is dispersed over the area where they reside. The study found that the US children have a significantly increased body burden of Hg in comparison to the body burden of Hg in the matched French children.

Hg Distribution and Persistence Following Exposure

Research studies in monkeys reported that significant, persistent Hg concentrations were present in the brain following administration of injection of thimerosal-containing childhood vaccines which mimicked the dosing schedule (weight and age adjusted) that US children received in the 1990s. In addition, a significant fraction of Hg observed in the infant monkey's brain following administration of thimerosal-containing childhood vaccines did not significantly decrease in concentration more than 120 days following the last dose administered to the infant monkeys. In addition, other investigators observed that significant Hg levels persisted in infant rat brains following administration of thimerosal mimicking the dosing that US children received and inorganic Hg exposure. Also, similar results were observed in infant monkey's brain following oral administration of low doses of methyl-Hg (Geier et al. 2008a, 2010). Some researchers have described that Hg may have the potential to remain in the brain from several years to decades following each exposure (Sugita 1978).

Biological Plausibility of Hg-Induced ASD

Patterns of increased and persistent brain Hg levels are of crucial importance because researchers have long recognized Hg is a neurodevelopmental poison (Clarkson et al. 1985). This means that Hg exposure can severely disrupt the normal neurodevelopmental processes in the human brain. As a result, Hg may cause problems in normal neuronal cell migration and division, as well as induce neuronal cell degeneration, and ultimately cell death. Based upon this knowledge, Nelson (1991) from the National Institute for Occupational Safety and Health (NIOSH) of the US CDC reported that organic Hg was among the compounds known to induce behavior disorders such as autism. Subsequently, other researchers reported the specific biological effects of Hg exposure on neuronal development to be consistent with the brain pathology observed in autism (Faustman et al. 2000). In addition, published case reports of patients have described developmental regressions with ASD symptoms following fetal and/or early childhood Hg exposure (Geier et al. 2008a, 2010).

Researchers reported that exposure to Hg can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with an ASD and that these similarities extend to neuroanatomy, neurotransmitters, and biochemistry (Geier et al. 2008a, 2010). Furthermore, as summarized in

Table 1 A summary comparison of the clinical traits of ASD and Hg poisoning

Overall category	Clinical symptoms
Psychiatric disturbances	Social deficits, shyness, social withdrawal
	Repetitive, preservative, stereotypic behaviors; obsessive – compulsive tendencies
	Mood swings, flat affect, impaired face recognition
	Anxiety, schizoid tendencies, irrational fears
	Irritability, aggression, temper tantrums
	Lacks eye contact, impaired visual fixation/problems in joint attention
Speech and language deficits	Loss of speech, delayed language, failure to develop speech
	Dysarthria, articulation problems
	Speech comprehension deficits
	Verbalizing and word retrieval problems, echolalia, word use and pragmatic errors
Sensory abnormalities	Abnormal sensation in mouth and extremities
	Sound sensitivity, mild to profound hearing loss
	Abnormal touch sensations, touch aversion
	Oversensitivity to light, blurred vision
Motor disorders	Flapping, myoclonal jerks, choreiform movements, circling, rocking, toe walking, unusual postures
	Deficits in eye – hand coordination, limb apraxia, intention tremors/ problems with movement or imitation
	Abnormal gait and posture, clumsiness, and incoordination; difficulties in sitting, lying, crawling, and walking; problem on one side of body
Cognitive impairments	Borderline intelligence, mental retardation
	Poor concentration, attention, response inhibition
	Uneven performance on intelligence quotient testing
	Deficits in understanding abstract ideas and symbolism/sequencing, planning, and organizing
Physical disturbances	Hyper- or hypotonia; abnormal reflexes; decreased muscle strength, especially upper body; incontinence problems; problems chewing swallowing
	Rashes, dermatitis, eczema, itching
	Diarrhea, abdominal pain/discomfort, constipation, colitis
	Anorexia, nausea/vomiting, poor appetite/restricted diet
	Lesions of ileum and colon, increased gut permeability

Table 1, from the work of Bernard et al. (2001), there is a direct overlap between the clinical symptoms of ASD and Hg intoxication.

Finally, a scientific consensus statement developed by the Collaborative on Health and the Environment's Learning Developmental Disabilities Initiative (2008) on environmental agents associated with neurodevelopmental disorders declared there was no doubt Hg exposure causes learning and developmental disorders including conditions such as an ASD.

Epidemiological Evidence of a Link Between Hg Exposure and a Subsequent ASD Diagnosis

While epidemiology is not intended to provide an absolute demonstration of drug safety or harm to an individual, the use of this academic discipline as a surveillance tool is appropriate in determining whether low levels of Hg exposure may have contributed to an increased risk for a child developing an ASD. A number of epidemiological studies linking Hg exposure with an increased risk of an ASD diagnosis have been previously reviewed (Geier et al. 2008a, 2010). The following are a few important examples of these studies.

In order to investigate the relationship between levels of Hg exposure from thimerosal-containing vaccines and a subsequent ASD diagnosis, a meta-analysis epidemiological study was performed on data from the Vaccine Adverse Event Reporting System (VAERS) database (Geier and Geier 2006). The VAERS is an epidemiological database that has been maintained by the US CDC since 1990 as a surveillance tool to evaluate vaccine safety. Using techniques developed and published by the US CDC, unique VAERS reports stating that a DTP or thimerosal-containing diphtheria – tetanus – acellular pertussis (DTaP) was administered were assigned to the Hg-exposed group, and the unique VAERS reports stating that diphtheria – tetanus – pertussis – *Haemophilus influenzae* type b (DTPH) or thimerosal-free DTaP was given were assigned to the less Hg-exposed group. The Biological Surveillance Summary reports from the US CDC, sorted by vaccine manufacturers, indicated that there were a total of 57,151,417 vaccine doses administered to children in the exposed group, those receiving additional doses of Hg from thimerosal-containing vaccines (i.e., the DTP or thimerosal-containing DTaP vaccines), and 47,985,230 vaccine doses administered to children in the less Hg-exposed group, those receiving lower doses of Hg from vaccines (i.e., the DTPH or thimerosal-free DTaP vaccines). The following numbers of study-associated neurodevelopmental disorder adverse events were identified in VAERS: autism (133 reports), speech disorders (115 reports), mental retardation (143 reports), personality disorders (124 reports), thinking abnormalities (41 reports), ataxia (41 reports), and neurodevelopmental disorders in general (374 reports). It was observed that there were significantly increased adjusted risk ratios for neurodevelopmental disorder adverse events reported to VAERS in the exposed group, when compared to the less Hg-exposed group, for the outcomes of autism, speech disorders, mental retardation, personality disorders, thinking abnormalities, ataxia, and neurodevelopmental disorders in general. By contrast, none of the control adverse events (note: these were selected on an a priori as not biologically plausibly linked to an increased risk following additional doses of Hg from thimerosal-containing vaccines) of conjunctivitis, febrile seizures, or lymphadenopathy reported to VAERS had a significantly increased risk ratio in the exposed group when compared to the unexposed group.

Young et al. (2008) examined possible associations between neurodevelopmental disorders and exposure to Hg from thimerosal-containing vaccines by evaluating automated medical records for patients in the US CDC's Vaccine Safety

Datalink (VSD) database. A total of 278,624 subjects were identified in birth cohorts from 1990 to 1996 that had received their first oral polio vaccination by 3 months of age in the VSD. The birth cohort prevalence rate of medically diagnosed International Classification of Disease, ninth revision (ICD-9), specific neurodevelopmental disorders (selected a priori as having a biologically plausible association with Hg exposure) and control outcomes (selected a priori as not having biologically plausible association with Hg exposure) were calculated. Exposure to Hg from thimerosal-containing childhood vaccines was calculated by birth cohort for specific exposure windows from "birth to 7 months" and "birth to 13 months" of age. Because adverse events are rare in the population, a Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from thimerosal-containing childhood vaccines. Consistent significantly increased rate ratios were observed for autism, ASDs, tic disorders, developmental disorder/learning disorder, attention-deficit disorder, and emotional disorders with Hg exposure from thimerosal-containing childhood vaccines. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from thimerosal-containing childhood vaccines.

Gallagher and Goodman (2010) investigated the relationship between Hg exposure from thimerosal-containing hepatitis B vaccination and the risk of an ASD diagnosis by undertaking a cross-sectional study using weighted probability samples obtained from the National Health Interview Survey (NHIS) 1997–2002 data sets. Vaccination status was determined from the vaccination record. Logistic regression was used to estimate the odds for autism diagnosis associated with neonatal hepatitis B vaccination among boys age 3–17 years, born before 1999 (when thimerosal was routinely present in hepatitis B vaccines at a preservative level), adjusted for race, maternal education, and two-parent household. Boys vaccinated as neonates had a threefold greater odds for subsequently being given an autism diagnosis compared to boys never vaccinated or vaccinated after the first month of life.

Another study evaluated the potential adverse effects of prenatal Hg exposure from thimerosal-containing Rho(D)-immune globulins routinely administered to Rh-negative mothers in the USA prior to 2002 (Geier et al. 2008b). It was hypothesized: (1) if prenatal Rho(D)-immune globulin preparation exposure was a risk factor for neurodevelopmental disorders, then more children with neurodevelopmental disorders would have Rh-negative mothers compared to controls; and (2) if thimerosal in the Rho(D)-immune globulin preparations was the ingredient associated with neurodevelopmental disorders, then following the removal of thimerosal from all manufactured Rho(D)-immune globulin preparations in 2002, the US frequency of maternal Rh negativity among children with neurodevelopmental disorders should be similar to control populations.

Maternal Rh negativity was assessed at two sites (Clinic A – Lynchburg, VA; Clinic B – Rockville and Baltimore, MD) among 298 Caucasian children with neurodevelopmental disorders and known Rh status. Maternal Rh-negativity frequency was determined from 124 Caucasian children (born 1987–2001) without neurodevelopmental disorders at Clinic A, and the Rh-negativity frequency

was determined from 1,021 Caucasian pregnant mothers that presented for prenatal genetic care at Clinic B (1980–1989). These two groups served as controls for their respective clinics. Additionally, 22 Caucasian patients with neurodevelopmental disorders born from 2002 onward (Clinics A and B) were assessed for maternal Rh negativity. There were significant and comparable increases in maternal Rh negativity among children with neurodevelopmental disorders (Clinic, A = 24.2 %), ASDs (Clinic, A = 28.3 %, B = 25.3 %), and attention-deficit disorder/attention-deficit hyperactivity disorder (Clinic, A = 26.3 %) observed at both clinics in comparison to both control groups (Clinic, A = 12.1 %, B = 13.9 %) employed. Interestingly, children with neurodevelopmental disorders born post-2001, after thimerosal was removed from Rho(D)-immune globulin preparations, had a maternal Rh-negativity frequency (13.6 %) similar to controls.

In yet another study, a prospective, blinded assessment was undertaken to examine the hypothesis that increased Hg exposure from maternal dental amalgams during pregnancy may significantly impact the severity of ASD diagnoses (Geier et al. 2009a). A total of 100 qualifying participants born from 1990 to 1999 and diagnosed with autism (severe) or ASD (mild) were prospectively recruited from patients presenting for outpatient genetic consultations at the Genetic Centers of America. Logistic regression analysis (age, gender, race, and region of residency adjusted) by quintile of maternal dental amalgams during pregnancy revealed the ratio of autism/ASD (severe/mild) was about 1 (no effect) for $\leq$5 amalgams and increased for $\geq$6 amalgams. Fitting the model with a binary maternal dental amalgam level during pregnancy revealed that subjects whose mothers had $\geq$6 amalgams during pregnancy were 3.2-fold significantly more likely to be diagnosed with autism (severe), in comparison to ASD (mild), than subjects whose mothers had $\leq$5 amalgams during pregnancy. This study concluded that elevated Hg exposure from maternal dental amalgams during pregnancy is associated with an elevated risk of increasing autism severity.

Finally, a series of epidemiological studies conducted in the USA (in California, Louisiana, and Texas) and in South America have all also found significant associations between environmental sources of Hg exposure and an increased risk of a subject being diagnosed with an ASD (Geier et al. 2008a, 2010). Among these studies, a recent study by Blanchard et al. (2011) is particularly compelling because it was demonstrated for Bexar County, Texas, and Santa Clara County, California, that the spatial structure of the occurrence of autism had a positive covariation with the spatial structure of the distribution of Hg in ambient air. Namely, these investigators demonstrated that the relative risk of autism was greater in the geographic areas of higher levels of ambient Hg and that higher levels of ambient Hg were geographically associated with point sources of Hg emission, such as coal-fired power plants and cement plants with coal-fired kilns.

Overall, Table 2 provides a summary of the specific Hg exposure sources associated with ASD from case reports and epidemiological studies. The sources include many common sources of Hg exposure for many fetuses/infants, and as a result, Hg continues to be a ubiquitous source of potential harm.

Table 2 A summary of significant sources of Hg exposure in fetuses/infants associated with ASD diagnoses

Sources of Hg exposure associated with ASD diagnoses
Vaccines
Fish
Coal-burning power plants
Dental amalgams
Factories (using Hg in industrial processes)
Immune globulin preparations
Hg thermometers

Animal Models of Hg-Induced Diagnosis of an ASD

Previously, a significant number of animal model studies were previously reviewed that revealed Hg exposure was able to induce clinical symptoms and brain pathological lesions consistent with those observed in subjects diagnosed with an ASD as summarized in Table 3 (Geier et al. 2008a, 2010). The following are a few important examples of these studies.

Burbacher et al. (1990) were among the first to make observations of the social behavior (consistent with the clinical symptoms observed in subjects diagnosed with an ASD) of *Macaca fascicularis* exposed in utero to low-dose methyl-Hg and nonexposed control infants. Infants were tested twice weekly from 2 weeks to 8 months of age. Data were summarized into six categories of social behavior and seven categories of nonsocial behavior. Analysis of the most prevalent behavior indicated that methyl-Hg-exposed offspring exhibited a decrease in social play behavior and a concomitant increase in nonsocial passive behavior. The methyl-Hg effect on social play behavior tended to decrease with age, while the group differences in nonsocial passive behavior tended to increase. The investigators concluded that, on the basis of their study, maternal intake of methyl-Hg during pregnancy can affect the social development of infant primates by suppressing social interactions and increasing nonsocial behavior.

Subsequently, Hornig et al. (2004) observed that administration of thimerosal to various mice strains mimicking the US childhood vaccine schedule of the 1990s resulted in a genetically sensitive mouse strain exhibiting many of the symptoms and altered brain structure observed in children diagnosed with an ASD, including growth delay, reduced locomotion, exaggerated response to novelty, decreased numbers of Purkinje cells, increased brain size, and densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters.

Similarly, Thomas Curtis (2011) reported on the administration of a different form of Hg (Hg chloride) to a different animal species (prairie vole). These investigators observed that 10 weeks of exposure at 60 parts-per-million of Hg chloride in drinking water resulted in increased social deficits and tumor necrosis factor-alpha protein expression in the cerebellum and hippocampus among males.

Table 3 A summary of example animal models of Hg-induced ASD pathology

Animal model system	Significant findings
Mice	A genetically sensitive mouse strain exhibited many of the symptoms and altered brain structures observed in subjects diagnosed with an ASD, including growth delay, reduced locomotion, exaggerated response to novelty, decreased numbers of Purkinje cells, increased brain size, and densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters
Hamster	A hamster strain exhibited many of the altered brain structures observed in ASD, including damage to the hippocampus, cerebral cortex, and cerebellum (Purkinje cells and granulose cells); with decrease in neuronal density, neuronal necrosis, axonal demyelinization, and gliosis
Rat	Adult male and female rats, which were exposed to Hg during the early postnatal life, manifested with many of the symptoms and altered brain structures observed in ASD, including impairments of locomotor activity and increased anxiety/neophobia in the open-field test. The frequency of prosocial interactions was reduced, while the frequency of asocial/antisocial interactions was increased in males, but decreased in females. Rats also manifested with alterations to the brain dopaminergic system. Males were more sensitive than females to some neurodisruptive/neurotoxic actions of Hg
Prairie vole	A prairie vole strain exhibited many of the symptoms and altered brain pathology observed in subjects diagnosed with an ASD, including social avoidance and increase in tumor necrosis factor-alpha protein expression in the cerebellum and hippocampus with males more sensitive than females
Monkey	Longitudinal structural and functional neuroimaging was undertaken to examine central effects on the developing brain in monkeys exposed to Hg. Consistent with altered brain structures observed in ASD, volumetric analyses showed that exposed animals did not undergo the maturational changes over time in amygdala volume. Further, Hg exposure was associated with significant delays in the acquisition of root, snout, and suck reflexes

Finally, Ida-Eto et al. (2013) investigated whether prenatal thimerosal exposure (single dose of 10 μg Hg/kg body weight) caused persistent impairment after birth. These investigators observed that analysis on postnatal day 50 showed significant increases in hippocampal serotonin and striatal dopamine following thimerosal administration on embryonic day 9. This observation is even more significant because these same authors previously reported that prenatal exposure in rats on embryonic day 9 to thalidomide or valproic acid, chemicals known to induce autism when exposed at embryonic day 9, also induced increased hippocampal serotonin in the adult brains of rats at postnatal day 50 and behavioral abnormalities in rats closely mimicking human autism.

Clinical Evidence of Toxicity from Hg in Subjects with an ASD Diagnosis

With animal models demonstrating the toxic effect of low-dose Hg exposure upon the developing brain, and epidemiological studies suggesting a correlation between

Table 4 A summary of the evidence showing an elevated body burden of Hg in subjects diagnosed with an ASD in comparison to controls

Evidence of elevated Hg body burden in subjects diagnosed with an ASD
Increased blood Hg levels
Increased brain Hg levels
Increased baby teeth Hg levels
Increased fingernail Hg levels
Increased urinary Hg levels
Increased fecal Hg levels
Increased hair Hg levels
Increased Hg-associated urinary porphyrin levels
Decreased hair Hg excretion levels (primarily in first baby haircuts, even with equal or greater Hg exposure)

the level of Hg exposure the risk of a subsequent ASD diagnosis, one must assess whether this association is theoretical or actual. This requires an assessment of Hg body burden and/or detoxification between children diagnosed with an ASD and neurotypical controls. Among individuals diagnosed with an ASD relative to controls, data have demonstrated increased brain Hg levels, increased blood Hg levels, increased Hg levels in baby teeth, increased Hg levels in hair samples, increased urinary porphyrins associated with Hg intoxication, increased Hg in urine/fecal samples, and decreased Hg through first baby haircuts (Geier et al. 2008a, 2010) as summarized in Table 4.

In addition to the aforementioned studies, Kern et al. (2011b) recently published the most careful study to date examining Hg body burden among subjects diagnosed with an ASD in comparison to controls. In the Kern et al. (2011b) study, urinary porphyrin biomarkers in a group of children (2–13 years of age) diagnosed with an ASD were compared to a matched (age, gender, race, location, and year tested) group of typically developing controls. The results of this study revealed significantly increased levels of Hg-associated urinary porphyrins in subjects diagnosed with an ASD in comparison to controls with no significant differences in non-Hg-associated urinary porphyrins.

Furthermore, another study by Kern et al. (2010) compared measurements of Hg body burden to independent assessments from a recognized quantitative measurement, the Autism Treatment Evaluation Checklist (ATEC), of the specific domains of autistic disorders symptoms (Speech/Language, Sociability, Sensory/Cognitive Awareness, and Health/Physical/Behavior) in a group of children having a clinical ASD diagnosis. The results of the study indicated that the participants' overall ATEC scores and their scores on each of the ATEC subscales (Speech/Language, Sociability, Sensory/Cognitive Awareness, and Health/Physical/Behavior) were linearly related to urinary porphyrins associated with Hg toxicity. The results showed an association between the apparent level of Hg toxicity as measured by recognized urinary porphyrin biomarkers of Hg toxicity and the magnitude of the specific hallmark features of autism as assessed by ATEC. In yet another study

using statistical regression analyses, the body burden of toxic metals, particularly Hg, as assessed by urinary excretion before and after detoxification therapy, was found to be significantly related to autism severity, as measured by a professional evaluation based on the Autism Diagnostic Observation Schedule (ADOS), among children diagnosed with an ASD (Adams et al. 2009).

Clinical Evidence of Susceptibility to Hg in Subjects Diagnosed with an ASD

In heavy metal toxicity, Hg binds to cysteine thiol (–SH) groups on intracellular proteins and inactivates their function. The cysteine-SH group of glutathione binds Hg and protects essential proteins from functional inactivation. The synthesis of glutathione has been directly linked to the rate of Hg excretion and cellular protection from Hg-induced damage. Given the preceding reality, individuals with genetic deficiencies in glutathione synthesis will be less able to excrete Hg and will be more sensitive to its adverse effects (Geier et al. 2008a, 2010).

As summarized in Table 5, several studies in patients diagnosed with an ASD relative to controls demonstrate transsulfuration pathway abnormalities, including significant reductions in plasma cysteine, plasma sulfate, plasma-reduced glutathione, and total glutathione levels (Geier et al. 2008a, 2010). Chauhan et al. (2012) have recently examined concentrations of glutathione in different regions of brains from subjects diagnosed with an ASD and age-matched control subjects. In the cerebellum and temporal cortex from subjects with autism, reduced glutathione levels were significantly decreased by 34.2 and 44.6 %, with a concomitant increase in the levels of oxidized glutathione by 38.2 and 45.5 %, respectively, as compared to the control group. There was also a significant decrease in the levels of total glutathione by 32.9 % in the cerebellum and by 43.1 % in the temporal cortex of subjects diagnosed with an ASD. In contrast, there was no significant change in reduced glutathione, oxidized glutathione, and total glutathione levels in the frontal, parietal, and occipital cortices in the ASD diagnosis group versus control group. The redox ratio of reduced glutathione to oxidized glutathione was also significantly decreased by 52.8 % in the cerebellum and by 60.8 % in the temporal cortex of subjects diagnosed with an ASD, suggesting glutathione redox imbalance in the brain of subjects diagnosed with an ASD. These investigators concluded that their

Table 5 A summary of the evidence showing decreased glutathione-dependent Hg excretion pathways in subjects diagnosed with an ASD in comparison to controls

Evidence of decreased glutathione-dependent Hg excretion in subjects diagnosed with an ASD
Decreased total glutathione levels
Decreased reduced glutathione levels
Elevated oxidized glutathione levels
Decreased cysteine levels
Decreased sulfate levels

findings indicate that ASD is associated with deficits in glutathione antioxidant defense in selective regions of the brain.

A series of studies showed that there were significant correlations between genetic changes associated with reduced functioning in Hg detoxification pathways (i.e., gene deletions/polymorphisms) in patients diagnosed with ASDs in comparison to controls. A significant increasing correlation was found between increased body burden of Hg, as measured by urinary porphyrins, and glutathione unavailability, as measured by plasma oxidized glutathione (Geier et al. 2008a, 2010).

Shandley and Austin (2011) further examined the role of genetic susceptibility to Hg intoxication as a risk factor for a subject being diagnosed with an ASD by examining ancestry of pink disease (infantile acrodynia) as a risk factor for an ASD diagnosis. Specifically, they described pink disease was prevalent in the first half of the twentieth century and was primarily attributed to Hg found in teething powers. Pink disease was observed in about one in 500 exposed children, and as a result it was described that these children were sensitive to Hg intoxication. The objective of the Shandley and Austin (2011) epidemiological study was to test the hypothesis that individuals with a known hypersensitivity to Hg (pink disease survivors) may be more likely to have descendants diagnosed with an ASD. Five hundred and 22 participants who had previously been diagnosed with pink disease completed a survey on the health outcomes of their descendants. The prevalence rates of ASD and a variety of other clinical conditions not associated with Hg and diagnosed in childhood (fragile X syndrome and Down syndrome) were compared to well-established general population prevalence rates. The results showed the prevalence rate of ASD among the grandchildren of pink disease survivors (1 in 22) to be significantly higher than the comparable general population prevalence rate (1 in 160), whereas other conditions not associated with Hg and diagnosed in childhood occurred at similar rates as well-established population prevalence rates. The results support the hypothesis that Hg sensitivity may be a heritable/genetic risk factor for an ASD diagnosis.

James et al. (2009) utilized lymphoblastoid cells (LCLs) derived from children diagnosed with autism and from unaffected controls. These cells were used to assess relative concentrations of reduced glutathione and oxidized glutathione in cell extracts and isolated mitochondria as a measure of intracellular redox capacity. The results indicated that the reduced glutathione/oxidized glutathione redox ratio was decreased and that the percentage of oxidized glutathione increased in both cytosol and mitochondria in the autism LCLs. Exposure to oxidative stress via thimerosal exposure resulted in a greater decrease in the reduced glutathione/oxidized glutathione ratio and an increase in free radical generation in those diagnosed with autism compared to control cells. Acute exposure to physiological levels of nitric oxide decreased mitochondrial membrane potential to a greater extent in the LCLs from those diagnosed with autism, although reduced glutathione/oxidized glutathione and adenosine triphosphate (ATP) concentrations were similarly decreased in both cell lines. These investigators concluded that it is plausible to hypothesize that exposures to prooxidant environmental toxins, including thimerosal, would have the greatest effect on individuals with a preexisting

fragile redox homeostasis or depleted glutathione reserves due to concurrent infection, or who are simultaneously exposed to other prooxidant contaminants that in combination can reach a toxic threshold. These potentially vulnerable subpopulations need to be identified and evaluated independently because large-population epidemiologic studies do not have the sensitivity to detect minor high-risk subpopulations.

Cellular Mechanisms of Hg-Induced ASD

In considering the potential for an exposure to induce a disorder, it is important to demonstrate that the exposure can induce the pathological findings characterizing the disorder in an in vitro model system. Furthermore, estimating the amount of exposure necessary to induce the pathology observed in the disorder and evaluating this in the context of other exposures that may produce and/or contribute to the disorder is essential.

Recent studies have demonstrated that Hg, and in particular thimerosal-induced significant mitochondrial dysfunction, reduced cellular oxidative–reduction activity, cell death, and cellular degeneration in a concentration- and time-dependent fashion (Geier et al. 2009b). The calculated lethal concentration (LC) to stop mitochondrial function for 50 % of the test cells for mitochondrial dysfunction following 24 h incubation with thimerosal (without serum) ranged between 9.7 and 337 nanomole (nM). Additionally, following 48 h of incubation with thimerosal-containing media, the LC50 for cell oxidative – reduction activity in the neuroblastoma cells studied was 7.6 nM (0.003 μg Hg/mL or 0.0015 parts-per-million Hg) thimerosal (without serum). Furthermore, different cell types showed different levels of susceptibility to thimerosal-induced cellular toxicity. The observed order of the sensitivity to thimerosal-induced cellular toxicity for the cell types studied was human fetal cells $>$ human neuroblastoma cells $>$ human astrocytoma cells. Similarly, other investigators reported the LC to kill 50 % of SH-SY5Y human neuroblastoma following incubation with thimerosal (without serum) at 38.7 (0.0077 μg Hg/mL or 0.0077 parts-per-million Hg) and 4.35 nM (0.00087 μg Hg/mL or 0.00087 parts-per-million Hg), at 24 and 48 h, respectively. In addition, other investigators demonstrated that thimerosal significantly increased cell death in neuroblastoma cells, in a concentration- and time-dependent manner, at nM concentrations down to 25 nM (0.005 μg Hg/mL or 0.005 parts-per-million Hg), the lowest concentration of thimerosal tested. These researchers demonstrated that the cell death induced by thimerosal was characterized by visual phenomena, including nuclear morphology of apoptosis, vacuolization, and chromatin condensation and shrinking. Further, investigators reported on mitochondrial-mediated thimerosal-induced apoptosis in SK-N-SH human neuroblastoma cells. These researchers visually observed that low-concentration thimerosal exposure rapidly induced neuronal cell degeneration characterized by alterations in membranes, characteristic of the cellular blebbing seen in apoptosis. In addition, cell shrinkage, and detachment were also observed. Finally, other researchers

showed that low-dose thimerosal exposure rapidly inhibited important neurodevelopmental pathways in neurons (Geier et al. 2008a, 2010).

The concentrations of thimerosal used in many of the aforementioned studies to induce significant neuronal cell toxicity are comparable with physiological levels known to be induced by fetal and early infant exposure to Hg from thimerosal-containing biologics and vaccines. The observed effects induced by thimerosal are also consistent with recently emerging evidence documenting the brain pathophysiology present in patients diagnosed with an ASD.

For example, it was shown that human neuroblastoma cells were significantly more susceptible to thimerosal-induced damage than human astrocytoma cells (Geier et al. 2008a, 2010).

Previously, Toimela and Tahti (2004) evaluated co-cultures of neuroblastoma and astrocytoma cells following organic and inorganic Hg exposures. In co-cultures of neuroblastoma and astrocytoma cells, researchers visually observed that neuroblastoma cells were significantly damaged at Hg concentrations which had little or no observed adverse effects on the astrocytoma cells. Consistent with these observations, Lopez-Hurtado and Prieto (2008) found striking differences in the density of glial cells, the density of neurons and the number of lipofuscin-containing neurons in brain regions associated with the production and processing of speech when samples from patients diagnosed with an ASD were compared to controls. Specifically, it was observed that the brains in the patients diagnosed with an ASD had significantly greater mean densities of glial cells in comparison to controls. By contrast, the density of neurons was significantly decreased in the brain samples of patients diagnosed with autistic disorders in comparison with controls. These researchers observed that patients diagnosed with an ASD had significantly increased numbers of lipofuscin-containing neurons comparison to controls. The presence of lipofuscin in neurons is significant with regard to Hg toxicity, because lipofuscin is a depot for heavy metals such as Hg. It is important to note that visual images obtained from the brain samples of patients diagnosed with an ASD were virtually identical in morphology with those observed in co-cultures of neuroblastoma and astrocytoma cells exposure to Hg (Geier et al. 2008a, 2010).

Finally, several studies observed that significant mitochondrial dysfunction and impaired oxidative–reduction are significant mechanisms underlying neuronal cell damage induced by thimerosal. Consistent with these observations, these studies identified evidence for mitochondrial dysfunction and impaired oxidative–reduction in patients diagnosed with an ASD (Geier et al. 2008a, 2010).

An Explanation of the Male/Female Ratio in Subjects Diagnosed with an ASD

ASD disproportionately affects male children (roughly, five males per one female). Hence, any causal factors for ASD must be able to explain this phenomenon. In animal models and in human poisonings, males were found to be significantly more susceptible to Hg toxicity than females (Geier et al. 2008a, 2010). Also, in a series

Table 6 Parallels between Hg effects on the brain and autism brain pathology

Hg effects on the brain and autism brain pathology
Large, long-range axon degeneration
Dendritic overgrowth
Neuroinflammation
Microglial/astrocytic activation
Brain immune response activation
Elevated GFAP
Oxidative stress and lipid peroxidation
Decreased reduced glutathione levels and elevated oxidized glutathione
Mitochondrial dysfunction
Disrupted calcium homeostasis and signaling
Inhibited glutamic acid decarboxylase activity
Disrupted GABAergic and glutamatergic homeostasis
Inhibited IGF-1 and methionine synthase activity
Impaired methylation
Vascular endothelial cell dysfunction and pathological changes of the blood vessels
Decreased cerebral/cerebellar blood flow
Increased amyloid precursor protein
Granule and Purkinje neuron loss in the cerebellum
Increased proinflammatory cytokine levels (TNF-α, IFN-γ, IL-1beta, IL-6, IL-8)
Aberrant nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

of tissue culture experiments, testosterone was able to potentiate the neuronal toxicity of Hg, whereas estrogen lessened the toxicity (Haley 2005).

Overall Parallels Between Hg Intoxication and the Brain Pathology in ASD

In compiling the cellular mechanisms of Hg neurotoxicity and the brain pathology in subjects diagnosed with an ASD, Kern et al. (2012), as summarized in Table 6, observed many parallels. The research evidence suggests that the pathologies are similar; in fact, there is no pathology found in the brains of those diagnosed with an ASD that cannot be explained by Hg intoxication.

Conclusion

The dramatic increase in the prevalence of diagnosed ASDs in recent years suggests external or environmental factors have contributed to causation. Examination of childhood vaccine schedules reveals a dramatic increase in Hg exposure beginning

for the most part in the 1990s. In addition to vaccines, other sources such as drugs, fish and other foods, dental amalgams, and air Hg sources (e.g., coal-burning power plants) can add to the exposure. Mercury is known to accumulate in the brain, persist, and be lethal to neurons. Several studies have shown that children with an ASD diagnosis have higher levels of Hg burden relative to neurotypical controls. Moreover, the neurological damage caused by Hg is consistent with the abnormalities found in the brains of children diagnosed with an ASD. Unfortunately, Hg not only directly kills brain cells but also disrupts the critical mechanisms and pathways needed to eliminate it. The evidence of a causal role for Hg in ASD pathogenesis is compelling.

Key Terms

Body burden. Body burden is the amount of toxic chemical compounds, elements, or their metabolites in the body. Often measurements of body burden are measured by the use of blood or urine. In addition, body burden can be indirectly measured by the effects of the compound on body biological process such the altered porphyrin profile found in urine samples from Hg-poisoned individuals whose light-protected urine samples have been subjected to porphyrin testing.

Glutathione. Glutathione is a tripeptide with a linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain and a normal peptide linkage between cysteine and glycine. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species, and is important to the body's natural excretion of Hg.

Mercury. Mercury (Hg) is a chemical element with an atomic number of 80. It is also known as quicksilver and is the only metal that is liquid at standard conditions for temperature and pressure. It is highly toxic by ingestion, inhalation, and other routes of administration.

Methylmercury. The methyl-Hg moiety is composed of a methyl group bonded to a mercury atom. It readily combines with anions such as chloride, hydroxide, and nitrate, and it also has a very high affinity particularly for the thiol (–SH) group on the amino acid cysteine and hence in proteins containing cysteine. In the past methyl-Hg compounds were produced directly and indirectly as part of several industrial processes but currently are mostly the indirect consequence of burning fuel containing inorganic Hg (i.e., coal). Methyl-Hg is formed from inorganic Hg by the action of anaerobic organisms that live in aquatic systems. Methyl-Hg is biomagnified in aquatic food chains from bacteria to plankton, through macroinvertebrates, to herbivorous fish and to fish-eating fish and fish-eating aquatic mammals (e.g., dolphins, seals, and whales). At each step in the food chain, the concentration of the methyl-Hg compounds stored increases, so that at top level aquatic predators can reach a level a million times higher than the background level of Hg in the water. In humans,

methyl-Hg compounds are apparently formed by demethylation of ethyl-Hg compounds degradatively formed from the metabolic breakdown of thimerosal in the human body.

Oxidative Stress. Oxidative stress represents an imbalance between reactive oxygen species found systemically and the ability to detoxify the reactive intermediates or to repair the resulting damage in a biological system. Mercury is known to significantly increase oxidative stress, and oxidative stress is known to induce significant cellular dysfunction by damaging all components of the cell, including proteins, lipids, and DNA.

Thimerosal is an organic Hg-containing compound that is about 50 % Hg by weight. It was originally developed by Eli Lilly and Corporation, and it has been used as a preservative in vaccines, immunoglobulin preparations, skin test antigens, antivenins, and ophthalmic and nasal products. Its use as a vaccine preservative is controversial, and it has been and/or is being phased out of vaccines in some countries. In the body, it is metabolized or degraded to ethyl-Hg species and thiosalicylate. Thimerosal is very toxic be inhalation, ingestion, and in contact with skin, with a danger of cumulative effects. It is also very toxic to aquatic organisms and may cause long-term adverse effects in aquatic environments.

Key Facts

- Hg, a heavy metal, is widespread and persistent in the environment and is a ubiquitous source of danger in fish, drugs, fungicides/herbicides, dental fillings, thermometers, vaccines, immune globulins, and many other products.
- Elevated Hg concentrations may remain in the brain from several years to decades following exposure and, as a result, may play an important etiological role in the brain for the development of childhood neurodevelopmental disorders such as ASD.
- Glutathione is a sulfur-bearing amino acid tripeptide that is deficient in many subjects diagnosed with an ASD and makes them significantly more susceptible to the toxic consequences of Hg exposure.
- ASD disproportionately affects male children, and the sexual dimorphism found in those with an ASD diagnosis may result from synergistic neurotoxicity caused by the interaction of testosterone and Hg; in contrast, estrogen is protective and mitigates the toxicity of Hg species.
- Case reports of patients have described developmental regressions with ASD symptoms following fetal and/or early childhood Hg exposure, and epidemiological studies have linked exposure to Hg with an elevated risk of a patient being diagnosed with an ASD.
- Animal models of fetal/infant exposure to Hg compounds have revealed the emergence of ASD symptoms.
- Clinical studies of subjects diagnosed with an ASD revealed elevated Hg body burden and significant correlations between quantitative measurements of ASD severity and Hg body burden.

- Immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASD were reported following Hg intoxication with similarities extending to neuroanatomy, neurotransmitters, and biochemistry.

Summary Points

- ASD is a behaviorally defined neurodevelopmental disorder that is usually diagnosed in early childhood following fetal/infant exposure to Hg from ubiquitous environmental sources.
- Developmental regressions with ASD symptoms have been described following fetal/infant Hg exposure in case reports and epidemiological studies.
- Experiments in mice, rats, hamsters, and monkeys have revealed that fetal/infant Hg exposure is associated with emergence of pathological brain lesions and behavioral characteristics consistent with those observed in subjects diagnosed with an ASD.
- Elevated levels of Hg were found in clinical studies of subjects diagnosed with an ASD by examining samples of brain, blood, urine, feces, hair, fingernails, baby teeth, and urinary orphyrins; and significant correlations were observed between quantitative measurements of ASD severity and Hg samples measured in subjects diagnosed with an ASD.
- Subjects diagnosed with an ASD were observed to have significant impairments in Hg excretion pathways, and further, synergistic neurotoxicity was observed in interactions between testosterone (the male hormone) and Hg species; in contrast, estrogen (the female hormone) was protective, mitigating the toxicity of Hg species.
- The neurological damage caused by Hg species is consistent with the abnormalities found in the brains of subjects diagnosed with an ASD.
- Overall, the evidence for a causal role for Hg species in ASD pathogenesis is compelling.

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