



Sensible Action For Ending Mercury-Induced
Neurological Disorders

Summary of Science Demonstrating the Harmful Nature of Mercury in Vaccines

2009 SCIENCE SUMMARY UPDATE

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Introduction

As part of the Food and Drug Administration (FDA) Modernization Act, an assessment of thimerosal use in vaccines was conducted from 1997 to 1999. The FDA investigation was unable to locate any clinical studies formally evaluating the use of thimerosal before its initial marketing in the 1930's. The only study found was from 1931 where thimerosal was administered to individuals suffering from meningitis. The study was not designed to specifically examine toxicity; no clinical assessments were described nor were laboratory studies reported. In the paper, the authors acknowledge the clinician who treated the meningitis patients was not convinced of its efficacy stating “beneficial effects of the drug were not definitely proven.” Industry scientists noted in 1930 that a “wide range of toxicity and injury tests should be done” but they were not.

Today, the scientific literature is flush with research that documents deleterious effects of thimerosal on numerous organ systems, including the immune, metabolic and nervous, in mammals and humans. These effects may vary depending on the dose, the genetics of the individual, and the timing of exposure. This research strongly suggests that ethyl mercury exposure from thimerosal containing vaccines given to infants or pregnant women has the potential to cause harmful effects.

Therefore, in the interest of precaution, removal of mercury from vaccines given to vulnerable populations is warranted. Actions that lead to removal of thimerosal, particularly given that sufficient supplies of mercury free vaccines are readily available, should be supported.

In addition, *all* of the recommendations for additional research from the Institute of Medicine Immunization Safety Review report: *Thimerosal Containing Vaccines and Neurodevelopmental Disorders*, 2001 should be conducted immediately. We note that the 2004 report from the Institute of Medicine in this regard, *Immunization Safety Review: Vaccines and Autism*, did not fulfill the recommendations from the 2001 report, regarding clinical and biological science, and relied heavily on epidemiological studies containing serious design flaws and conflicts of interest.

This document is a brief summary of recently published science, conducted in the many fields of research recommended in the initial report by the Institute of Medicine in 2001, regarding thimerosal at doses which correspond to levels found in vaccines, or at concentrations that are likely to result from vaccine administration.

A brief summary of research supporting other forms of mercurials and their role in autism, autism behaviors and known biological anomalies have been included, as mercury from all vectors is known to impact human development.

Human & Infant Research

IA TROGENIC EXPOSURE TO MERCURY AFTER HEPATITIS B VACCINATION IN PRETERM INFANTS

Stajich GV, Lopez GP, Harry SW, Sexson, SW. *J Pediatr.* 2000 May; 136(5):679-81

Stajich measured blood mercury levels in low birth weight and term newborns administered the Hepatitis B vaccine containing 12.5 µg ethyl mercury. The investigation documented elevated post-immunization concentrations relative to pre-immunization levels in all neonates studied. Levels of blood mercury after exposure in low birth weight infants were 7.36 (± 4.99) µg/L. Note: One infant was found to have developed a mercury level of 23.6 µg/L, thus meeting the CDC criteria as a case of chemical poisoning from mercury defined as a blood level of 10µg/L or greater.

MERCURY CONCENTRATIONS AND METABOLISM IN INFANTS RECEIVING VACCINES CONTAINING THIMEROSAL: A DESCRIPTIVE STUDY

Pichichero ME, Cernichiari E, Lopreiato J and Treanor J. *Lancet.* 2002; 360:1737-41.

Pichichero reported a mercury blood level in a 2-month-old infant of 20.55 nmol/L five days after the infant received a 37.5 µg dose of ethylmercury (the amount contained in one DTaP and one Hepatitis B vaccine). Many infants, however, beginning in the early 1990's and for the next decade, received a 62.5 µg dose of ethylmercury (adding in the Haemophilus influenzae type b (Hib) vaccine) at the 2-month well baby visit. A vaccine expert from the Johns Hopkins Institute for Vaccine Safety estimated that these infants may have experienced peak blood mercury levels of 48.3 nmol/L; well above the presumed EPA safety threshold of 29.0 nmol/L. As a reference point, the CDC recently defined a toxic exposure to mercury in an adult as a blood mercury level of >10µg /L (50 nmol/L) -- approximately the same blood level that some infants experienced at two months of age.

HAIR MERCURY IN BREAST-FED INFANTS EXPOSED TO THIMEROSAL-PRESERVED VACCINES

Marques RC, Dorea JG, Fonseca MF, Bastos WR, Malm O. *Eur J Pediatr.* 2007 Jan 20; [Epub ahead of print]

Marques investigated the impact of thimerosal on the total mercury content of hair in breast fed infants receiving thimerosal containing vaccines and found exposure to vaccine-EtHg represents 80% of that expected from total breast milk-Hg in the first month but only 40% of the expected exposure integrated in the 6 months of breastfeeding. However, the Hg exposure corrected for body weight at the day of immunization was much higher from thimerosal- EtHg (5.7 to 11.3 mugHg/kg b.w.) than from breastfeeding (0.266 mugHg/kg b.w.). While mothers showed a relative decrease (-57%) in total hair-mercury during the 6 months lactation there was substantial increase in the infant's hair-mercury (446%).

MERCURY LEVELS IN NEWBORNS AND INFANTS AFTER RECEIPT OF THIMEROSAL-CONTAINING VACCINES

Pediatrics. 2008 Feb;121(2):e208-14

Pichichero ME, Gentile A, Giglio N, Umido V, Clarkson T, Cernichiari E, Zareba G, Gotelli C, Gotelli M, Yan L, Treanor J Dept of Microbiology/Immunology, Pediatrics, & Medicine, University of Rochester

CONCLUSIONS: The blood half-life of intramuscular ethyl mercury from thimerosal in vaccines in infants is substantially shorter than that of oral methyl mercury in adults. Increased mercury levels were detected in stools after vaccination, suggesting that the gastrointestinal tract is involved in ethyl mercury elimination. Because of the differing pharmacokinetics of ethyl and methyl mercury, exposure guidelines based on oral methyl mercury in adults may not be accurate for risk assessments in children who receive thimerosal-containing vaccines.

CAN CHILDREN WITH AUTISM RECOVER? IF SO, HOW?

Helt M, Kelley E, Kinsbourne M, Pandey J, Boorstein H, Herbert M, Fein D. Department of Psychology, University of Connecticut, Storrs, CT 06268, USA. Neuropsychol Rev. 2008 Dec;18(4):339-66. Epub 2008 Nov 14.

Although Autism Spectrum Disorders (ASD) are generally assumed to be lifelong, we review evidence that between 3% and 25% of children reportedly lose their ASD diagnosis and enter the normal range of cognitive, adaptive and social skills. Predictors of recovery include relatively high intelligence, receptive language, verbal and motor imitation, and motor development, but not overall symptom severity. Earlier age of diagnosis and treatment, and a diagnosis of Pervasive Developmental Disorder-Not Otherwise Specified are also favorable signs. The presence of seizures, mental retardation and genetic syndromes are unfavorable signs, whereas head growth does not predict outcome. Controlled studies that report the most recovery came about after the use of behavioral techniques. Residual vulnerabilities affect higher-order communication and attention. Tics, depression and phobias are frequent residual co-morbidities after recovery. Possible mechanisms of recovery include: normalizing input by forcing attention outward or enriching the environment; promoting the reinforcement value of social stimuli; preventing interfering behaviors; mass practice of weak skills; reducing stress and stabilizing arousal. Improving nutrition and sleep quality is non-specifically beneficial.

HEPATITIS B TRIPLE SERIES VACCINE AND DEVELOPMENTAL DISABILITY IN US CHILDREN AGED 1–9 YEARS

Carolyn Gallagher* and Melody Goodman
Toxicological & Environmental Chemistry
Vol. 90, No. 5, September–October 2008, 997–1008

This study investigated the association between vaccination with the Hepatitis B triple series vaccine prior to 2000 and developmental disability in children aged 1–9 years (n=1824), proxied by parental report that their child receives early intervention or special education services (EIS). National Health and Nutrition Examination Survey 1999–2000

data were analyzed and adjusted for survey design by Taylor Linearization using SAS version 9.1 software, with SAS callable SUDAAN version 9.0.1. The odds of receiving EIS were approximately nine times as great for vaccinated boys (n¹/446) as for unvaccinated boys (n¹/47), after adjustment for confounders. This study found statistically significant evidence to suggest that boys in United States who were vaccinated with the triple series Hepatitis B vaccine, during the time period in which vaccines were manufactured with thimerosal, were more susceptible to developmental disability than were unvaccinated boys.

MERCURY AND HUMAN GENOTOXICITY: CRITICAL CONSIDERATIONS AND POSSIBLE MOLECULAR MECHANISMS.

Crespo-López ME, Macêdo GL, Pereira SI, Arrifano GP, Picanço-Diniz DL, Nascimento JL, Herculano AM.

Laboratório de Farmacologia Molecular, Brazil.

Pharmacol Res. 2009 Mar 9. [Epub ahead of print]

Mercury compounds versatility explains their numerous applications in diverse areas of industry. The growing use of this metal has resulted in a significant increase of environment contamination and episodes of human intoxication, arousing the concern of international organisms. Meanwhile, consequences of these intoxication outbreaks are still not fully understood, especially if we consider long-term effects of chronic exposure to relatively low levels of mercury compounds. In the present manuscript, studies about the genotoxicity of mercury compounds, performed in vitro, in vivo, and/or including epidemiologic studies of human populations were reviewed. Some mercury compounds are known as teratogenic agents, especially affecting the normal development of the central nervous system; however, the connection between mercury exposure and carcinogenesis remains controversial. Since 1990s, epidemiological studies have begun to include an increasing number of human subjects, making the results more reliable: thus, increased genotoxicity was demonstrated in human populations exposed to mercury through diet, occupation or by carrying dental fillings. In fact, concentrations of methylmercury causing significant genotoxic alterations in vitro below both safety limit and concentration were associated with delayed psychomotor development with minimal signs of methylmercury poisoning. Based on mercury's known ability to bind sulfhydryl groups, several hypotheses were raised about potential molecular mechanisms for the metal genotoxicity. Mercury may be involved in four main processes that lead to genotoxicity: generation of free radicals and oxidative stress, action on microtubules, influence on DNA repair mechanisms and direct interaction with DNA molecules. All data reviewed here contributed to a better knowledge of the widespread concern about the safety limits of mercury exposure.

NEONATE EXPOSURE TO THIMEROSAL MERCURY FROM HEPATITIS B VACCINES.

Dórea JG, Marques RC, Brandão KG.

Universidade de Brasília, Brasília, DF, Brazil.

[Am J Perinatol.](#) 2009 Mar 12. [Epub ahead of print]

Infant exposure to ethylmercury (EtHg) has not only increased but is starting earlier as a result of the current immunization schedule that uses thimerosal-containing vaccines (TCVs). Although vaccination schedule varies considerably between countries, infants in less-developed countries continue to be exposed to EtHg derived from more affordable TCVs. We studied the exposure of newborns to EtHg from hepatitis B vaccines; hospital

records (21,685) were summarized for the years 2001 to 2005 regarding date of birth, vaccination date, and birth weight. Most of the vaccinations occurred in the first 24 hours postdelivery; over the 5 years, there was an increase in vaccinations within hours of birth (same day), from 7.4% (2001) to 87.8% (2005). Nearly 94.6% of infants are now being vaccinated within the first 24hours. Range of mercury exposure spread from 4.2 to 21.1 mug mercury/kg body weight for those receiving TCVs with the highest thimerosal concentration; these exposure levels are conservative for 2% of children receiving vaccines within 2 to 3 postnatal days, when they are still going through physiological postnatal weight loss. Because of the particular timing (transitioning from in utero to ex utero metabolism) and specific aspects of exposure (i.e., parenteral mode, bypassing gastroenteric barriers) and dose (related to vaccine manufacturer and with variation in birth weight), this study reveals critical issues that can modulate toxicokinetics and toxicodynamics of organomercurials in neonates.

Infant Primate Research

COMPARISON OF BLOOD AND BRAIN MERCURY LEVELS IN INFANT MONKEYS EXPOSED TO METHYLMERCURY OR VACCINES CONTAINING THIMEROSAL

*Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T.
Environmental Health Perspectives. 2005 Aug;113(8):1015-21.*

Burbacher compared brain mercury levels in infant *Macaca fascicularis* primates exposed to injected ethylmercury (thimerosal) and equal amounts of ingested methylmercury. The ethylmercury more rapidly converted to inorganic mercury in the brains of the primates which resulted in increasing levels of inorganic mercury and the primates exposed to ethylmercury retained at least twice as much inorganic mercury in their brains compared to the primates exposed to methylmercury. The relative concentrations in monkeys with detectable levels of inorganic mercury were 16 ng/g in thimerosal-treated monkeys and 7 ng/g in the methylmercury-treated monkeys in which inorganic mercury levels were detectable. Inorganic mercury was below detectable levels in 8 out of 17 of the methylmercury-treated monkeys. Exposures to mercury during these critical periods of development disrupt the growth and migration of neurons, with the potential to cause irreversible damage to the central nervous system. Prior primate studies found inorganic mercury in the brain was associated with microgliosis and neuroinflammation, recent finding also documented in autistic brain.

PEDIATRIC VACCINES INFLUENCE PRIMATE BEHAVIOR, AND AMYGDALA GROWTH AND OPIOID LIGAND BINDING

Friday, May 16, 2008: IMFAR

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Background: Macaques are commonly used in pre-clinical vaccine safety testing, but the combined childhood vaccine regimen, rather than individual vaccines, has not been studied. Childhood vaccines are a possible causal factor in autism, and abnormal behaviors and anomalous amygdala growth are potentially inter-related features of this condition.

Objectives: The objective of this study was to compare early infant cognition and behavior with amygdala size and opioid binding in rhesus macaques receiving the recommended childhood vaccines (1994-1999), the majority of which contained the bactericidal preservative ethylmercurithiosalicylic acid (thimerosal).

Methods: Macaques were administered the recommended infant vaccines, adjusted for age and thimerosal dose (exposed; N=13), or saline (unexposed; N=3). Primate development, cognition and social behavior were assessed for both vaccinated and unvaccinated infants using standardized tests developed at the Washington National Primate Research Center. Amygdala growth and binding were measured serially by MRI and by the binding of the non-selective opioid antagonist [¹¹C]diprenorphine, measured by PET, respectively, before (T1) and after (T2) the administration of the measles-mumps-rubella vaccine (MMR).

Results: Compared with unexposed animals, significant neurodevelopmental deficits were evident for exposed animals in survival reflexes, tests of color discrimination and reversal, and learning sets. Differences in behaviors were observed between exposed and unexposed animals and within the exposed group before and after MMR vaccination. Compared with unexposed animals, exposed animals showed attenuation of amygdala growth and differences in the amygdala binding of [¹¹C]diprenorphine. Interaction models identified significant associations between specific aberrant social and non-social behaviors, isotope binding, and vaccine exposure.

Conclusions: This animal model, which examines for the first time, behavioral, functional, and neuromorphometric consequences of the childhood vaccine regimen, mimics certain neurological abnormalities of autism. The findings raise important safety issues while providing a potential model for examining aspects of causation and disease pathogenesis in acquired disorders of behavior and development.

PEDIATRIC VACCINES INFLUENCE PRIMATE BEHAVIOR, AND BRAIN STEM VOLUME AND OPIOID LIGAND BINDING

Saturday, IMFAR

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Background: Abnormal brainstem structure and function have been reported in children with autism. Opioid receptors play key roles in neuro-ontogeny, are present in brainstem nuclei, and may influence aspects of autism. Childhood vaccines are a possible causal factor in autism and while primates are used in pre-clinical vaccine safety testing, the recommended infant regimen (1994-1999) has not been tested.

Objectives: The objective of this study was to compare brain stem volume and opioid binding in rhesus infants receiving the recommended infant vaccine regimen.

Methods: Rhesus macaques were administered vaccines adjusted for age and thimerosal dose (exposed; N=13), or placebo (unexposed; N=3) from birth onwards. Brainstem volume was measured by quantitative MRI, and binding of the non-selective opioid antagonist [11C]diprenorphine (DPN) was measured by PET, at 2 (T1) and 4 (T2) months of age. Neonatal reflexes and sensorimotor responses were measured in standardized tests for 30 days.

Results: Kaplan-Meier survival analyses revealed significant differences between exposed and unexposed animals, with delayed acquisition of root, suck, clasp hand, and clasp foot reflexes. Interaction models examined possible relationships between time-to-acquisition of reflexes, exposure, [3C]DPN binding, and volume. Statistically significant interactions between exposure and time-to-acquisition of reflex on overall levels of binding at T1 and T2 were observed for all 18 reflexes. For all but one (snout), this involved a mean increase in time-to-acquisition of the reflex for exposed animals. In each model there was also a significant interaction between exposure and MRI volume on overall binding.

Conclusions: This animal model examines the neurological consequences of the childhood vaccine regimen. Functional and neuromorphometric brainstem anomalies were evident in vaccinated animals that may be relevant to some aspects of autism. The findings raise important safety issues while providing a potential animal model for examining aspects of causation and disease pathogenesis in acquired neurodevelopmental disorders.

MICROARRAY ANALYSIS OF GI TISSUE IN A MACAQUE MODEL OF THE EFFECTS OF INFANT VACCINATION

Saturday, May 17, 2008 IMFAR

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Background: There has been considerable debate regarding the question of an interaction between childhood vaccinations and adverse sequelae in the gastrointestinal tract, immune system, and central nervous system of some recipients. These systems, either singly or in combination, appear to be adversely affected in many ASD children. Although pre-clinical tests of individual vaccines routinely find the risk/benefit ratio to be low, previously there has not been a study to examine the effects of the comprehensive vaccination regime currently in use for infants.

Objectives: This study was designed to evaluate potential alterations in normal growth and development resulting from the vaccine regimen that was in use from 1994-1999. Specifically, this portion of the study was to compare the gene expression profiles obtained from gastrointestinal tissue from vaccinated and unvaccinated infants.

Methods: Infant male macaques were vaccinated (or given saline placebo) using the human vaccination schedule. Dosages and times of administration were adjusted for differences between macaques and humans. Biopsy tissue was collected from the animals at three time points: (1) 10 weeks [pre-MMR1], (2) 14 weeks [post-MMR1] and, (3) 12-15 months [at necropsy]. Whole genome microarray analysis was performed on RNA extracted from the GI tissue from 7 vaccinated and 2 unvaccinated animals at each of these 3 time points (27 samples total).

Results: Histopathological examination revealed that vaccinated animals exhibited progressively severe chronic active inflammation, whereas unexposed animals did not. Gene expression comparisons between the groups (vaccinated versus unvaccinated) revealed only 120 genes differentially expressed ($fc > 1.5$; $\log \text{ratio } p < 0.001$) at 10 weeks, whereas there were 450 genes differentially expressed at 14 weeks, and 324 differentially expressed genes between the 2 groups at necropsy.

Conclusions: We have found many significant differences in the GI tissue gene

expression profiles between vaccinated and unvaccinated animals. These differences will be presented and discussed.

Animal Research

COMPARISON OF ORGANIC AND INORGANIC MERCURY DISTRIBUTION IN SUCKLING RATS

Orct T, Blanusa M, Lazarus M, Varnai VM, Kostial K. J. Appl. Toxicol. 2006; 26: 536-539.

Orct compared body distribution of organic mercury (thimerosal) and inorganic mercury in suckling rats imitating the vaccination schedule of infants. The levels of mercury were higher in the liver and kidney of the inorganic group and the thimerosal group demonstrated higher levels in the blood and brain tissue. Brain retention of mercury in the thimerosal group was 1.5 times higher than the inorganic mercury group, which confirms the fact that thimerosal more easily crosses the blood-brain barrier and may result in significant accumulation with repeated exposure.

IMMUNOSUPPRESSIVE AND AUTOIMMUNE EFFECTS OF THIMEROSAL IN MICE

Havarinasab S, Haggqvist B, Bjorn E, Pollard KM Hultman P. Toxicol Appl Pharmacol. 2005 Apr 15;204(2):109-21

Havarinasab studied the effect of thimerosal by treating A.SW (H-2S) mice, susceptible to induction of autoimmunity by heavy metals, with thimerosal in drinking water developed antinuclear antibodies (ANoA) whereas mice sharing background genes with the A.SW and B10.S strain, but with a different H-2 haplotype, did not develop ANoA, linking the susceptibility to H-2. They concluded that thimerosal has initial immunosuppressive effects similar to those of MeHg. However, in contrast to MeHg, thimerosal treatment leads in genetically susceptible mice to a second phase with strong immunostimulation and autoimmunity, which is T-cell dependent, H-2 linked and may at least partly be due to the inorganic mercury derived from the metabolism of ethyl mercury.

NEUROTOXIC EFFECTS OF POSTNATAL THIMEROSAL ARE MOUSE STRAIN DEPENDENT

Hornig M, Chian D, Lipkin WI. Molecular Psychiatry. 2004 Sep;9(9):833-45.

Hornig exposed autoimmune-prone infant mice with thimerosal-containing vaccines at the dose given to human infants adjusted for mouse weight. This investigation reported a number of observable effects including growth delay; reduced locomotion; exaggerated response to novelty; and densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Strains resistant to autoimmunity were not susceptible. These findings implicate genetic influences and provide a model for investigating thimerosal-related neurotoxicity.

EFFECT OF THIMEROSAL, A PRESERVATIVE IN VACCINES, ON INTRACELLULAR CA²⁺ CONCENTRATION OF RAT CEREBELLAR NEURONS

Ueha-Ishibashi T, Oyama Y, Nakao H, Umebayashi C, Nishizaki Y, Tatsuishi T, Iwase K, Murao K, Seo H. Toxicology 2004 Jan 15;195(1):77-84.

Ueha-Ishibashi investigated the effect of thimerosal on cerebellar neurons dissociated from 2-week-old rats was compared with those of methylmercury. Both agents at 1 microM or more similarly decreased the cellular content of glutathione in a concentration-dependent manner, suggesting an increase in oxidative stress and increased intercellular concentrations of Ca²⁺. Thimerosal was also found to exert cytotoxic actions on cerebellar granule neurons and its potency was similar to that of methylmercury. The FDA and EPA use methylmercury as their toxicity standard, so demonstration of equivalence shows the potential of thimerosal to cause the same harm as methylmercury, for which more research exists.

THIMEROSAL DISTRIBUTION AND METABOLISM IN NEONATAL MICE: COMPARISON WITH METHYL MERCURY

Neurotoxicology. 2007 Feb 23; : 17382399

Grazyna Zareba, Elsa Cernichiari, Rieko Hojo, Scott Mc Nitt, Bernard Weiss, Moiz M Mumtaz, Dennis E Jones, Thomas W Clarkson

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Thimerosal, which releases the ethyl mercury radical as the active species, has been used as a preservative in many currently marketed vaccines throughout the world. Because of concerns that its toxicity could be similar to that of methyl mercury, it is no longer incorporated in many vaccines in the United States. There are reasons to believe, however, that the disposition and toxicity of ethyl mercury compounds, including thimerosal, may differ substantially from those of the methyl form. The current study sought to compare, in neonatal mice, the tissue concentrations, disposition and metabolism of thimerosal with that of methyl mercury. ICR mice were given single intramuscular injections of thimerosal or methyl mercury (1.4 mg Hg kg⁻¹) on postnatal day 10 (PND 10). Tissue samples were collected daily on PND 11-14. Most analysed tissues demonstrated different patterns of tissue distribution and a different rate of mercury decomposition. The mean organic mercury in the brain and kidneys was significantly lower in mice treated with thimerosal than in the methyl mercury-treated group. In the brain, thimerosal-exposed mice showed a steady decrease of organic mercury levels following the initial peak, whereas in the methyl mercury-exposed mice, concentrations peaked on day 2 after exposure. In the kidneys, thimerosal-exposed mice retained significantly higher inorganic mercury levels than methyl mercury-treated mice. In the liver both organic and inorganic mercury concentrations were significantly higher in thimerosal-exposed mice than in the methyl mercury group. Ethyl mercury was incorporated into growing hair in a similar manner to methyl mercury. The data showing significant kinetic differences in tissue distribution and metabolism of mercury species challenge the assumption that ethyl mercury is toxicologically identical to methyl mercury. Copyright (c) 2007 John Wiley & Sons, Ltd.

GENDER-SELECTIVE TOXICITY OF THIMEROSAL

Exp Toxicol Pathol. 2008 Sep 2. [Epub ahead of print]

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A recent report shows a correlation of the historical use of thimerosal in therapeutic immunizations with the subsequent development of autism; however, this association remains controversial. Autism occurs approximately four times more frequently in males compared to females; thus, studies of thimerosal toxicity should take into consideration gender-selective effects. The present study was originally undertaken to determine the maximum tolerated dose (MTD) of thimerosal in male and female CD1 mice. However, during the limited MTD studies, it became apparent that thimerosal has a differential MTD that depends on whether the mouse is male or female. At doses of 38.4-76.8mg/kg using 10% DMSO as diluent, seven of seven male mice compared to zero of seven female mice tested succumbed to thimerosal. Although the thimerosal levels used were very high, as we were originally only trying to determine MTD, it was completely unexpected to observe a difference of the MTD between male and female mice. Thus, our studies, although not directly addressing the controversy surrounding thimerosal and autism, and still preliminary due to small numbers of mice examined, provide, nevertheless, the first report of gender-selective toxicity of thimerosal and indicate that any future studies of thimerosal toxicity should take into consideration gender-specific differences.

INDUCTION OF METALLOTHIONEIN IN MOUSE CEREBELLUM AND CEREBRUM WITH LOW-DOSE THIMEROSAL INJECTION.

Minami T, Miyata E, Sakamoto Y, Yamazaki H, Ichida S.

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Cell Biol Toxicol. 2009 Apr 9. [Epub ahead of print]

Thimerosal, an ethyl mercury compound, is used worldwide as a vaccine preservative. We previously observed that the mercury concentration in mouse brains did not increase with the clinical dose of thimerosal injection, but the concentration increased in the brain after the injection of thimerosal with lipopolysaccharide, even if a low dose of thimerosal was administered. Thimerosal may penetrate the brain, but is undetectable when a clinical dose of thimerosal is injected; therefore, the induction of metallothionein (MT) messenger RNA (mRNA) and protein was observed in the cerebellum and cerebrum of mice after thimerosal injection, as MT is an inducible protein. MT-1 mRNA was expressed at 6 and 9 h in both the cerebrum and cerebellum, but MT-1 mRNA expression in the cerebellum was three times higher than that in the cerebrum after the injection of 12 microg/kg thimerosal. MT-2 mRNA was not expressed until 24 h in both organs. MT-3 mRNA was expressed in the cerebellum from 6 to 15 h after the injection, but not in the cerebrum until 24 h. MT-1 and MT-3 mRNAs were expressed in the cerebellum in a dose-dependent manner. Furthermore, MT-1 protein was detected from 6 to 72 h in the cerebellum after 12 microg/kg of thimerosal was injected and peaked at 10 h. MT-2 was detected in the cerebellum only at 10 h. In the cerebrum, little MT-1 protein was detected at 10 and 24 h,

and there were no peaks of MT-2 protein in the cerebrum. In conclusion, MT-1 and MT-3 mRNAs but not MT-2 mRNA are easily expressed in the cerebellum rather than in the cerebrum by the injection of low-dose thimerosal. It is thought that the cerebellum is a sensitive organ against thimerosal. As a result of the present findings, in combination with the brain pathology observed in patients diagnosed with autism, the present study helps to support the possible biological plausibility for how low-dose exposure to mercury from thimerosal-containing vaccines may be associated with autism.

An earlier study by the same lab related to the above article:

***EFFECTS OF LIPOPOLYSACCHARIDE AND CHELATOR ON MERCURY CONTENT
IN THE CEREBRUM OF THIMEROSAL-ADMINISTERED MICE***

Takeshi Minami, Keisuke Oda, Naoya Gima, Hideo Yamazaki
Environmental Toxicology and Pharmacology
Volume 24, Issue 3, November 2007, Pages 316-320

Thimerosal is one of the best-known preservative agents for vaccines in the world but a relationship between its use and autism has long been suspected so that its effects on the brain need more detailed research. We here examined the influence of lipopolysaccharide injury to the blood–brain barrier on the penetration of mercury from thimerosal into mouse cerebrums, as well as the effect of chelator of heavy metals on cerebrum mercury content. Mercury can be expected to be detected in the cerebrum of normal mice, because the metal is present in standard mouse chow. When 60 µg/kg of thimerosal was subcutaneously injected into the mouse, the mercury content in the cerebrum was significantly higher 48 h after the thimerosal injection with a maximum peak after 72 h. In addition, mercury content in the cerebrum was still higher on day 7 than in the control group. When lipopolysaccharide was pre-injected into mice to induce damage on blood–brain barrier, the mercury content in the cerebrum was significantly higher at 24 and 72 h after the injection of 12 µg/kg of thimerosal compared to the control group, this dose alone does not cause any increase. The mercury content in the cerebrums of mice was decreased to the control group level on day 7 when a chelator, dimercaprol, was administered once a day from days 3 to 6 after a 60 µg/kg, s.c. injection. In addition, d-penicillamine as a chelator decreased the mercury contents in the cerebrum after the high dose administration. In conclusion, a physiological dose of thimerosal did not increase the content of mercury in the cerebrum, but levels were increased when damage to the blood–brain barrier occurred in mice injected with thimerosal. In addition, a chelator of heavy metals may be useful to remove mercury from the cerebrum.

EFFECTS OF INTERMITTENT, VACCINATION-LIKE SCHEME, THIMEROSAL ADMINISTRATION ON RAT DEVELOPMENT AND BEHAVIOUR.

Olczak M., Duszczyk M., Mierzejewski P. & Majewska M. D.
Dept. Pharmacol. Inst. Psychiatry & Neurology, Warsaw, Poland
Publication ref.: *FENS Abstr.*, vol.4, 083.19, 2008

Mercury from thimerosal, which was added to many child vaccines, is one of the agents suspected to be responsible for autism epidemics observed in the past two decades. Data analysis from Vaccine Adverse Event Reporting System of the Center for Disease Control and Prevention (USA) documented that children immunized with vaccines containing thimerosal were several times more likely to develop autism and other neurodevelopmental diseases/disorders than those, who did not receive thimerosal. In this study we examined the potential neurotoxic effects of different cumulative doses of thimerosal, from 0.040 mg/kg to 25 mg/kg, administered to rats s.c. or i. m. in four doses on postnatal days 7-14. Three strains of rats were tested: Wistar, Lewis and Brown Norway. Development and behaviour of the experimental animals was monitored. At different developmental stages (between weeks 4 and 22 of age) several behavioral tests were conducted, which included open field locomotor activity, motor coordination, pain reaction (hot plate), water maze learning and memory test, prepulse inhibition, and social interaction test. Brains of thimerosal treated rats accumulated a significant amount of mercury. They were examined for histopathological changes. Generally, rats appeared to be quite resistant to overt neurotoxic effects of thimerosal at doses tested, although higher doses of this drug caused subtle changes on some behavioral measures, which appear to be species and sex dependent. Significant thimerosal effects on pain reaction, certain learning parameters and prepulse inhibition were observed. Also some aspects of social interactions were altered. Behavioural and histopathological data will be presented in the context of putative rat model of mercury-mediated neurodevelopmental pathologies. Funded by EC grant MEXC-CT-2006-42371 to M. D. Majewska.

EFFECTS OF POSTNATAL ADMINISTRATION OF THIMEROSAL ON RAT DEVELOPMENT AND BEHAVIOR.

Michalina Duszczyk, Mieszko Olczak, Pawe Mierzejewski, Dorota M. Majewska.
Department of Pharmacology and Physiology of the Central Nervous System, Institute of Psychiatry and Neurology, Warsaw, Poland.
Pharmacological Reports. 2008 60; p261-262

Numerous clinical findings support hypothesis that mercury, which was added to many infant vaccines in the form of thimerosal between 2000–2004, may be one of the factors responsible for autism epidemics currently observed all over the world. Data from Adverse Event Reporting of the Center for Disease Control and Prevention (USA) provide strong epidemiological evidence for a link between vaccine-thimerosal exposure and autism or other neurodevelopmental disorders/diseases. The onset of autistic symptoms in children often follows the administration of vaccine thimerosal and symptom emergence is consistent with the expression of developmental mercury toxicity.

In this study, we examined potential neurodevelopmental outcomes following postnatal exposure of rats to thimerosal (Sigma-Aldrich), administered *sc* or *im* from 0.040 mg/kg to 50 mg/kg in four equal doses on days 7–14 after birth. Three strains of rats were used in this experiment: Wistar, Lewis and Brown Norway. Development and behavior of experimental animals was observed. Various behavioral tests were carried out, which evaluated: open field locomotor and exploratory activity, motor coordination, pain reaction (hot plate), learning and memory (water maze), prepulse inhibition, sociability (social interaction test). Growth of animals was monitored and after animal sacrifice, weigh of brains was measured. Thimerosal had variable, often biphasic, effects on different measured behaviors, which were strain- and dose-dependent, but no dramatic behavioral impairments were observed at doses tested. Data will be discussed in the context of rodent model of autism following postnatal exposure to mercury. [Note: autism is 4 times more prevalent in boys than girls, and no one has been able to identify why. The differential gender effects of thimerosal and mercury might explain why.]

GENDER-SELECTIVE TOXICITY OF THIMEROSAL.

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Exp Toxicol Pathol. 2009 Mar;61(2):133-6. Epub 2008 Sep 3.

A recent report shows a correlation of the historical use of thimerosal in therapeutic immunizations with the subsequent development of autism; however, this association remains controversial. Autism occurs approximately four times more frequently in males compared to females; thus, studies of thimerosal toxicity should take into consideration gender-selective effects. The present study was originally undertaken to determine the maximum tolerated dose (MTD) of thimersosal in male and female CD1 mice. However, during the limited MTD studies, it became apparent that thimerosal has a differential MTD that depends on whether the mouse is male or female. At doses of 38.4-76.8mg/kg using 10% DMSO as diluent, seven of seven male mice compared to zero of seven female mice tested succumbed to thimerosal. Although the thimerosal levels used were very high, as we were originally only trying to determine MTD, it was completely unexpected to observe a difference of the MTD between male and female mice. Thus, our studies, although not directly addressing the controversy surrounding thimerosal and autism, and still preliminary due to small numbers of mice examined, provide, nevertheless, the first report of gender-selective toxicity of thimerosal and indicate that any future studies of thimerosal toxicity should take into consideration gender-specific differences.

IDENTIFICATION OF GENES MEDIATING THYROID HORMONE ACTION IN THE DEVELOPING MOUSE CEREBELLUM.

Takahashi, Masaki; Negishi, Takayuki; Tashiro, Tomoko
Journal of Neurochemistry. 104(3):640-652, February 2008.
[Noteto article below – *nlg3* is *neuroigin 3*.]

Abstract:

Despite the indispensable role thyroid hormone (TH) plays in brain development, only a small number of genes have been identified to be directly regulated by TH and its precise mechanism of action remains largely unknown, partly because most of the previous studies have been carried out at postnatal day 15 or later. In the present study, we screened for TH-responsive genes in the developing mouse cerebellum at postnatal day 4 when morphological alterations because of TH status are not apparent. Among the new candidate genes selected by comparing gene expression profiles of experimentally hypothyroid, hypothyroid with postnatal thyroxine replacement, and control animals using oligoDNA microarrays, six genes were confirmed by real-time PCR to be positively (*orc11*, *galr3*, *sort1*, *nlg3*, *cdk5r2*, and *zfp367*) regulated by TH. Among these, *sort1*, *cdk5r2*, and *zfp367* were up-regulated already at 1 h after a single injection of thyroxine to the hypothyroid or control animal, suggesting them to be possible primary targets of the hormone. Cell proliferation and apoptosis examined by BrdU incorporation and terminal deoxynucleotide transferase-mediated dUTP nick-end labeling assay revealed that hypothyroidism by itself did not enhance apoptosis at this stage, but rather increased cell survival, possibly through regulation of these newly identified genes.

Cellular Research

THIMEROSAL INDUCES TH2 RESPONSES VIA INFLUENCING CYTOKINE SECRETION BY HUMAN DENDRITIC CELLS

Agrawal A, Kaushal P, Agrawal S, Gollapudi S, Gupta S. *J Leukoc Biol.* 2007 Feb;81(2):474-82.

Agrawal documented that thimerosal exercised TH2-promoting effects through modulation of functions of human dendritic cells (DC) by inhibition of LPS induced proinflammatory cytokines TNF-alpha, IL-6, and IL-12p70 resulting in an increase TH2 (IL-5, IL-13 and decreased TH1 (IFN-gamma). Thimerosal exposure of DC led to depletion of intracellular glutathione (GSH) and the addition of exogenous GSH to DC abolished the TH2 promoting effect of thimerosal. (Note James has documented that children with autism have low levels of plasma glutathione)

MITOCHONDRIAL DYSFUNCTION, IMPAIRED OXIDATIVE-REDUCTION ACTIVITY, DEGENERATION, AND DEATH IN HUMAN NEURONAL AND FETAL CELLS INDUCED BY LOW-LEVEL EXPOSURE TO THIMEROSAL AND OTHER METAL COMPOUNDS

D.A. Geier et al.

Toxicological & Environmental Chemistry. 2009, 1–15, iFirst

Thimerosal (ethylmercurithiosalicylic acid), an ethylmercury (EtHg)-releasing compound (49.55% mercury (Hg)), was used in a range of medical products for more than 70 years. Of particular recent concern, routine administering of Thimerosal-containing biologics/childhood vaccines have become significant sources of Hg exposure for some fetuses/infants. This study was undertaken to investigate cellular damage among in vitro human neuronal (SH-SY-5Y neuroblastoma and 1321N1 astrocytoma) and fetal (nontransformed) model systems using cell vitality assays and microscope-based digital image capture techniques to assess potential damage induced by Thimerosal and other metal compounds (aluminum (Al) sulfate, lead (Pb)(II) acetate, methylmercury (MeHg) hydroxide, and mercury (Hg)(II) chloride) where the cation was reported to exert adverse effects on developing cells. Thimerosal-associated cellular damage was also evaluated for similarity to pathophysiological findings observed in patients diagnosed with autistic disorders (ADs). Thimerosal-induced cellular damage as evidenced by concentration- and time-dependent mitochondrial damage, reduced oxidative–reduction activity, cellular degeneration, and cell death in the in vitro human neuronal and fetal model systems studied. Thimerosal at low nanomolar (nM) concentrations induced significant cellular toxicity in human neuronal and fetal cells. Thimerosal-induced cytotoxicity is similar to that observed in AD pathophysiologic studies. Thimerosal was found to be significantly more toxic than the other metal compounds examined. Future studies need to be conducted to evaluate additional mechanisms underlying Thimerosal-induced cellular damage and assess potential co-exposures to other compounds that may increase or decrease Thimerosal-mediated toxicity.

THIMEROSAL INDUCES APOPTOSIS IN A NEUROBLASTOMA MODEL VIA THE cJUN N-TERMINAL KINASE PATHWAY

Herdman ML, Marcelo A, Huang Y, Niles RM, Dhar S, Kinningham KK. *Toxicol Sci.* 2006 Jul;92(1):246-53.

Herdman notes that cJun N-terminase kinase (JNK)-signaling pathway activation has been implicated in neuronal apoptosis. Herdman investigated the role that the JNK pathway plays in neurotoxicity caused by thimerosal. SK-N-SH cells treated with thimerosal (0-10 microM) showed an increase in the phosphorylated (active) form of JNK and cJun with 5 and 10 microM thimerosal treatment at 2 and 4 h.. To assess which components are essential to apoptosis, cells were treated with a cell-permeable JNK inhibitor and the downstream effectors of apoptosis were analyzed. Results indicate that thimerosal-induced neurotoxicity occurs through the JNK-signaling pathway, independent of cJun activation, leading to apoptotic cell death.

UNCOUPLING OF ATP-MEDIATED CALCIUM SIGNALING AND DYSREGULATION INTERLEUKIN-6 SECRETION IN DENDRITIC CELLS BY NANOMOLAR THIMEROSAL

Goth SR, Chu RA, Gregg JP, Cherednichenko G, Pessah IN. *Environ Health Perspect.* 2006 Jul;114(7):1083-91.

Goth investigated adenosine triphosphate (ATP) mediated Ca²⁺ responses in dendritic cells (responsible for initiating primary immune responses) exposed briefly to nanomolar concentrations (100nM, 5 min) of thimerosal and found that dendritic cells were exquisitely sensitive to thimerosal resulting in uncoupling of the positive and negative regulation of Ca²⁺ + signals.

THIMEROSAL INDUCES NEURONAL CELL DEATH BY CAUSING CYTOCHROME C AND APOPTOSIS-INDUCING FACTOR RELEASE FROM MITOCHONDRIA.

Yel L, Brown LE, Su K, Gollapudi S, Gupta S. *Int J Mol Med.* 2005 Dec;16(6):971-7.

Yel demonstrated that thimerosal, at nanomolar concentrations, induced neuronal cell death through the mitochondrial pathway. The thimerosal induced apoptosis was associated with depolarization of mitochondrial membranes, generation of reactive oxygen species and release of cytochrome c and apoptosis-inducing factor, suggesting that thimerosal cause apoptosis in neuroblastoma cells by altering the mitochondrial microenvironment.

IN VITRO UPTAKE OF GLUTAMATE IN GLAST AND GLT-1 TRANSFECTED MUTANT CHO-K1 CELLS IS INHIBITED BY THE ETHYLMERCURY-CONTAINING PRESERVATIVE THIMEROSAL

Mutkus L, Aschner JL, Syversen T, Shanker G, Sonnewald U, Aschner M. *Bio Trace Elem Res.* 2005 Summer;105(1-3):71-86

Mutkus determined that thimerosal caused significant and selective changes in both glutamate transporter mRNA and protein expression in the CHO-K1 cell line. This study suggests that thimerosal accumulation in the central nervous system might contribute to dysregulation of glutamate homeostasis. Glutamate is a neurotransmitter and is necessary for proper brain functioning. Note: Yip (2007) documented decreased levels of glutamate in autistic cerebral brain tissue and Hornig (2004) noted altered glutamate receptors in thimerosal exposed mice.

THIMEROSAL INDUCES DNA BREAKS, CASPASE-3 ACTIVATION, MEMBRANE DAMAGE, AND CELL DEATH IN CULTURED HUMAN NEURONS AND FIBROBLASTS

Baskin DS, Ngo H, Didenko VV. *Toxicological Sciences.* 2003 Aug;74(2):361-8.

Baskin documented that thimerosal disrupts cell membranes, damages DNA and alters cell shape at concentrations only 4 times those expected from vaccines. Greater effects were seen as the length of time of exposure grew, suggesting that under real conditions the concentration needed for the observed alterations would be much lower. It has been documented in subsequent research that exposure of cells to nanomolar levels of thimerosal after 24 hours results in cell alterations.

MITOCHONDRIAL MEDIATED THIMEROSAL-INDUCED APOPTOSIS IN A HUMAN NEUROBLASTOMA CELL LINE (SK-N-SH)

Humphrey ML, Cole MP, Pendergrass JC, Kinningham KK. Neurotoxicology. 2005 Jun;26(3):407-16.

Humphrey noted that after only short (2 hour) exposures to thimerosal at 5 micromolar concentrations in a human neuroblastoma cell line caused morphological changes including membrane alterations and cell shrinkage leading to cell death. Cytochrome C was shown to leak from the mitochondria followed by caspase 9 cleavage. These findings support deleterious effects on cellular cytoarchitecture and initiation of mitochondrial-mediated apoptosis induced by thimerosal.

THIMEROSAL NEUROTOXICITY IS ASSOCIATED WITH GLUTATHIONE DEPLETION: PROTECTION WITH GLUTATHIONE PRECURSORS

JAMES SJ, SLIKKER W 3RD, MELNYK S, NEW E, POGRIBNA M, JERNIGAN S. NEUROTOXICOLOGY. 2005 JAN;26(1):1-8.

James note that the viability of neuronal cell lines was decreased after just 3 hour exposure to 2.5 micromolar concentrations of thimerosal. Also noted was that cultured neuroblastoma cells were found to have lower levels of GSH and increased sensitivity to thimerosal in comparison to glioblastoma cells that contain higher levels of GSH. Furthermore, pretreatment with glutathione ethyl ester or NAC prevented cytotoxicity with exposure up to 15 micromolar thimerosal.

BIOCHEMICAL AND MOLECULAR BASIS OF THIMEROSAL-INDUCED APOPTOSIS IN T CELLS: A MAJOR ROLE OF MITOCHONDRIAL PATHWAY

Makani S, Gollapudi S, Yel L, Chiplunkar S, Gupta S. Genes & Immunity. 2002 Aug;3(5):270-8.

Makani found thimerosal, in micromolar concentration, causes cell death (apoptosis) in immune cells (T cells). The data also suggested that the thimerosal induced apoptosis in T cells occurred via mitochondrial pathways by inducing oxidative stress and depletion of glutathione.

EFFECTS OF THIMEROSAL ON NGF SIGNAL TRANSDUCTION AND CELL DEATH IN NEUROBLASTOMA CELLS

Parran DK, Barker A, Ehrlich M. Toxicological Sciences. 2005 Jul;86(1):132-40. Parran documented that thimerosal causes DNA fragmentation of neuronal cells and disrupts neuronal growth factor signaling at micromolar and even nanomolar concentrations. With and without NGF, thimerosal caused elevated levels of fragmented DNA appearing at 0.01 microM (apoptosis) to decrease at concentrations >1 microM (necrosis). These data demonstrate that thimerosal could alter NGF-induced signaling in neurotrophin-treated cells at concentrations lower than those responsible for cell death.

ACTIVATION OF METHIONINE SYNTHASE BY INSULIN-LIKE GROWTH FACTOR-1 AND DOPAMINE: A TARGET FOR EURODEVELOPMENTAL TOXINS AND THIMEROSAL

Waly M, Olteanu H, Banerjee R, Choi SW, Mason JB, Parker BS, Sukumar S, Shim S, Sharma A, Benzecry JM, Power-Charnitsky VA, Deth RC. *Molecular Psychiatry*. 2004 Apr;9(4):358-70.

Waly noted that thimerosal inhibits critical DNA methylation and attentional pathways at nanomolar concentrations, leading to alterations in brain function. Thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC(50) of 1 nM and eliminated MS activity which can lead to alterations in brain function. A novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation was also identified.

THIMEROSAL INDUCES MICRONUCLEI IN THE CYTOCHALASIN B BLOCK MICRONUCLEUS TEST WITH HUMAN LYMPHOCYTES

Westphal GA, Asgari S, Schulz TG, Bünger J, Müller M, Hallier E. *Archives of Toxicology*. 2003 Jan; 77(1):50 – 55.

Significant induction of micronuclei was seen at concentrations of thimerosal between 0.05-0.5 µg/ml in 14 out of 16 experiments. Thus, genotoxic effects were seen even at concentrations which can occur at the injection site. Toxicity and toxicity-related elevation of micronuclei was seen at and above 0.6 µg/ml thimerosal. Marked individual and intraindividual variations in the in vitro response to thimerosal among the different blood donors occurred. However, there was no association observed with any of the glutathione S-transferase polymorphism investigated. In conclusion, thimerosal is genotoxic in the cytochalasin B block micronucleus test with human lymphocytes (immune cells). These data raise some concern on the widespread use of thimerosal.

ZINC IONS CAUSE THE THIMEROSAL-INDUCED SIGNAL OF FLUORESCENT CALCIUM PROBES IN LYMPHOCYTES

Cell Calcium. 2008 Oct 31. [Epub ahead of print]

Haase H, Hebel S, Engelhardt G, Rink L.,

Institute of Immunology, RWTH Aachen University Hospital, Aachen, Germany.

Most fluorescent probes for the investigation of calcium signaling also detect zinc ions. Consequently, changes in the intracellular zinc concentration could be mistaken for calcium signals. Thimerosal (TMS) is used as a calcium-mobilizing agent and we analyzed the contribution of zinc ions to the signal observed with fluorescent calcium probes after TMS stimulation. Our findings show that the fluorescent signal in lymphocytes is entirely due to zinc release. Experiments in the T lymphocyte cell line Jurkat and primary human lymphocytes show that TMS and its active metabolite, ethyl mercury, cause an increase in signal intensity with probes designed for the detection of either calcium or zinc ions. The TMS/ethyl mercury-induced signal of the calcium probes Fluo-4 and FURA-2 was completely absent when the zinc chelator TPEN [N,N,N',N'-tetrakis-(2-pyridyl-methyl)ethylenediamine] was added. In contrast, the signal caused by thapsigargin-induced release of calcium from the endoplasmic reticulum was unaffected by TPEN. In light of these observations, zinc may also contribute to calcium signals

caused by mercury-containing compounds other than TMS, and a potential involvement of zinc release in the immunomodulatory effects of these substances should be considered.

GENOTOXICITY OF THIMEROSAL IN CULTURED HUMAN LYMPHOCYTES WITH AND WITHOUT METABOLIC ACTIVATION SISTER CHROMATID EXCHANGE ANALYSIS PROLIFERATION INDEX AND MITOTIC INDEX

Eke D, Celik A.

Mersin University, Faculty of Science and Letters, Department of Biology, 33343 Mersin, Turkey.

Toxicol In Vitro. 2008 Jun;22(4):927-34. Epub 2008 Feb 1.

Thimerosal is an antiseptic containing 49.5% of ethyl mercury that has been used for years as a preservative in many infant vaccines and in flu vaccines. Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. In this study, we evaluated the genotoxic effect of thimerosal in cultured human peripheral blood lymphocytes using sister chromatid exchange analysis in culture conditions with and without S9 metabolic activation. This study is the first report investigating the genotoxic effects of thimerosal in cultured human peripheral blood lymphocyte cells using sister chromatid exchange analysis. An analysis of variance test (ANOVA) was performed to evaluate the results. Significant induction of sister chromatid exchanges was seen at concentrations between 0.2 and 0.6 microg/ml of thimerosal compared with negative control. A significant decrease ($p < 0.001$) in mitotic index (MI) and proliferation index (PRI) as well as an increase in SCE frequency ($p < 0.001$) was observed compared with control cultures. Our results indicate the genotoxic and cytotoxic effect of TH in cultured human peripheral blood lymphocytes at tested doses in cultures with/without S9 fraction.

CELLULAR AND MITOCHONDRIAL GLUTATHIONE REDOX IMBALANCE IN LYMPHOBLASTOID CELLS DERIVED FROM CHILDREN WITH AUTISM.

James SJ, Rose S, Melnyk S, Jernigan S, Blossom S, Pavliv O, Gaylor DW.

Department of Pediatrics; and Department of Biostatistics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas, USA.

FASEB J. 2009 Mar 23. [Epub ahead of print]

Research into the metabolic phenotype of autism has been relatively unexplored despite the fact that metabolic abnormalities have been implicated in the pathophysiology of several other neurobehavioral disorders. Plasma biomarkers of oxidative stress have been reported in autistic children; however, intracellular redox status has not yet been evaluated. Lymphoblastoid cells (LCLs) derived from autistic children and unaffected controls were used to assess relative concentrations of reduced glutathione (GSH) and oxidized disulfide glutathione (GSSG) in cell extracts and isolated mitochondria as a measure of intracellular redox capacity. The results indicated that the GSH/GSSG redox ratio was decreased and percentage oxidized glutathione increased in both cytosol and mitochondria in the autism LCLs. Exposure to oxidative stress via the sulfhydryl reagent thimerosal resulted in a greater decrease in the GSH/GSSG ratio and increase in free

radical generation in autism compared to control cells. Acute exposure to physiological levels of nitric oxide decreased mitochondrial membrane potential to a greater extent in the autism LCLs, although GSH/GSSG and ATP concentrations were similarly decreased in both cell lines. These results suggest that the autism LCLs exhibit a reduced glutathione reserve capacity in both cytosol and mitochondria that may compromise antioxidant defense and detoxification capacity under prooxidant conditions.

GENETIC VARIANT OF GLUTATHIONE PEROXIDASE 1 IN AUTISM.

Ming X, Johnson WG, Stenroos ES, Mars A, Lambert GH, Buyske S. Department of Neurosciences and Neurology, UMDNJ-New Jersey Medical School, 90 Bergen Street, DOC 8100, Newark, NJ 07103, USA.

Brain Dev. 2009 Feb 3. [Epub ahead of print]

Genetic factors can contribute to autistic disorder (AD). Abnormal genes of oxidative stress pathways and increased oxidative stress have been reported in autism spectrum disorders. Polymorphisms of genes involved in glutathione metabolism, e.g. GSTP1 and GSTM1 are reportedly associated with autistic disorder. We investigated a GCG repeat polymorphism of a human glutathione peroxidase (GPX1) polyalanine repeat (ALA5, ALA6 and ALA7) in 103 trios of AD (proband and parents) using the transmission disequilibrium test. Significant transmission disequilibrium ($p=0.044$) was found in the overall transmission of the three alleles. The ALA6 allele was under transmitted ($p=0.017$). These results suggest that possessing this ALA6 allele may be protective for AD. Future study of interaction of the GPX1 GCG repeat and other gene polymorphisms such as the MnSOD ALA16 or the GPX1 Pro198Leu polymorphism in this cohort of AD families may shed light in whether the combination of the ALA6 allele with another polymorphism of antioxidant allele contributes to the increased oxidative stress in autism.

Earlier pre-2009 studies relevant to the previous new study on glutathione, autism and mercury:

GLUTATHIONE-S-TRANSFERASE POLYMORPHISM, METALLOTHIONEIN EXPRESSION, AND MERCURY LEVELS AMONG STUDENTS IN AUSTRIA

Gundacker C, Komarnicki G, Jagiello P, Gencikova A, Dahmen N, Wittmann KJ, Gencik M.

Sci Total Environ. 2007 Oct 15;385(1-3):37-47.

BACKGROUND: Detoxification is an essential process in all living organisms. Humans accumulate heavy metals primarily as a result of lifestyle and environmental contamination. However, not all humans experience the estimated individual exposure. This suggests the presence of genetic regulatory mechanisms.

OBJECTIVE: In order to identify genetic factors underlying the inter-individual variance in detoxification capacity for the heavy metal mercury, 192 students were investigated. We focused on the relationship between polymorphisms in glutathione-S-transferase (GST) genes and mercury

concentrations in blood, urine, and hair. The correlation between blood mercury levels, GSTT1 and GSTM1 polymorphism, and gene expression of certain metallothionein subgroups (MT1, MT3) was evaluated in a further group of students (N=30).

METHODS: Mercury levels in acid digested samples were measured by cold vapor AAS. Genotyping of the GSTT1 and GSTM1-gene deletion polymorphism was performed by means of PCR. Gene expression of several MT genes was analyzed in lymphocytes from fresh peripheral blood by semiquantitative RT-PCR.

RESULTS: The following was noted: a) hair mercury concentrations are significantly increased in persons with the double deleted genotype (GSTT1-/- and GSTM1-/-) as compared to persons with the intact genotype, and b) MT1X expression is higher in persons with the intact genotype (GSTT1+/+ and GSTM1+/+).

CONCLUSIONS: We conclude that the epistatic effect of the GSTT1 and the GSTM1 deletion polymorphism is a risk factor for increased susceptibility to mercury exposure. The relationship between MT gene expression and GST gene polymorphisms needs further investigation. If MT expression depends on GST polymorphisms it would have important implications on the overall metal detoxification capability of the human organism.

RISK OF AUTISTIC DISORDER IN AFFECTED OFFSPRING OF MOTHERS WITH A GLUTATHIONE S-TRANSFERASE P1 HAPLOTYPE.

Williams TA, Mars AE, Buyske SG, Stenroos ES, Wang R, Factura-Santiago MF, Lambert GH, Johnson WG.

Department of Neurology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA.

[Arch Pediatr Adolesc Med.](#) 2007 Apr;161(4):356-61.

OBJECTIVE: To test whether polymorphisms of the glutathione S-transferase P1 gene (GSTP1) act in the mother during pregnancy to contribute to the phenotype of autistic disorder (AD) in her fetus.

DESIGN: Transmission disequilibrium testing (TDT) in case mothers and maternal grandparents. **SETTING:** Autistic disorder may result from multiple genes and environmental factors acting during pregnancy and afterward. Teratogenic alleles act in mothers during pregnancy to contribute to neurodevelopmental disorders in their offspring; however, only a handful have been identified. GSTP1 is a candidate susceptibility gene for AD because of its tissue distribution and its role in oxidative stress, xenobiotic metabolism, and JNK regulation. **PARTICIPANTS:** We genotyped GSTP1*G313A and GSTP1*C341T polymorphisms in 137 members of 49

families with AD. All probands received a clinical diagnosis of AD by Autism Diagnostic Interview-Revised and Autism Diagnostic Observation Schedule-Generic testing. MAIN OUTCOME

MEASURES: Association of haplotypes with AD was tested by the TDT-Phase program, using the expectation-maximization (EM) algorithm for uncertain haplotypes and for incomplete parental genotypes, with standard measures of statistical significance. RESULTS: The GSTP1*A haplotype was overtransmitted to case mothers ($P = .01$ [$P = .03$ using permutation testing]; odds ratio, 2.67 [95% confidence interval, 1.39-5.13]). Results of the combined haplotype and genotype analyses suggest that the GSTP1-313 genotype alone determined the observed haplotype effect.

CONCLUSIONS: Overtransmission of the GSTP1*A haplotype to case mothers suggests that action in the mother during pregnancy likely increases the likelihood of AD in her fetus. If this is confirmed and is a result of a gene-environment interaction occurring during pregnancy, these findings could lead to the design of strategies for prevention or treatment.

ANALYSIS OF CASE-PARENT TRIOS AT A LOCUS WITH A DELETION ALLELE: ASSOCIATION OF GSTM1 WITH AUTISM.

Buyske S, Williams TA, Mars AE, Stenroos ES, Ming SX, Wang R, Sreenath M, Fatura MF, Reddy C, Lambert GH, Johnson WG.
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BMC Genet. 2006 Feb 10;7:8.

BACKGROUND: Certain loci on the human genome, such as glutathione S-transferase M1 (GSTM1), do not permit heterozygotes to be reliably determined by commonly used methods. Association of such a locus with a disease is therefore generally tested with a case-control design. When subjects have already been ascertained in a case-parent design however, the question arises as to whether the data can still be used to test disease association at such a locus. RESULTS: A likelihood ratio test was constructed that can be used with a case-parents design but has somewhat less power than a Pearson's chi-squared test that uses a case-control design. The test is illustrated on a novel dataset showing a genotype relative risk near 2 for the homozygous GSTM1 deletion genotype and autism.

CONCLUSION: Although the case-control design will remain the mainstay for a locus with a deletion, the likelihood ratio test will be useful for such a locus analyzed as part of a larger case-parent study design. The likelihood ratio test has the advantage that it can incorporate complete and incomplete case-parent trios as well as independent cases and controls. Both analyses support ($p = 0.046$ for the proposed test, $p = 0.028$ for the case-control

analysis) an association of the homozygous GSTM1 deletion genotype with autism.

ABERRATIONS IN FOLATE METABOLIC PATHWAY AND ALTERED SUSCEPTIBILITY TO AUTISM.

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Psychiatr Genet. 2009 May 13. [Epub ahead of print]

OBJECTIVE: To investigate whether genetic polymorphisms are the underlying causes for aberrations in folate pathway that was reported in autistic children.

BASIC METHODS: A total of 138 children diagnosed as autistic based on Diagnostic and Statistical Manual of Mental Disorders, fourth edition criteria and Autism Behavior Checklist scoring and 138 age and sex matched children who are nonautistic were tested for five genetic polymorphisms, that is, cytosolic serine hydroxyl methyl transferase (SHMT1 C1420T), methylene tetrahydrofolate reductase (MTHFR C677T and MTHFR A1298C), methionine synthase reductase (MTRR A66G), methionine synthase (MS A2756G) using PCR-restriction fragment length polymorphism methods. Fisher's exact test and logistic regression analysis were used for statistical analyses.

RESULTS: MTHFR 677T-allele frequency was found to be higher in autistic children compared with nonautistic children (16.3 vs. 6.5%) with 2.79-fold increased risk for autism [95% confidence interval (CI): 1.58-4.93]. The frequencies of MTRR 66A allele (12.7 vs. 21.0%) and SHMT 1420T allele (27.9 vs. 45.3%) were lower in autistic group compared with nonautistic group with odds ratios 0.55 (95% CI: 0.35-0.86) and 0.44 (95% CI: 0.31-0.62), respectively, indicating reduced risk. MTHFR 1298C-allele frequency was similar in both the groups (53.3 vs. 53.6%) and hence individually not associated with any risk. However, this allele was found to act additively in the presence of MTHFR 677T allele as evidenced by 8.11-fold (95% CI: 2.84-22.92) risk associated with MTHFR 677CT+TT/1298AC+CC genotypes cumulatively.

CONCLUSION: MTHFR C677T is a risk factor, whereas MTRR A66G and SHMT C1420T polymorphisms reduce risk for autism. MTHFR A1298C acts additively in increasing the risk for autism.

Epidemiological Research

EARLY THIMEROSAL EXPOSURE & NEUROPSYCHOLOGICAL OUTCOME AT 7 TO 10 YEARS

New England Journal of Medicine; 9/27/07, vol. 357 no. 13

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It has been hypothesized that early exposure to thimerosal, a mercury-containing preservative used in vaccines and immune globulin preparations, is associated with neuropsychological deficits in children. 1047 children between the ages of 7 and 10 years were enrolled and administered standardized tests assessing 42 neuropsychological outcomes. Exposure to mercury from thimerosal was determined from computerized immunization records, medical records, personal immunization records, and parent interviews. Information on potential confounding factors was obtained from the interviews and medical charts. The association between current neuropsychological performance and exposure to mercury was assessed during the prenatal period, the neonatal period (birth to 28 days), and the first 7 months of life.

Among the 42 neuropsychological outcomes, boys receiving thimerosal were 2 ½ times more likely to have motor and phonic tics, which can be debilitating. Additionally, this study revealed that children receiving thimerosal were more likely to have deficits in attention, behavior control and verbal IQ.

AN EPIDEMIOLOGICAL ANALYSIS OF THE ‘AUTISM AS MERCURY POISONING’ HYPOTHESIS

International Journal of Risk & Safety in Medicine 20 (2008) 135-142

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Abstract. Where direct experimental research into a causal hypothesis of a disease is impossible due to ethical and practical considerations, epidemiological inference is the accepted route to establishing cause. Therefore, to examine the *autism as mercury poisoning* hypothesis, this paper reviews the existing scientific literature within the context of established epidemiological criteria and finds that the evidence for a causal relationship is compelling. Exposure to mercury (via vaccines and maternal dental amalgam) *in utero* and during infant years is confirmed; mercury poisoning is known to cause symptoms consistent with autism; animal modeling supports the link and, critically, mercury levels are higher in both the urine and blood of autistic children than in non-autistic peers. Analogous to epidemiological evidence of the smoking-lung cancer relationship, a mercury-autism relationship is confirmed. The *precautionary principle*

demands that health professionals not take an action if there is suspicion that the action may cause severe or lifelong health effects: it does not require certainty. Therefore, given the severity, devastating lifelong impact and extremely high prevalence of autism, it would be negligent to continue to expose pregnant and nursing mothers and infant children to an amount of avoidable mercury.

NEURODEVELOPMENTAL DISORDERS, MATERNAL RH-NEGATIVITY, AND RHO(D) IMMUNE GLOBULINS: A MULTI-CENTER ASSESSMENT

Neuro Endocrinol Lett. 2008 Apr;29(2):272-80.

Geier DA, Mumper E, Gladfelter B, Coleman L, Geier MR.

The Institute of Chronic Illnesses, Inc., Silver Spring, MD

BACKGROUND: Many formulations of Thimerosal (49.55% mercury by weight)-containing Rho(D) immune globulins (TCRs) were routinely administered to Rh-negative mothers in the US prior to 2002. **OBJECTIVES:** It was hypothesized: (1) if prenatal Rho(D)-immune globulin preparation exposure was a risk factor for neurodevelopmental disorders (NDs) then more children with NDs would have Rh-negative mothers compared to controls; and (2) if Thimerosal in the Rho(D)-immune globulin preparations was the ingredient associated with NDs, following the removal of Thimerosal from all manufactured Rho(D)-immune globulin preparations from 2002 in the US the frequency of maternal Rh-negativity among children with NDs should be similar to control populations.

METHODS: Maternal Rh-negativity was assessed at two sites (Clinic A-Lynchburg, VA; Clinic B-Rockville and Baltimore, MD) among 298 Caucasian children with NDs and known Rh-status. As controls, maternal Rh-negativity frequency was determined from 124 Caucasian children (born 1987-2001) without NDs 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). Additionally, 22 Caucasian patients with NDs born from 2002 onwards (Clinics A and B) were assessed for maternal Rh-negativity.

RESULTS: There were significant and comparable increases in maternal Rh-negativity among children with NDs (Clinic: A=24.2%), autism spectrum disorders (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. Children with NDs born post-2001 had a maternal Rh-negativity frequency (13.6%) similar to controls.

CONCLUSION: This study associates TCR exposure with some NDs in children.

THIMEROSAL EXPOSURE IN INFANTS AND NEURODEVELOPMENTAL DISORDERS: AN ASSESSMENT OF COMPUTERIZED MEDICAL RECORDS IN THE VACCINE SAFETY DATALINK

J Neurol Sci. 2008 Aug 15;271(1-2):110-8. Epub 2008 May 15.

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The George Washington University School of Public Health and Health Services,
Department of Epidemiology and Biostatistics, United States.

The study evaluated possible associations between neurodevelopmental disorders (NDs) and exposure to mercury (Hg) from Thimerosal-containing vaccines (TCVs) by examining the automated Vaccine Safety Datalink (VSD). A total of 278,624 subjects were identified in birth cohorts from 1990-1996 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, 9th revision (ICD-9) specific NDs and control outcomes were calculated. Exposures to Hg from TCVs were calculated by birth cohort for specific exposure windows from birth-7 months and birth-13 months of age. Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from TCVs. Consistent significantly increased rate ratios were observed for autism, autism spectrum disorders, tics, attention deficit disorder, and emotional disturbances with Hg exposure from TCVs. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs. Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be conducted to further evaluate the relationship between Hg exposure and NDs.

OCKHAM'S RAZOR AND AUTISM: THE CASE FOR DEVELOPMENTAL NEUROTOXINS CONTRIBUTING TO A DISEASE OF NEURODEVELOPMENT

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Neurotoxicology. 2009 May;30(3):331-7. Epub 2009 Mar 21.

Much professional awareness regarding environmental triggers for ASD has been narrowly focused on a single possible exposure pathway (vaccines). Meanwhile, empirical support for environmental toxins as a broad class has been quietly accumulating. Recent research has shown that persons with ASD have comparatively higher levels of various toxins and are more likely to have reduced detoxifying ability, and, that rates of ASD may be higher in areas with greater pollution. This report documents that within the state with the highest rate of ASD, the rate is higher for schools near EPA Superfund sites, $t(332)=3.84, p=.0001$. The reasons for the rise in diagnoses likely involve genetically predisposed individuals being exposed to various environmental triggers at higher rates than in past generations.

HEPATITIS B VACCINE AND THE RISK OF CNS INFLAMMATORY DEMYELINATION IN CHILDHOOD

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NEUROLOGY 2009;72:873-880

Background: The risk of CNS inflammatory demyelination associated with hepatitis B (HB) vaccine is debated, with studies reporting conflicting findings.

Methods: We conducted a population-based case-control study where the cases were children with a first episode of acute CNS inflammatory demyelination in France (1994–2003). Each case was matched on age, sex, and geographic location to up to 12 controls, randomly selected from the general population. Information on vaccinations was confirmed by a copy of the vaccination certificate. The odds ratios (ORs) of CNS inflammatory demyelination associated with HB vaccination were estimated using conditional logistic regression.

Results: The rates of HB vaccination in the 3 years before the index date were 24.4% for the 349 cases and 27.3% for their 2,941 matched controls. HB vaccination within this period was not associated with an increase in the rate of CNS inflammatory demyelination (adjusted OR, 0.74; 0.54–1.02), neither >3 years nor as a function of the number of injections or brand type. When the analysis was restricted to subjects compliant with vaccination, HB vaccine exposure >3 years before index date was associated with an increased trend (1.50; 0.93–2.43), essentially from the Engerix B vaccine (1.74; 1.03–2.95). The OR was particularly elevated for this brand in patients with confirmed multiple sclerosis (2.77; 1.23–6.24).

Conclusions: Hepatitis B vaccination does not generally increase the risk of CNS inflammatory demyelination in childhood. However, the Engerix B vaccine appears to increase this risk, particularly for confirmed multiple sclerosis, in the longer term. Our results require confirmation in future studies.

A REVIEW OF EVENTS THAT EXPOSE CHILDREN TO ELEMENTAL MERCURY IN THE UNITED STATES

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Environ Health Perspect 117:871–878 (2009).

Abstract

Objective: Concern for children exposed to elemental mercury prompted the Agency for Toxic Substances and Disease Registry and the Centers for Disease Control and Prevention to review the sources of elemental mercury exposures in children, describe the location and proportion of children affected, and make recommendations on how to prevent these exposures. In this review, we excluded mercury exposures from coal-burning facilities, dental amalgams, fish consumption, medical waste incinerators, or thimerosal-containing vaccines.

Data Sources: We reviewed federal, state, and regional programs with information on mercury releases along with published reports of children exposed to elemental mercury in the United States. We selected all mercury-related events that were documented to expose (or potentially expose) children. We then explored event characteristics (i.e., the exposure source, location) .

Data Synthesis: Primary exposure locations were at home, at school, and at other locations such as industrial property not adequately remediated or medical facilities. Exposure to small spills from broken thermometers was the most common scenario ; however, reports of such exposures are declining.

Discussion and Conclusions: Childhood exposures to elemental mercury often result from inappropriate handling or cleanup of spilled mercury. The information reviewed suggests that most releases do not lead to demonstrable harm if the exposure period is short and the mercury is properly cleaned up.

Recommendations: Primary prevention should include health education and policy initiatives. For larger spills, better coordination among existing surveillance systems would assist in understanding the risk factors and in developing effective prevention efforts.

Related Autism-Mercury Research

MUTATION RESEARCH/FUNDAMENTAL AND MOLECULAR MECHANISMS OF MUTAGENESIS

Leticia Bucio, Cecilia Garca, Verranica Souza, Elizabeth Hernandez, Cristina Gonzalez, Miguel Betancourt and Ma. Concepcian Gutierrez-Ruiz
Volume 423, Issues 1-2, 25 January 1999, Pages 65-72

Uptake, cellular distribution and DNA damage produced by mercuric chloride in a human fetal hepatic cell line. Abstract: A human hepatic cell line (WRL-68 cells) was employed to investigate the uptake of the toxic heavy metal mercury. Hg accumulation in WRL-68 cells is a time and concentration dependent process. A rapid initial phase of uptake was followed by a second slower phase. The transport does not require energy and at low HgCl₂ concentrations (<50 nM) Hg transport occurs by temperature-insensitive processes. Subcellular distribution of Hg was: 48% in mitochondria, 38% in nucleus and only 8% in cytosolic fraction and 7% in microsomes. Little is known at the molecular level concerning the genotoxic effects following the acute exposure of eucaryotic cells to low concentrations of Hg. Our results showed that Hg induced DNA single-strand breaks or alkali labile sites using the single-cell gel electrophoresis assay (Comet assay). The percentage of damaged nucleus and the average length of DNA migration increased as metal concentration and time exposure increased. Lipid peroxidation, determined as malondialdehyde production in the presence of thiobarbituric acid, followed the same tendency, increased as HgCl₂ concentration and time of exposure increased. DNA damage recovery took 8 h after partial metal removed with PBS+EGTA.

A CASE-CONTROL STUDY OF MERCURY BURDEN IN CHILDREN WITH AUTISTIC SPECTRUM DISORDERS

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Journal of American Physicians & Surgeons, Fall, 2003, Vol. 8 No. 3

Abstract: Large autism epidemics have recently been reported in the United States and the United Kingdom. Emerging epidemiologic evidence and biologic plausibility suggest an association between autistic spectrum disorders and mercury exposure. This study compares mercury excretion after a three-day treatment with an oral chelating agent, meso-2,3-dimercaptosuccinic acid (DMSA), in children with autistic spectrum disorders and a matched control population. Overall, urinary mercury concentrations were significantly higher in 221 children with autistic spectrum disorders than in 18 normal controls (Relative Increase (RI)=3.15; P < 0.0002). Additionally, vaccinated cases showed a significantly higher urinary mercury concentration than did vaccinated controls (RI=5.94; P < 0.005). Similar urinary mercury concentrations were observed among matched vaccinated and unvaccinated controls, and no association was found between urinary cadmium or lead concentrations and autistic spectrum disorders. The observed urinary concentrations of mercury could plausibly have resulted from thimerosal in childhood vaccines, although other environmental sources and thimerosal in Rh (D)

immune globulin administered to mothers may be contributory. Regardless of the mechanism by which children with autistic spectrum disorders have high urinary mercury concentrations, the DMSA treatment described in this study might be useful to diagnose their present burden of mercury.

AUTISM SPECTRUM DISORDERS IN RELATION TO DISTRIBUTION OF HAZARDOUS AIR POLLUTANTS IN THE SAN FRANCISCO BAY AREA

Gayle C. Windham, Lixia Zhang, Robert Gunier, Lisa A. Croen, and Judith K. Grether
Environ Health Perspect. 2006 September; 114(9): 1438–1444

Objective. To explore possible associations between autism spectrum disorders (ASD) and environmental exposures, we linked the California autism surveillance system to estimated hazardous air pollutant (HAP) concentrations compiled by the U.S. Environmental Protection Agency.

Methods. Subjects included 284 children with ASD and 657 controls, born in 1994 in the San Francisco Bay area. We assigned exposure level by census tract of birth residence for 19 chemicals we identified as potential neurotoxicants, developmental toxicants, and/or endocrine disruptors from the 1996 HAPs database. Because concentrations of many of these were highly correlated, we combined the chemicals into mechanistic and structural groups, calculating summary index scores. We calculated ASD risk in the upper quartiles of these group scores or individual chemical concentrations compared with below the median, adjusting for demographic factors.

Results. The adjusted odds ratios (AORs) were elevated by 50% in the top quartile of chlorinated solvents and heavy metals [95% confidence intervals (CIs), 1.1–2.1], but not for aromatic solvents. Adjusting for these three groups simultaneously led to decreased risks for the solvents and increased risk for metals (AORs for metals: fourth quartile = 1.7; 95% CI, 1.0–3.0; third quartile = 1.95; 95% CI, 1.2–3.1). The individual compounds that contributed most to these associations included mercury, cadmium, nickel, trichloroethylene, and vinyl chloride.

Conclusions. Our results suggest a potential association between autism and estimated metal concentrations, and possibly solvents, in ambient air around the birth residence, requiring confirmation and more refined exposure assessment in future studies.

PRENATAL METHYLMERCURY EXPOSURE HAMPERS GLUTATHIONE ANTIOXIDANT SYSTEM ONTOGENESIS AND CAUSES LONG-LASTING OXIDATIVE STRESS IN THE MOUSE BRAIN.

Toxicol Appl Pharmacol. 2008 Feb 15;227(1):147-54. Epub 2007 Oct 22.

Stringari J, Nunes AK, Franco JL, Bohrer D, Garcia SC, Dafre AL, Milatovic D, Souza DO, Rocha JB, Aschner M, Farina M.

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During the perinatal period, the central nervous system (CNS) is extremely sensitive to metals, including methylmercury (MeHg). Although the mechanism(s) associated with

MeHg-induced developmental neurotoxicity remains obscure, several studies point to the glutathione (GSH) antioxidant system as an important molecular target for this toxicant. To extend our recent findings of MeHg-induced GSH dyshomeostasis, the present study was designed to assess the developmental profile of the GSH antioxidant system in the mouse brain during the early postnatal period after in utero exposure to MeHg. Pregnant mice were exposed to different doses of MeHg (1, 3 and 10 mg/l, diluted in drinking water, ad libitum) during the gestational period. After delivery, pups were killed at different time points - postnatal days (PND) 1, 11 and 21 - and the whole brain was used for determining biochemical parameters related to the antioxidant GSH system, as well as mercury content and the levels of F(2)-isoprostane. In control animals, cerebral GSH levels significantly increased over time during the early postnatal period; gestational exposure to MeHg caused a dose-dependent inhibition of this developmental event. Cerebral glutathione peroxidase (GPx) and glutathione reductase (GR) activities significantly increased over time during the early postnatal period in control animals; gestational MeHg exposure induced a dose-dependent inhibitory effect on both developmental phenomena. These adverse effects of prenatal MeHg exposure were corroborated by marked increases in cerebral F(2)-isoprostanes levels at all time points. Significant negative correlations were found between F(2)-isoprostanes and GSH, as well as between F(2)-isoprostanes and GPx activity, suggesting that MeHg-induced disruption of the GSH system maturation is related to MeHg-induced increased lipid peroxidation in the pup brain. In utero MeHg exposure also caused a dose-dependent increase in the cerebral levels of mercury at birth. Even though the cerebral mercury concentration decreased to nearly basal levels at postnatal day 21, GSH levels, GPx and GR activities remained decreased in MeHg-exposed mice, indicating that prenatal exposure to MeHg affects the cerebral GSH antioxidant systems by inducing biochemical alterations that endure even when mercury tissue levels decrease and become indistinguishable from those noted in pups born to control dams. This study is the first to show that prenatal exposure to MeHg disrupts the postnatal development of the glutathione antioxidant system in the mouse brain, pointing to an additional molecular mechanism by which MeHg induces pro-oxidative damage in the developing CNS. Moreover, our experimental observation corroborates previous reports on the permanent functional deficits observed after prenatal MeHg exposure.

BLOOD LEVELS OF MERCURY ARE RELATED TO DIAGNOSIS OF AUTISM: A REANALYSIS OF AN IMPORTANT DATA SET

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Journal of Child Neurology, Vol. 22, No. 11, 1308-1311 (2007)

The question of what is leading to the apparent increase in autism is of great importance. Like the link between aspirin and heart attack, even a small effect can have major health implications. If there is any link between autism and mercury, it is absolutely crucial that the first reports of the question are not falsely stating that no link occurs. We have reanalyzed the data set originally reported by Ip et al. in 2004 and have found that the original *p* value was in error and that a significant relation does exist between the blood levels of mercury and diagnosis of an autism spectrum disorder. Moreover, the hair

sample analysis results offer some support for the idea that persons with autism may be less efficient and more variable at eliminating mercury from the blood.

A significant relationship does exist between the blood levels of mercury and diagnosis of an autism spectrum disorder.

HOW ENVIRONMENTAL AND GENETIC FACTORS COMBINE TO CAUSE AUTISM: A REDOX/METHYLATION HYPOTHESIS

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Neurotoxicology. 2008 Jan;29(1):190-201. Epub 2007 Oct 13. Review.

Recently higher rates of autism diagnosis suggest involvement of environmental factors in causing this developmental disorder, in concert with genetic risk factors. Autistic children exhibit evidence of oxidative stress and impaired methylation, which may reflect effects of toxic exposure on sulfur metabolism. We review the metabolic relationship between oxidative stress and methylation, with particular emphasis on adaptive responses that limit activity of cobalamin and folate-dependent methionine synthase. Methionine synthase activity is required for dopamine-stimulated phospholipid methylation, a unique membrane-delimited signaling process mediated by the D4 dopamine receptor that promotes neuronal synchronization and attention, and synchrony is impaired in autism. Genetic polymorphisms adversely affecting sulfur metabolism, methylation, detoxification, dopamine signaling and the formation of neuronal networks occur more frequently in autistic subjects. On the basis of these observations, a "redox/methylation hypothesis of autism" is described, in which oxidative stress, initiated by environment factors in genetically vulnerable individuals, leads to impaired methylation and neurological deficits secondary to reductions in the capacity for synchronizing neural networks.

PROXIMITY TO POINT SOURCES OF ENVIRONMENTAL MERCURY RELEASE AS A PREDICTOR OF AUTISM PREVALENCE.

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Health Place. 2009 Mar;15(1):18-24. Epub 2008 Feb 12.

The objective of this study was to determine if proximity to sources of mercury pollution in 1998 were related to autism prevalence in 2002. Autism count data from the Texas Educational Agency and environmental mercury release data from the Environmental Protection Agency were used. We found that for every 1000 pounds of industrial release, there was a corresponding 2.6% increase in autism rates ($p < .05$) and a 3.7% increase associated with power plant emissions ($P < .05$). Distances to these sources were independent predictors after adjustment for relevant covariates. For every 10 miles from industrial or power plant sources, there was an associated decreased autism Incident Risk of 2.0% and 1.4%, respectively ($p < .05$). While design limitations preclude interpretation

of individual risk, further investigations of environmental risks to child development issues are warranted.

EVIDENCE OF OXIDATIVE STRESS IN AUTISM DERIVED FROM ANIMAL MODELS

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American Journal of Biochemistry and Biotechnology 4 (2): 218-225, 2008

Abstract: Autism is a pervasive neurodevelopmental disorder that leads to deficits in social interaction, communication and restricted, repetitive motor movements. Autism is a highly heritable disorder, however, there is mounting evidence to suggest that toxicant-induced oxidative stress may play a role. The focus of this article will be to review our animal model of autism and discuss our evidence that oxidative stress may be a common underlying mechanism of neurodevelopmental damage. We have shown that mice exposed to either methylmercury (MeHg) or valproic acid (VPA) in early postnatal life display aberrant social, cognitive and motor behavior. Interestingly, early exposure to both compounds has been clinically implicated in the development of autism. We recently found that Trolox, a water-soluble vitamin E derivative, is capable of attenuating a number of neurobehavioral alterations observed in mice postnatally exposed to MeHg. In addition, a number of other investigators have shown that oxidative stress plays a role in neural injury following MeHg exposure both *in vitro* and *in vivo*. New data presented here will show that VPA-induced neurobehavioral deficits are attenuated by vitamin E as well and that the level of glial fibrillary acidic protein (GFAP), a marker of astrocytic neural injury, is altered following VPA exposure. Collectively, these data indicate that vitamin E and its derivative are capable of protecting against neurobehavioral deficits induced by both MeHg and VPA. This antioxidant protection suggests that oxidative stress may be a common mechanism of injury leading to aberrant behavior in both our animal model as well as in the human disease state.

A PROSPECTIVE STUDY OF TRANSSULFURATION BIOMARKERS IN AUTISTIC DISORDERS

Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Geier MR.
Institute of Chronic Illnesses, Inc., Silver Spring, MD, USA.
Neurochem Res. 2008 Jul 9.

The goal of this study was to evaluate transsulfuration metabolites in participants diagnosed with autism spectrum disorders (ASDs). Transsulfuration metabolites, including: plasma reduced glutathione (GSH), plasma oxidized glutathione (GSSG), plasma cysteine, plasma taurine, plasma sulfate, and plasma free sulfate among participants diagnosed with ASDs (n = 38) in comparison to age-matched neurotypical controls were prospectively evaluated. Testing was conducted using Vitamin Diagnostics, Inc. (CLIA-approved). Participants diagnosed with ASDs had significantly (P < 0.001) decreased plasma reduced GSH, plasma cysteine, plasma taurine, plasma sulfate, and plasma free sulfate relative to controls. By contrast, participants diagnosed with ASDs had significantly (P < 0.001) increased plasma GSSG relative to controls. The present observations are compatible with increased oxidative stress and a decreased

detoxification capacity, particularly of mercury, in patients diagnosed with ASDs. Patients diagnosed with ASDs should be routinely tested to evaluate transsulfuration metabolites, and potential treatment protocols should be evaluated to potentially correct the transsulfuration abnormalities observed.

BIOMARKERS OF ENVIRONMENTAL TOXICITY AND SUSCEPTIBILITY IN AUTISM

J Neurol Sci. 2008 Sep 24.

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Autism spectrum disorders (ASDs) may result from a combination of genetic/biochemical susceptibilities in the form of a reduced ability to excrete mercury and/or increased environmental exposure at key developmental times. Urinary porphyrins and transsulfuration metabolites in participants diagnosed with an ASD were examined. A prospective, blinded study was undertaken to evaluate a cohort of 28 participants with an ASD diagnosis for Childhood Autism Rating Scale (CARS) scores, urinary porphyrins, and transsulfuration metabolites. Testing was conducted using Vitamin Diagnostics, Inc. (CLIA-approved) and Laboratoire Philippe Auguste (ISO-approved). Participants with severe ASDs had significantly increased mercury intoxication-associated urinary porphyrins (pentacarboxyporphyrin, precoproporphyrin, and coproporphyrin) in comparison to participants with mild ASDs, whereas other urinary porphyrins were similar in both groups. Significantly decreased plasma levels of reduced glutathione (GSH), cysteine, and sulfate were observed among study participants relative to controls. In contrast, study participants had significantly increased plasma oxidized glutathione (GSSG) relative to controls. Mercury intoxication-associated urinary porphyrins were significantly correlated with increasing CARS scores and GSSG levels, whereas other urinary porphyrins did not show these relationships. The urinary porphyrin and CARS score correlations observed among study participants suggest that mercury intoxication is significantly associated with autistic symptoms. The transsulfuration abnormalities observed among study participants indicate that mercury intoxication was associated with increased oxidative stress and decreased detoxification capacity.

AN INVESTIGATION OF PORPHYRINURIA IN AUSTRALIAN CHILDREN WITH AUTISM.

J Toxicol Environ Health A. 2008;71(20):1349-51.

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Two recent studies, from France (Nataf et al., 2006) and the United States (Geier & Geier, 2007), identified atypical urinary porphyrin profiles in children with an autism spectrum disorder (ASD). These profiles serve as an indirect measure of environmental toxicity generally, and mercury (Hg) toxicity specifically, with the latter being a variable proposed as a causal mechanism of ASD (Bernard et al., 2001; Mutter et al., 2005). To examine whether this phenomenon occurred in a sample of Australian children with ASD, an analysis of urinary porphyrin profiles was conducted. A consistent trend in abnormal porphyrin levels was evidenced when data was compared with those previously reported

in the literature. The results are suggestive of environmental toxic exposure impairing heme synthesis. Three independent studies from three continents have now demonstrated that porphyrinuria is concomitant with ASD, and that Hg may be a likely xenobiotic to produce porphyrin profiles of this nature.

FEEDING MICE WITH DIETS CONTAINING MERCURY-CONTAMINATED FISH FLESH FROM FRENCH GUIANA: A MODEL FOR THE MERCURIAL INTOXICATION OF THE WAYANA AMERINDIANS

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Environmental Health 2008, **7**:53doi:10.1186/1476-069X-7-53

In 2005, 84% of Wayana Amerindians living in the upper marshes of the Maroni River in French Guiana presented a hair mercury concentration exceeding the limit set up by the World Health Organization (10 ug/g). To determine whether this mercurial contamination was harmful, mice have been fed diets prepared by incorporation of mercury-polluted fish from French Guiana.

Methods: Four diets containing 0, 0.1, 1, and 7.5% fish flesh, representing 0, 5, 62, and 520 ng methylmercury per g, respectively, were given to four groups of mice for a month. The lowest fish regimen led to a mercurial contamination pressure of 1 ng mercury per day per g of body weight, which is precisely that affecting the Wayana Amerindians.

Results: The expression of several genes was modified with mercury intoxication in liver, kidneys, and hippocampus, even at the lowest tested fish regimen. A net genetic response could be observed for mercury concentrations accumulated within tissues as weak as 0.15 ppm in the liver, 1.4 ppm in the kidneys, and 0.4 ppm in the hippocampus.

This last value is in the range of the mercury concentrations found in the brains of chronically exposed patients in the Minamata region or in brains from heavy fish consumers. Mitochondrial respiratory rates showed a 35-40% decrease in respiration for the three contaminated mice groups.

In the muscles of mice fed the lightest fish-containing diet, cytochrome c oxidase activity was decreased to 45% of that of the control muscles. When mice behavior was assessed in a cross maze, those fed the lowest and mid-level fish-containing diets developed higher anxiety state behaviors compared to mice fed with control diet.

Conclusions: We conclude that a vegetarian diet containing as little as 0.1% of mercury-contaminated fish is able to trigger in mice, after only one month of exposure, disorders presenting all the hallmarks of mercurial contamination.