safeMinds

Sensible Action For Ending Mercury-Induced Neurological Disorders

Summary of Supportive Science Regarding Thimerosal & Vaccines

Coalition of SafeMinds

Contents

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 development.

Human & Infant Research

IATROGENIC EXPOSURE TO MERCURY AFTER HEPATITIS B VACCINATION IN PRETERM INFANTS (STAJICH 2000)

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 dministered the Hepatitis B vaccine containing 12.5 □g 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) □g/L. Note: One infant was found to have developed a mercury level of 23.6 □g/L, thus meeting the CDC criteria as a case of chemical poisoning from mercury defined as a blood level of 10□g/L or greater.

MERCURY CONCENTRATIONS AND METABOLISM IN INFANTS RECEIVING VACCINES CONTAINING THIMEROSAL: A DESCRIPTIVE STUDY (PICHICHERO 2002)

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 □g 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 □g 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 >10□g /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 2007)

Marques RC, Dorea JG, Fonseca MF, Bastos WR, Malm O. Eur J Pediatr. 2007 Jan 20;

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 (PICHICHERO 2008)

Pichichero ME, Gentile A, Giglio N, Umido V, Clarkson T, Cernichiari E, Zareba G, Gotelli C, Gotelli M, Yan L, Treanor J Pediatrics. 2008 Feb;121(2):e208-14

OBJECTIVES: Thimerosal is a mercurial preservative that was widely used in multidose vaccine vials in the United States and Europe until 2001 and continues to be used in many countries throughout the world. We conducted a pharmacokinetic study to assess blood levels and elimination of ethyl mercury after vaccination of infants with thimerosal-containing vaccines. METHODS: Blood, stool, and urine samples were obtained before vaccination and 12 hours to 30 days after vaccination from 216 healthy children: 72 newborns (group 1), 72 infants aged 2 months (group 2), and 72 infants aged 6 months (group 3). Total mercury levels were measured by atomic absorption. Blood mercury pharmacokinetics were calculated by pooling the data on the group and were based on a 1-compartment first-order pharmacokinetics model. RESULTS: For groups 1, 2, and 3, respectively, (1) mean +/- SD weights were 3.4 +/- 0.4, 5.1 +/- 0.6, and 7.7 +/- 1.1 kg; (2) maximal mean +/- SD blood mercury levels were 5.0 +/- 1.3, 3.6 +/- 1.5, and 2.8 +/- 0.9 ng/mL occurring at 0.5 to 1 day after vaccination; (3) maximal mean +/- SD stool mercury levels were 19.1 +/- 11.8, 37.0 +/- 27.4, and 44.3 +/- 23.9 ng/g occurring on day 5 after vaccination for all groups; and (4) urine mercury levels were mostly nondetectable. The blood mercury half-life was calculated to be 3.7 days and returned to prevaccination levels by day 30. 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.

HEPATITIS B TRIPLE SERIES VACCINE AND DEVELOPMENTAL DISABILITY IN US CHILDREN AGED 1-9 YEARS (GALLAGHER 2008)

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 (n¼1824), 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 (n¼46) as for unvaccinated boys (n¼7), 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.

NEONATE EXPOSURE TO THIMEROSAL MERCURY FROM HEPATITIS B VACCINES (DOREA 2009)

Dórea JG, Marques RC, Brandão KG. Universidade de Brasília, Brasília, DF, Brazil. Am J Perinatol. 2009 Mar 12.

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.

BLOOD LEVELS OF MERCURY ARE RELATED TO DIAGNOSIS OF AUTISM: A REANALYSIS OF AN IMPORTANT DATA SET (DESOTO 2007)

M. Catherine DeSoto, PhD , Robert Hitlan, Ph.D. Department of Psychology, University of Northern Iowa, Cedar Falls, Iowa, 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.

BIOMARKERS OF ENVIRONMENTAL TOXICITY AND SUSCEPTIBILITY IN AUTISM (GEIER 2008)

J Neurol Sci. 2008 Sep 24. Geier DA, Kern JK, Garver CR, Adams JB, Audhya T, Nataf R, Geier MR. Institute of Chronic Illnesses, Inc., Silver Spring, MD; CoMeD, Inc., Silver Spring, MD.

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 AUSTRAILIAN CHILDREN WITH AUTISM (AUSTIN 2008)

J Toxicol Environ Health A. 2008;71(20):1349-51. Austin DW, Shandley K. Swinburne Autism Bio-Research Initiative (SABRI), Faculty of Life and Social Sciences, Swinburne University of Technology, Melbourne, Australia.

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.

CELLULAR AND MITOCHONDRIAL GLUTATHIONE REDOX IMBALANCE IN LYMPHOBLASTOID CELLS DERIVED FROM CHILDREN WITH AUTISM (JAMES 2009)

James SJ, Rose S, Melnyk S, Jernigan S, Blossom S, Pavliv O, Gaylor DW. FASEB J. 2009 Aug;23(8):2374-83. Epub 2009 Mar 23.

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.

NEONATE EXPOSURE TO THIMEROSAL MERCURY FROM HEPATITIS B VACCINES (DOREA 2009)

Dórea JG, Marques RC, Brandão KG. Am J Perinatol. 2009 Aug;26(7):523-7. Epub 2009 Mar 12.

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 24 hours. Range of mercury exposure spread from 4.2 to 21.1 microg 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.

A CASE SERIES OF CHILDREN WITH APPARENT MERCURY TOXIC ENCEPHALOPATHIES MANIFESTING WITH CLINICAL SYMPTOMS OF REGRESSIVE AUTISTIC DISORDERS. (GEIER 2007)

Geier DA, Geier MR. J Toxicol Environ Health A. 2007 May 15;70(10):837-51.

Impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns characterize autism spectrum disorders (ASDs). It is clear that while genetic factors are important to the pathogenesis of ASDs, mercury exposure can induce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or

associated with ASDs. The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, U.S. Department of Health and Human Services, IRB number IRB00005375) approved the present study. A case series of nine patients who presented to the Genetic Centers of America for a genetic/developmental evaluation are discussed. Eight of nine patients (one patient was found to have an ASD due to Rett's syndrome) (a) had regressive ASDs; (b) had elevated levels of androgens; (c) excreted significant amounts of mercury post chelation challenge; (d) had biochemical evidence of decreased function in their glutathione pathways; (e) had no known significant mercury exposure except from Thimerosal

Primate Infant Research

COMPARISON OF BLOOD AND BRAIN MERCURY LEVELS IN INFANT MONKEYS EXPOSED TO METHYLMERCURY OR VACCINES CONTAINING THIMEROSAL (BURBACHER 2005)

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 (HEWITSON 2008)

L. Hewitson B. Lopresti , C. Stott, J. Tomko , L. Houser , E. Klein , G. Sackett , S. Gupta , D. Atwood , L. Blue , E. R. White , A. Wakefield IMFAR (May 2008)

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 thylmercurithiosalicylic 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 (WAKEFIELD, 2008)

A Wakefield , C. Stott , B. Lopresti , J. Tomko , L. Houser , G. Sackett , L. Hewitson IMFAR (May 2008)

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 (WALKER 2008)

S. J. Walker , E. K. Lobenhofer , A. Wakefield , L. Hewitson IMFAR (May 2008)

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 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.

DELAYED ACQUISITION OF NEONATAL REFLEXES IN NEWBORN PRIMATES RECEIVING A THIMEROSAL-CONTAINING HEPATITIS B VACCINE: INFLUENCE OF GESTATIONAL AGE AND BIRTH WEIGHT. (HEWITSON 2010)

Hewitson L, Houser LA, Stott C, Sackett G, Tomko JL, Atwood D, Blue L, White ER. J Toxicol Environ Health A. 2010 Jan;73(19):1298-313.

This study examined whether acquisition of neonatal reflexes in newborn rhesus macaques was influenced by receipt of a single neonatal dose of hepatitis B vaccine containing the preservative thimerosal (Th). Hepatitis B vaccine containing a weight-adjusted Th dose was administered to male macaques within 24 h of birth (n = 13). Unexposed animals received saline placebo (n = 4) or no injection (n = 3). Infants were tested daily for acquisition of nine survival, motor, and sensorimotor reflexes. In exposed animals there was a significant delay in the acquisition of root, snout, and suck reflexes, compared with unexposed animals. No neonatal responses were significantly delayed in unexposed animals. Gestational age (GA) and birth weight (BW) were not significantly correlated. Cox regression models were used to evaluate main effects and interactions of exposure with BW and GA as independent predictors and time-invariant covariates. Significant main effects remained for exposure on root and suck when controlling for GA and BW, such that exposed animals were relatively delayed in time-to-criterion. Interaction models indicated there were various interactions between exposure, GA, and BW and that inclusion of the relevant interaction terms significantly improved model fit. This, in turn, indicated that lower BW and/or lower GA exacerbated the adverse effects following vaccine exposure. This primate model provides a possible means of assessing adverse neurodevelopmental outcomes from neonatal Th-containing hepatitis B vaccine exposure,

particularly in infants of lower GA or BW. The mechanisms underlying these effects and the requirements for Th requires further study

INFLUENCE OF PEDIATRIC VACCINES ON AMYGDALA GROWTH AND OPIOID LIGAND BINDING IN RHESUS MACAQUE INFANTS: A PILOT STUDY. (HEWITSON 2010)

Hewitson L, Lopresti BJ, Stott C, Mason NS, Tomko J. Acta Neurobiol Exp (Wars). 2010;70(2):147-64.

This longitudinal, case-control pilot study examined amygdala growth in rhesus macaque infants receiving the complete US childhood vaccine schedule (1994-1999). Longitudinal structural and functional neuroimaging was undertaken to examine central effects of the vaccine regimen on the developing brain. Vaccine-exposed and saline-injected control infants underwent MRI and PET imaging at approximately 4 and 6 months of age, representing two specific timeframes within the vaccination schedule. Volumetric analyses showed that exposed animals did not undergo the maturational changes over time in amygdala volume that was observed in unexposed animals. After controlling for left amygdala volume, the binding of the opioid antagonist [(11)C]diprenorphine (DPN) in exposed animals remained relatively constant over time, compared with unexposed animals, in which a significant decrease in [(11)C]DPN binding occurred. These results suggest that maturational changes in amygdala volume and the binding capacity of [(11)C]DPN in the amygdala was significantly altered in infant macaques receiving the vaccine schedule. The macaque infant is a relevant animal model in which to investigate specific environmental exposures and structural/functional neuroimaging during neurodevelopment.

DELAYED ACQUISITION OF NEONATAL REFLEXES IN NEWBORN PRIMATES RECEIVING A THIMEROSAL-CONTAINING HEPATITIS B VACCINE: INFLUENCE OF GESTATIONAL AGE AND BIRTH WEIGHT.

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birth weight (BW) were not significantly correlated. Cox regression models were used to evaluate main effects and interactions of exposure with BW and GA as independent predictors and time-invariant covariates. Significant main effects remained for exposure on root and suck when controlling for GA and BW, such that exposed animals were relatively delayed in time-to-criterion. Interaction models indicated there were various interactions between exposure, GA, and BW and that inclusion of the relevant interaction terms significantly improved model fit. This, in turn, indicated that lower BW and/or lower GA exacerbated the adverse effects following vaccine exposure. This primate model provides a possible means of assessing adverse neurodevelopmental outcomes from neonatal Th-containing hepatitis B vaccine exposure, particularly in infants of lower GA or BW. The mechanisms underlying these effects and the requirements for Th requires further study.

Animal Research

COMPARISON OF ORGANIC AND INORGANIC MERCURY DISTRIBUTION IN SUCKLING RAT (ORCT 2006)

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 2005)

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 2004)

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 2004)

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 methymercury 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 (ZAREBA 2007)

Grazyna Zareba, Elsa Cernichiari, Rieko Hojo, Scott Mc Nitt, Bernard Weiss, Moiz M Mumtaz, Dennis E Jones, Thomas W Clarkson Neurotoxicology. 2007 Feb 23; : 17382399

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.

GENDER-SELECTIVE TOXICITY OF THIMEROSAL (BRANCH 2008)

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

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.

INDUCTION OF METALLOTHIONEIN IN MOUSE CEREBELLUM AND CEREBRUM WITH LOW-DOSE THIMEROSAL INJECTION (MINAMI 2009)

Minami T, Miyata E, Sakamoto Y, Yamazaki H, Ichida S. Department of Life Sciences, School of Science & Engineering, Kinki University, 3-4-1 Kowakae, Higashi-osaka, Osaka, 577-8502, Japan, minamita@life.kindai.ac.jp. Cell Biol Toxicol. 2009 Apr 9.

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 (MINAMI 2007)

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 aftera 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 BEHAVIOR (OLCZAK 2008)

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 do 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 or 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 ON THIMEROSAL ON RAT DEVELOPMENT AND BEHAVIOR (DUSZCZYK 2008)

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, weight 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.]

PRENATAL METHYLMERCURY EXPOSURE HAMPERS GLUTATHIONE ANTIOXIDANT SYSTEM ONTOGENSIS AND CAUSES LONG-LASTING OXIDATIVE STRESS IN THE MOUSE BRRAIN (STRINGARI 2008)

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. Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, SC, Brazil.

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.

NEONATAL ADMINISTRATION OF A VACCINE PRESERVATIVE, THIMEROSAL, PRODUCES LASTING IMPAIRMENT OF NOCICEPTION AND APPARENT ACTIVATION OF OPOID SYSTEM IN RATS (OLCZAK 2009)

Olczak M, Duszczyk M, Mierzejewski P, Majewska MD. Brain Res. 2009 Dec 8;1301:143-51. Epub 2009 Sep 9.

Thimerosal (THIM), an organomercury preservative added to many child vaccines is a suspected factor in pathogenesis of neurodevelopmental disorders. We examined the pharmacokinetics of Hg in the brain, liver and kidneys after i.m. THIM injection in suckling rats and we tested THIM effect on nociception. THIM solutions were injected to Wistar and Lewis rats in a vaccination-like mode on PN days 7, 9, 11 and 15 in four equal doses. For Wistar rats these were: 12, 48, 240, 720, 1440, 2160, 3000 microg Hg/kg and for Lewis: 54, 216, 540 and 1080 microg Hg/kg. Pharmacokinetic analysis revealed that Hg from THIM injections accumulates in the rat brain in significant amounts and remains there longer than 30 days after the injection. At the 6th week of age animals were examined for pain sensitivity using the hot plate test. THIM treated rats of both strains and sexes manifested statistically significantly elevated pain threshold (latency for paw licking, jumping) on a hot plate (56 degrees C). Wistar rats were more sensitive to this effect than Lewis rats. Protracted THIM-induced hypoalgesia was reversed by naloxone (5 mg/kg, i.p.) injected before the hot plate test, indicative of involvement of endogenous opioids. This was confirmed by augmented catalepsy after morphine (2.5 mg/kg, s.c.) injection. Acute THIM injection to 6-week-old rats also produced hypoalgesia, but this effect was transient and was gone within 14 days. Present findings show that THIM administration to suckling or adult rats impairs sensitivity to pain, apparently due to activation the endogenous opioid system.

MERCURY TOXICOKINETICS – DEPENDENCY ON STRAIN AND GENDER (EKSTRAND 2010)

Ekstrand J, Nielsen JB, Havarinasab S, Zalups RK, Söderkvist P, Hultman P. Toxicol Appl Pharmacol. 2010 Mar 15;243(3):283-91. Epub 2009 Sep 2.

Mercury (Hg) exposure from dental amalgam fillings and thimerosal in vaccines is not a major health hazard, but adverse health effects cannot be ruled out in a small and more susceptible part of the exposed population. Individual differences in toxicokinetics may explain susceptibility to mercury. Inbred, H-2-congenic A.SW and B10.S mice and their F1- and F2-hybrids were given HgCl2 with 2.0 mg Hg/L drinking water and traces of (203)Hg. Whole-body retention (WBR) was monitored until steady state after 5 weeks, when the organ Hg content

was assessed. Despite similar Hg intake, A.SW males attained a 20-30% significantly higher WBR and 2- to 5-fold higher total renal Hg retention/concentration than A.SW females and B10.S mice. A selective renal Hg accumulation but of lower magnitude was seen also in B10.S males compared with females. Differences in WBR and organ Hg accumulation are therefore regulated by non-H-2 genes and gender. Lymph nodes lacked the strain- and gender-dependent Hg accumulation profile of kidney, liver and spleen. After 15 days without Hg A.SW mice showed a 4-fold higher WBR and liver Hg concentration, but 11-fold higher renal Hg concentration, showing the key role for the kidneys in explaining the slower Hg elimination in A.SW mice. The trait causing higher mercury accumulation was not dominantly inherited in the F1 hybrids. F2 mice showed a large inter-individual variation in Hg accumulation, showing that multiple genetic factors influence the Hg toxicokinetics in the mouse. The genetically heterogeneous human population may therefore show a large variation in mercury toxicokinetics.

INCREASE IN INTRACELLULAR ZN2+ CONCENTRATION BY THIMEROSAL IN RAT THYMOCYTES: INTRACELLULAR ZN2+ RELEASE INDUCED BY OXIDATIVE STRESS (HASHIMOTO 2009)

Hashimoto E, Oyama TB, Oyama K, Nishimura Y, Oyama TM, Ueha-Ishibashi T, Okano Y, Oyama Y. Toxicol In Vitro. 2009 Sep;23(6):1092-9. Epub 2009 Jun 2.

Thimerosal (TMR), an ethylmercury-containing preservative in pharmaceutical products, was recently reported to increase intracellular Zn(2+) concentration. Therefore, some health concerns about the toxicity of TMR remain because of physiological and pathological roles of Zn(2+). To reveal the property of TMR-induced increase in intracellular Zn(2+) concentration, the effect of TMR on FluoZin-3 fluorescence, an indicator of intracellular Zn(2+), of rat thymocytes was examined. TMR at concentrations ranging from 0.3 microM to 10 microM increased the intensity of FluoZin-3 fluorescence in a concentration-dependent manner under external Ca(2+)- and Zn(2+)-free condition. The threshold concentration was 0.3-1 microM. The increase in the intensity was significant when TMR concentration was 1 microM or more. N,N,N',N'-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), a chelator for intracellular Zn(2+), completely attenuated the TMR-induced augmentation of FluoZin-3 fluorescence. Hydrogen peroxide (H(2)O(2)) and N-ethylmaleimide, reducing cellular thiol content, significantly increased FluoZin-3 fluorescence intensity and decreased 5-chloromethylfluorescein (5-CMF) fluorescence intensity, an indicator for cellular thiol. The correlation coefficient between TMR-induced augmentation of FluoZin-3 fluorescence and attenuation of 5-CMF fluorescence was -0.882. TMR also attenuated the 5-CMF fluorescence in the presence of TPEN. Simultaneous application of H(2)O(2) and TMR synergistically augmented the FluoZin-3 fluorescence. It is

suggested that TMR increases intracellular Zn(2+) concentration via decreasing cellular thiol content.

NEONATAL ADMINISTRATION OF THIMEROSAL CAUSES PERSISTENT CHANGES IN MU OPIOID RECEPTORS IN THE RAT BRAIN (OLCZAK 2010)

Olczak M, Duszczyk M, Mierzejewski P, Bobrowicz T, Majewska MD. Neurochem Res. 2010 Aug 28.

Thimerosal added to some pediatric vaccines is suspected in pathogenesis of several neurodevelopmental disorders. Our previous study showed that thimerosal administered to suckling rats causes persistent, endogenous opioid-mediated hypoalgesia. Here we examined, using immunohistochemical staining technique, the density of μ-opioid receptors (MORs) in the brains of rats, which in the second postnatal week received four i.m. injections of thimerosal at doses 12, 240, 1,440 or 3,000 μg Hg/kg. The periaqueductal gray, caudate putamen and hippocampus were examined. Thimerosal administration caused dose-dependent statistically significant increase in MOR densities in the periaqueductal gray and caudate putamen, but decrease in the dentate gyrus, where it was accompanied by the presence of degenerating neurons and loss of synaptic vesicle marker (synaptophysin). These data document that exposure to thimerosal during early postnatal life produces lasting alterations in the densities of brain opioid receptors along with other neuropathological changes, which may disturb brain development.

Cellular Research

THIMEROSAL INDUCES TH2 RESPONSES VIA INFLUENCING CYTOKINE SECRETION BY HUMAN DENDRITIC CELLS (AGRAWAL 2007)

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 (GEIER 2009)

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 cytoxicity 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 2006)

Herdman ML, Marcelo A, Huang Y, Niles RM, Dhar S, Kiningham 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 NANAMOLAR THIMEROSAL (GOTH 2006)

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 nanamolar 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 2005)

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 nanamolar 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 2005)

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 2003)

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 CELLINE (SK-N-SH)(HUMPHREY 2009)

Humphrey ML, Cole MP, Pendergrass JC, Kiningham 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 2005)

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

James notes 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 2002)

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 2005)

Parran DK, Barker A, Ehrich 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 2004)

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 2003)

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 (HAASE 2008)

Haase H, Hebel S, Engelhardt G, Rink L., Institute of Immunology, RWTH Aachen University Hospital, Aachen, Germany. Cell Calcium. 2008 Oct 31.

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.

CHARACTERIZATION OF EARLY EVENTS INVOLVED IN HUMAN DENDRITIC CELL MATURATION INDUCED BY SENSITIZERS: CROSS TALK BETWEEN MAPK SIGNALLING PATHWAYS (TROMPEZINSKI 2008)

Trompezinski S, Migdal C, Tailhardat M, Le Varlet B, Courtellemont P, Haftek M, Serres M. Toxicol Appl Pharmacol. 2008 Aug 1;230(3):397-406. Epub 2008 Apr 8.

Dendritic cells (DCs), efficient-antigen presenting cells play an important role in initiating and regulating immune responses. DC maturation following exposure to nickel or DNCB induced an up-regulation of phenotypic markers and inflammatory cytokine secretion. Early intracellular mechanisms involved in DC maturation required to be precise. To address this purpose, DCs

derived from human monocytes were treated with sensitizers (nickel, DNCB or thimerosal) in comparison with an irritant (SDS). Our data confirming the up-regulation of CD86, CD54 and cytokine secretion (IL-8 and TNFalpha) induced by sensitizers but not by SDS, signalling transduction involved in DC maturation was investigated using these chemicals. Kinase activity measurement was assessed using two new sensitive procedures (Facetrade mark and CBA) requiring few cells. SDS did not induce changes in signalling pathways whereas NiSO(4), DNCB and thimerosal markedly activated p38 MAPK and JNK, in contrast Erk1/2 phosphorylation was completely inhibited by DNCB or thimerosal and only activated by nickel. A pre-treatment with p38 MAPK inhibitor (SB203580) suppressed Erk1/2 inhibition induced by DNCB or thimerosal demonstrating a direct interaction between p38 MAPK and Erk1/2. A pre-treatment with an antioxidant, N-acetyl-L-cysteine (NAC) markedly reduced Erk1/2 inhibition and p38 MAPK phosphorylation induced by DNCB and thimerosal, suggesting a direct activation of p38 MAPK via an oxidative stress and a regulation of MAPK signalling pathways depending on chemicals. Because of a high sensitivity of kinase activity measurements, these procedures will be suitable for weak or moderate sensitizer screening.

EVALUATION OF CYTOTOXICITY ATTRIBUTED TO THIMEROSAL ON MURINE AND HUMAN KIDNEY CELLS.

Park EK, Mak SK, Kültz D, Hammock BD. J Toxicol Environ Health A. 2007 Dec;70(24):2092-5.

Renal inner medullary collecting duct cells (mIMCD3) and human embryonic kidney cells (HEK293) were used for cytoscreening of thimerosal and mercury chloride (HgCl2). Thimerosal and HgCl2 acted in a concentration-dependent manner. In mIMCD3 cells the 24-h LC50 values for thimerosal, thiosalicylic acid, 2,2-dithiosalicylic acid, and 2-sulfobenzoic acid were 2.9, 2200, >1000, and >10,000 microM, respectively. The 24-h LC50 value for HgCl2 in mIMCD3 cells was 40 microM. In HEK293 cells, the 24-h LC50 value for thimerosal was 9.5 microM. These data demonstrate that the higher cytotoxicity produced by thimerosal on renal cells with respect to similar compounds without Hg may be related to this metal content. The present study also establishes mIMCD3 cells as a valuable model for evaluation of cytotoxicity of nephrotoxic compounds.

Genetic Research

GENOTOXICITY OF THIMEROSAL IN CULTURED HUMAN LYMPHOCYTES WITH AND WITHOUT METABOLIC ACTIVATION SISTER CHROMATID EXCHANGE ANALYSIS PROLIFERATION INDEX AND MITOTIC INDEX (EKE 2008)

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 2009)

James SJ, Rose S, Melnyk S, Jernigan S, Blossom S, Pavliv O, Gaylor DW. Department of Pediatrics; andDepartment of Biostatistics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas, USA. FASEB J. 2009 Mar 23.

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 2009)

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.

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 (probands 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 2007)

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 2007)

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 2006)

Buyske S, Williams TA, Mars AE, Stenroos ES, Ming SX, Wang R, Sreenath M, Factura MF, Reddy C, Lambert GH, Johnson WG. Departments of Statistics and Genetics, 110 Frelinghuysen Rd, Rutgers University, Piscataway, NJ 08854, USA. buyske@stat.rutgers.edu 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 (MOHAMMAD 2009)

Mohammad NS, Jain JM, Chintakindi KP, Singh RP, Naik U, Akella RR. Center for DNA Fingerprinting and Diagnostics bInstitute of Child Health, Niloufer Hospital, Hyderabad, India. Psychiatr Genet. 2009 May 13.

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.

MUTATION RESEARCH/FUNDAMENTAL AND MOLECULAR MECHANISMS OF MUTAGENESIS (BUCIO 1999)

Leticia Bucio, Cecilia Garca, Verranica Souza, Elizabeth Hernandez, Cristina Gonzalez, Miguel Betancourt and Ma. Concepcian Gutiarrez-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 Î¼M) 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 PBSâ€“EGTA.

THIOL-MODULATED MECHANISMS OF THE CYTOTOXICITY OF THIMEROSAL AND INHIBITION OF DNA TOPOISOMERASE II ALPHA (WU 2008)

Wu X, Liang H, O'Hara KA, Yalowich JC, Hasinoff BB. Chem Res Toxicol. 2008 Feb;21(2):483-93. Epub 2008 Jan 16.

Thimerosal is an organic mercury compound that is widely used as a preservative in vaccines and other solution formulations. The use of thimerosal has caused concern about its ability to cause neurological abnormalities due to mercury accumulation during a normal schedule of childhood vaccinations. While the chemistry and the biological effects of methylmercury have been well-studied, those of thimerosal have not. Thimerosal reacted rapidly with cysteine, GSH, human serum albumin, and single-stranded DNA to form ethylmercury adducts that were detectable by mass spectrometry. These results indicated that thimerosal would be quickly metabolized in vivo because of its reactions with protein and nonprotein thiols. Thimerosal also potently inhibited the decatenation activity of DNA topoisomerase II alpha, likely through reaction with critical free cysteine thiol groups. Thimerosal, however, did not act as a topoisomerase II poison and the lack of cross-resistance with a K562 cell line with a decreased level of topoisomerase II alpha (K/VP.5 cells) suggested that inhibition of topoisomerase II alpha was not a significant mechanism for the inhibition of cell growth. Depletion of intracellular GSH with buthionine sulfoximine treatment greatly increased the K562 cell growth inhibitory effects of thimerosal, which showed that intracellular glutathione had a major role in protecting cells from thimerosal. Pretreatment of thimerosal with glutathione did not, however, change its K562 cell growth inhibitory effects, a result consistent with the rapid exchange of the ethylmercury adduct among various thiol-containing cellular reactants. Thimerosal-induced single and double strand breaks in K562 cells were consistent with a rapid induction of apoptosis. In conclusion, these studies have elucidated some of the chemistry and biological activities of the interaction of thimerosal with topoisomerase II alpha and protein and nonprotein thiols and with DNA.

Epidemiological Research

EARLY THIMEROSAL EXPOSURE AND NEUROPSYCHOLOGICAL OUTCOME AT 7 TO 10 YEARS (THOMPSON 2007)

New England Journal of Medicine; 9/27/07, vol. 357 no. 13 William W. Thompson, Ph.D., Cristofer Price, Sc.M., Barabara Goodson, Ph.D., David K. Shay, M.D., M.P.H., Pattie Benson, M.P.H., Virginia L. Hinrichsen, M.S., M.P.H, Edwin Lewis, M.P.H., Eileen Eriksen, M.P.H., Paula Ray, M.P.H., S. Michael Marcy, M.D., John Dunn, M.D., M.P.H., Lisa A. Jackson, M.D., M.P.H., Tracy A. Lieu, M.D., M.P.H, Steve Black, M.D., Gerrie Stewart, M.A., Eric S. Weintraub, M.P.H., Robert L. Davis, M.D., M.P.H., and Frank DeStefano, M.D., M.P.H., for the Vaccine Safety Datalink Team

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 (LIFE 2008)

International Journal of Risk & Safety in Medicine 20 (2008) 135-142 David Austin Life and Social Sciences, Swinburne University of Technology, Melbourne, Australia

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 RH0(D) IMMUNE GLOBULINS: A MULTI-CENTER ASSESSMENT (GEIER 2008)

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 (GEIER 2008)

J Neurol Sci. 2008 Aug 15;271(1-2):110-8. Epub 2008 May 15. Young HA, Geier DA, Geier MR. 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 (DESOTO 2009)

Desoto MC. Department of Psychology, University of Northern Iowa, Baker Hall, Cedar Falls, IA 50614-0505, United States. 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.

Related Autism-Mercury Research

HOW ENVIRONMENTAL AND GENETIC FACTORS COMBINE TO CAUSE AUTISM: A REDOX/METHYLATION HYPOTHESIS (DETH 2007)

Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M. Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 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.

HEPATITIS B VACCINE AND THE RISK OF CNS INFLAMMATORY DEMYELINATION IN CHILDHOOD (MIKAELOFF 2009)

Yann Mikaeloff, MD, PhD, Guillaume Caridade, MSc, Samy Suissa, PhD and Marc Tardieu, MD, PhD 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 CASE-CONTROL STUDY OF MERCURY BURDEN IN CHILDREN WITH AUTISTIC SPECTRUM DISORDERS (BRADSTREET 2003)

Jeff Bradstreet, M.D.; David A. Geier, B.A.; Jerold J. Kartzinel, M.D.; James B. Adams, Ph.D.; Mark R. Geier, M.D., Ph.D. 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 (WINDHAM 2006)

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.

PROXIMITY TO POINT SOURCES OF ENVIRONMENTAL MERCURY RELEASE AS A PREDICTOR OF AUTISM PREVALENCE (PALMER 2008)

Palmer RF, Blanchard S, Wood R University of Texas Health Science Center, San Antonio Department of Family and Community Medicine, San Antonio Texas. 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 (MING 2008)

Xue Ming, Michelle A. Cheh, Carrie L. Yochum, Alycia K. Halladay, George C. Wagner Pediatric Neuroscience, UMDNJ, Newark, NJ; Psychology, Rutgers University, New Brunswick, NJ; Autism Speaks, Princeton, NJ American Journal of Biochemistry and Biotechnology 4 (2): 218-225, 2008

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.