Summary of Supportive Science Regarding Thimerosal Removal

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Contents

Environmental Impact

A PILOT SCALE EVALUATION OF REMOVAL OF MERCURY FROM PHARMACEUTICAL WASTEWATER USING GRANULAR ACTIVATED CARBON (CYR 2002)

Cyr PJ, Suri RP, Helmig ED. Water Res. 2002 Nov;36(19):4725-34.

Thimerosal (an organic mercury compound) is widely used in the pharmaceutical industry and hospitals. This study examines the removal of mercury (thimerosal and Hg(II)) from a pharmaceutical wastewater using F-400 granular activated carbon (GAC) at bench and pilot scales. Bench scale dynamic column tests are conducted with 30, 60, 90 and 120 min empty bed contact times (EBCTs). The pilot scale study is conducted using two GAC columns-in-series each of 30 min EBCT. The capital and operational cost analysis for the treatment system is performed. Simultaneous removal of copper, turbidity, phenol, and color from the wastewater by the pilot scale system is also reported.

BIODEGRADATION OF THIOMERSAL CONTAINING EFFLUENTS BY A MERCURY RESISTANT PSEUDOMONAS PUTIDA STRAIN (FORTUNATO 2005)

Raquel Fortunato, João G. Crespo and M.A.M. Water Research Volume 39, Issue 15, September 2005, Pages 3511-3522

Thiomersal, a toxic organomercurial with a strong bactericidal effect, is the most widely used preservative in vaccine production. As a result, vaccine production wastewaters are frequently polluted with thiomersal concentrations above the European limit for mercury effluent discharges for which there is, presently, no remediation technology available. This work proposes a biotechnological process for the remediation of vaccine production wastewaters based on the biological degradation of thiomersal to metallic mercury, under aerobic conditions, by a mercury resistant bacterial strain. The kinetics of thiomersal degradation by a pure culture of Pseudomonas putida spi3 was firstly investigated in batch reactors using a thiomersal amended mineral medium. Subsequently, a continuous stirred tank reactor fed with the same medium was operated at a dilution rate of 0.05 h−1, and the bioreactor performance and robustness was evaluated when exposed to thiomersal shock loads. In a second stage, the bioreactor was fed directly with a real vaccine wastewater contaminated with thiomersal and the culture ability to grow in the wastewater and remediate it was evaluated for dilution rates ranging from 0.022 to 0.1 h−1.

USE OF ADSORPTION PROCESS TO REMOVE ORGANIC MERCURY THIMEROSAL FROM INDUSTRIAL PROCESS WASTEWATER (VELICU 2007)

Velicu M, Fu H, Suri RP, Woods K. J Hazard Mater. 2007 Sep 30;148(3):599-605. Epub 2007 Mar 12.

Carbon adsorption process is tested for removal of high concentration of organic mercury (thimerosal) from industrial process wastewater, in batch and continuously flow through column systems. The organic mercury concentration in the process wastewater is about 1123 mg/L due to the thimerosal compound. Four commercially available adsorbents are tested for mercury removal and they are: Calgon F-400 granular activated carbon (GAC), CB II GAC, Mersorb GAC and an ion-exchange resin Amberlite GT73. The adsorption capacity of each adsorbent is described by the Freundlich isotherm model at pH 3.0, 9.5 and 11.0 in batch isotherm experiments. Acidic pH was favorable for thimerosal adsorption onto the GACs. Columns-in-series experiments are conducted with 30-180 min empty bed contact times (EBCTs). Mercury breakthrough of 30 mg/L occurred after about 47 h (96 Bed Volume Fed (BVF)) of operation, and 97 h (197 BVF) with 120 min EBCT and 180 min EBCT, respectively. Most of the mercury removal is attributed to the 1st adsorbent column. Increase in contact time by additional adsorbent columns did not lower the effluent mercury concentration below 30 mg/L. However, at a lower influent wastewater pH 3, the mercury effluent concentration decreased to less than 7 mg/L for up to 90 h of column operation (183 BVF).

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 mcg 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) mcg/L. Note: One infant was found to have developed a mercury level of 23.6 mcg/L, thus meeting the CDC criteria as a case of chemical poisoning from mercury defined as a blood level of 10mcg/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 mcg 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 mcg 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 >10mcg /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.

URINARY PORPHYRIN EXCRETION IN NEUROTYPICAL AND AUTISTIC CHILDREN (WOODS 2010)

James S. Woods; Sarah E. Armel; Denise I. Fulton; Jason Allen; Kristine Wessels; P. Lynne Simmonds; Doreen Granpeesheh; Elizabeth Mumper; J. Jeffrey Bradstreet; Diana Echeverria; Nicholas J. Heyer; James P.K. Rooney From Environmental Health Perspectives

Background: Increased urinary concentrations of pentacarboxyl-, precopro- and copro-porphyrins have been associated with prolonged mercury (Hg) exposure in adults, and comparable increases have been attributed to Hg exposure in children with autism (AU). Objectives: This study was designed to measure and compare urinary porphyrin concentrations in neurotypical (NT) children and same-age children with autism, and to examine the association between porphyrin levels and past or current Hg exposure in children with autism. Methods: This exploratory study enrolled 278 children 2–12 years of age. We evaluated three groups: AU, pervasive developmental disorder-not otherwise specified (PDD-NOS), and NT. Mothers/caregivers provided information at enrollment regarding medical, dental, and dietary exposures. Urine samples from all children were acquired for analyses of porphyrin, creatinine, and Hg. Differences between groups for mean porphyrin and Hg levels were evaluated. Logistic

regression analysis was conducted to determine whether porphyrin levels were associated with increased risk of autism. Results: Mean urinary porphyrin concentrations are naturally high in young children and decline by as much as 2.5-fold between 2 and 12 years of age. Elevated copro- ($p < 0.009$), hexacarboxyl- ($p < 0.01$) and pentacarboxyl- ($p < 0.001$) porphyrin concentrations were significantly associated with AU but not with PDD-NOS. No differences were found between NT and AU in urinary Hg levels or in past Hg exposure as determined by fish consumption, number of dental amalgam fillings, or vaccines received. Conclusions: These findings identify disordered porphyrin metabolism as a salient characteristic of autism. Hg exposures were comparable between diagnostic groups, and a porphyrin pattern consistent with that seen in Hg-exposed adults was not apparent.

EMBRYONIC EXPOSURE TO THIMEROSAL, AN ORGANOMERCURY COMPOUND, CAUSES ABNORMAL EARLY DEVELOPMENT OF SEROTONERGIC NEURONS. (IDA-ETO 2011)

Ida-Eto M, Oyabu A, Ohkawara T, Tashiro Y, Narita N, Narita M. Neurosci Lett. 2011 Nov 14;505(2):61-4. doi: 10.1016/j.neulet.2011.05.053. Epub 2011 Jun 6.

Even though neuronal toxicity due to organomercury compounds is well known, thimerosal, an organomercury compound, is widely used in pediatric vaccine preservation. In the present study, we examined whether embryonic exposure to thimerosal affects early development of serotonergic neurons. Thimerosal (1mg Hg/kg) was intramuscularly administered to pregnant rats on gestational day 9 (susceptible time window for development of fetal serotonergic system), and fetal serotonergic neurons were assessed at embryonic day 15 using anti-serotonin antibodies. A dramatic increase in the number of serotonergic neurons localized to the lateral portion of the caudal raphe was observed in thimerosal group (1.9-fold increase, $p<0.01$ compared to control). These results indicate that embryonic exposure to thimerosal affects early development of serotonergic neurons.

SPECIATION OF METHYL- AND ETHYL-MERCURY IN HAIR OF BREASTFED INFANTS ACUTELY EXPOSED TO THIMEROSAL-CONTAINING VACCINES. (DOREA 2011)

Dórea JG, Bezerra VL, Fajon V, Horvat M. Clin Chim Acta. 2011 Aug 17;412(17-18):1563-6. doi: 10.1016/j.cca.2011.05.003. Epub 2011 May 7.

BACKGROUND: Different chemical forms of mercury occur naturally in human milk. The most controversial aspect of early post-natal exposure to organic mercury is ethylmercury (EtHg) in thimerosal-containing vaccines (TCV) still being used in many countries. Thus exclusively breastfed infants can be exposed to both, fish derived methylmercury (MeHg) in maternal diets and to EtHg from TCV. The aim of the study is to evaluate a new analytical method for ethyl and methyl mercury in hair samples of breastfed infants who had received the recommended schedule of TCV. METHODS: The hair of infants (<12 months) that had been exposed to TCV

(Hepatitis B and DTaP) was analysed. A method coupling isothermal gas chromatography with cold-vapor atomic fluorescence spectrometry was used for MeHg which can also speciate EtHg in biological matrices. RESULTS: In 20 samples of infants' hair, all but two samples showed variable amounts of MeHg (10.3 to 668 ng/g), while precise and reliable concentrations of EtHg (3.7 to 65.0 ng/g) were found in 15 of the 20 samples. A statistically significant inverse association (r=-05572; p=0.0384) was found between hair-EtHg concentrations and the time elapsed after the last TCV shot. CONCLUSIONS: The analytical method proved sensitive enough to quantify EtHg in babies' hair after acute exposure to thimerosal in vaccine shots. Provided that the mass of hair was above 10mg, organic-mercury exposure during early life can be speciated, and quantified in babies' first hair, thus opening opportunities for clinical and forensic studies.

INTEGRATING EXPERIMENTAL (IN VITRO AND IN VIVO) NEUROTOXICITY STUDIES OF LOW-DOSE THIMEROSAL RELEVANT TO VACCINES. (DOREA 2011)

Dórea JG. Neurochem Res. 2011 Jun;36(6):927-38. doi: 10.1007/s11064-011-0427-0. Epub 2011 Feb 25.

There is a need to interpret neurotoxic studies to help deal with uncertainties surrounding pregnant mothers, newborns and young children who must receive repeated doses of Thimerosal-containing vaccines (TCVs). This review integrates information derived from emerging experimental studies (in vitro and in vivo) of low-dose Thimerosal (sodium ethyl mercury thiosalicylate). Major databases (PubMed and Web-of-science) were searched for in vitro and in vivo experimental studies that addressed the effects of low-dose Thimerosal (or ethylmercury) on neural tissues and animal behaviour. Information extracted from studies indicates that: (a) activity of low doses of Thimerosal against isolated human and animal brain cells was found in all studies and is consistent with Hg neurotoxicity; (b) the neurotoxic effect of ethylmercury has not been studied with co-occurring adjuvant-Al in TCVs; (c) animal studies have shown that exposure to Thimerosal-Hg can lead to accumulation of inorganic Hg in brain, and that (d) doses relevant to TCV exposure possess the potential to affect human neuro-development. Thimerosal at concentrations relevant for infants' exposure (in vaccines) is toxic to cultured human-brain cells and to laboratory animals. The persisting use of TCV (in developing countries) is counterintuitive to global efforts to lower Hg exposure and to ban Hg in medical products; its continued use in TCV requires evaluation of a sufficiently nontoxic level of ethylmercury compatible with repeated exposure (co-occurring with adjuvant-Al) during early life.

NEONATAL EXPOSURE TO THIMEROSAL FROM VACCINES AND CHILD DEVELOPMENT IN THE FIRST 3YEARS OF LIFE. (MROZEK-BUDZYN 2012)

Mrozek-Budzyn D, Majewska R, Kieltyka A, Augustyniak M. Neurotoxicol Teratol. 2012 Nov-Dec;34(6):592-7. doi: 10.1016/j.ntt.2012.10.001. Epub 2012 Oct 13.

BACKGROUND: Despite the common use of Thimerosal as a preservative in childhood vaccines since the 1930s, there are not many studies on ethylmercury toxicokinetics and toxicodynamics in infants. The knowledge of ethylmercury's potential adverse effects is derived mostly from parallel methylmercury research or from animal and theoretical models. AIM OF THE STUDY: This study was designed to examine the relationship between neonatal exposure to Thimerosal-containing vaccine (TCV) and child development. MATERIAL AND METHODS: The study sample consisted of 196 infants born between January 2001 and March 2003 to mothers attending ambulatory prenatal clinics in the first and second trimesters of pregnancy in Krakow. Vaccination history (date and the type of the vaccine) was extracted from physicians' records. Child development was assessed using the Bayley Scales of Infant Development (BSID-II) measured in one-year intervals over 3years. General Linear Model (GLM) and Generalized Estimating Equation (GEE) models adjusted for potential confounders were used to assess the association. RESULTS: An adverse effect of neonatal TCV exposure was observed for the psychomotor development index (PDI) only in the 12th and 24th months of life (β=-6.44, $p<0.001$ and β=-5.89, $p<0.001$). No significant effect of neonatal TCV exposure was found in the 36th month. The overall deficit in the PDI attributable to neonatal TCV exposure measured over the course of the three-year follow-up (GEE) was significantly higher in TCV group (β=-4.42, $p=0.001$). MDI scores did not show the adverse association with neonatal TCV exposure.

BREAST-FEEDING AND RESPONSES TO INFANT VACCINES: CONSTITUTIONAL AND ENVIRONMENTAL FACTORS. (DOREA 2012)

Dórea JG. Am J Perinatol. 2012 Nov;29(10):759-75. doi: 10.1055/s-0032-1316442. Epub 2012 Jul 6.

Neonates and nursing infants are special with regard to immune development and vulnerability to infectious diseases. Although breast-feeding is essential to modulate and prime immune defenses, vaccines (an interventional prophylaxis) are crucial to prevent and control infectious diseases. During nursing, the type of feeding influences infants' natural defenses (including gut colonization) and their response to vaccines, both through cell-mediated immunity and specific antibody production. Given the variety and combination of vaccine components (antigens and excipients, preservative thimerosal, and aluminum adjuvants) and route of administration, there is a need to examine the role of infant feeding practices in intended and nonintended outcomes of vaccination. Maternal factors related to milk constituents (nutrients and

pollutants) and feeding practices can affect response to vaccines. Collectively, studies that compared type of feeding (or used breast-feeding-adjusted statistical models) showed significant influence on some vaccines taken during infancy. Nurslings deprived of the full benefit of breast-feeding could have altered immune responses affecting vaccine outcome. In the absence of studies elucidating neurodevelopment (including excitoxicity) and immunotoxicity issues, vaccination practices should promote and support breast-feeding.

THIMEROSAL EXPOSURE IN EARLY LIFE AND NEUROPSYCHOLOGICAL OUTCOMES 7-10 YEARS LATER. (BARILE 2012)

Barile JP, Kuperminc GP, Weintraub ES, Mink JW, Thompson WW. J Pediatr Psychol. 2012 Jan-Feb;37(1):106-18. doi: 10.1093/jpepsy/jsr048. Epub 2011 Jul 23.

OBJECTIVE: The authors used a public use data set to investigate associations between the receipt of thimerosal-containing vaccines and immune globulins early in life and neuropsychological outcomes assessed at 7-10 years. METHODS: The data were originally created by evaluating 1,047 children ages 7-10 years and their biological mothers. This study developed seven latent neuropsychological factors and regressed them on a comprehensive set of covariates and thimerosal exposure variables. RESULTS: The authors found no statistically significant associations between thimerosal exposure from vaccines early in life and six of the seven latent constructs. There was a small, but statistically significant association between early thimerosal exposure and the presence of tics in boys. CONCLUSIONS: This finding should be interpreted with caution due to limitations in the measurement of tics and the limited biological plausibility regarding a causal relationship.

Non-Human 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.

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.

Animal Research

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.

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.

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.

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.]

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.

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.

IDENTIFICATION AND DISTRIBUTION OF MERCURY SPECIES IN RAT TISSUES FOLLOWING ADMINISTRATION OF THIMEROSAL OR METHYLMERCURY. (RODRIGUES 2010)

Rodrigues JL, Serpeloni JM, Batista BL, Souza SS, Barbosa F Jr. Arch Toxicol. 2010 Nov;84(11):891-6. Epub 2010 Apr 13.

Methylmercury (Met-Hg) is one the most toxic forms of Hg, with a considerable range of harmful effects on humans. Sodium ethyl mercury thiosalicylate, thimerosal (TM) is an ethylmercury (Et-Hg)-containing preservative that has been used in manufacturing vaccines in many countries. Whereas the behavior of Met-Hg in humans is relatively well known, that of ethylmercury (Et-Hg) is poorly understood. The present study describes the distribution of mercury as (-methyl, -ethyl and inorganic mercury) in rat tissues (brain, heart, kidney and liver) and blood following administration of TM or Met-Hg. Animals received one dose/day of Met-Hg or TM by gavage (0.5 mg Hg/kg). Blood samples were collected after 6, 12, 24, 48, 96 and 120 h of exposure. After 5 days, the animals were killed, and their tissues were collected. Total blood

mercury (THg) levels were determined by ICP-MS, and methylmercury (Met-Hg), ethylmercury (Et-Hg) and inorganic mercury (Ino-Hg) levels were determined by speciation analysis with LC-ICP-MS. Mercury remains longer in the blood of rats treated with Met-Hg compared to that of TM-exposed rats. Moreover, after 48 h of the TM treatment, most of the Hg found in blood was inorganic. Of the total mercury found in the brain after TM exposure, 63% was in the form of Ino-Hg, with 13.5% as Et-Hg and 23.7% as Met-Hg. In general, mercury in tissues and blood following TM treatment was predominantly found as Ino-Hg, but a considerable amount of Et-Hg was also found in the liver and brain. Taken together, our data demonstrated that the toxicokinetics of TM is completely different from that of Met-Hg. Thus, Met-Hg is not an appropriate reference for assessing the risk from exposure to TM-derived Hg. It also adds new data for further studies in the evaluation of TM toxicity.

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.

CHRONIC METALS INGESTION BY PRAIRIE VOLES PRODUCES SEX-SPECIFIC DEFICITS IN SOCIAL BEHAVIOR: AN ANIMAL MODEL OF AUTISM. (CURTIS 2010)

Curtis JT, Hood AN, Chen Y, Cobb GP, Wallace DR. Behav Brain Res. 2010 Nov 12;213(1):42-9. Epub 2010 Apr 28.

We examined the effects of chronic metals ingestion on social behavior in the normally highly social prairie vole to test the hypothesis that metals may interact with central dopamine systems to produce the social withdrawal characteristic of autism. Relative to water-treated controls, 10 weeks of chronic ingestion of either Hg(++) or Cd(++) via drinking water significantly reduced social contact by male voles when they were given a choice between isolation or contact with an unfamiliar same-sex conspecific. The effects of metals ingestion were specific to males: no effects of metals exposure were seen in females. Metals ingestion did not alter behavior of males allowed to choose between isolation or their familiar cage-mates, rather than strangers. We also examined the possibility that metals ingestion affects central dopamine functioning by testing the voles' locomotor responses to peripheral administration of amphetamine. As with the social behavior, we found a sex-specific effect of metals on amphetamine responses. Males that consumed Hg(++) did not increase their locomotor activity in response to amphetamine, whereas similarly treated females and males that ingested only water significantly increased their locomotor activities. Thus, an ecologically relevant stimulus, metals ingestion, produced two of the hallmark characteristics of autism - social avoidance and a male-oriented bias. These results suggest that metals exposure may contribute to the development of autism, possibly by interacting with central dopamine function, and support the use of prairie voles as a model organism in which to study autism.

NEUROLIGIN-DEFICIENT MUTANTS OF C. ELEGANS HAVE SENSORY PROCESSING DEFICITS AND ARE HYPERSENSITIVE TO OXIDATIVE STRESS AND MERCURY TOXICITY. (HUNTER 2010)

Hunter JW, Mullen GP, McManus JR, Heatherly JM, Duke A, Rand JB. Dis Model Mech. 2010 May-Jun;3(5-6):366-76. Epub 2010 Jan 18.

Neuroligins are postsynaptic cell adhesion proteins that bind specifically to presynaptic membrane proteins called neurexins. Mutations in human neuroligin genes are associated with autism spectrum disorders in some families. The nematode Caenorhabditis elegans has a single neuroligin gene (nlg-1), and approximately a sixth of C. elegans neurons, including some sensory neurons, interneurons and a subset of cholinergic motor neurons, express a neuroligin transcriptional reporter. Neuroligin-deficient mutants of C. elegans are viable, and they do not appear deficient in any major motor functions. However, neuroligin mutants are defective in a subset of sensory behaviors and sensory processing, and are hypersensitive to oxidative stress and mercury compounds; the behavioral deficits are strikingly similar to traits frequently associated with autism spectrum disorders. Our results suggest a possible link between genetic

defects in synapse formation or function, and sensitivity to environmental factors in the development of autism spectrum disorders.

LASTING NEUROPATHOLOGICAL CHANGES IN RAT BRAIN AFTER INTERMITTENT NEONATAL ADMINISTRATION OF THIMEROSAL. (OLCZAK 2010)

Olczak M, Duszczyk M, Mierzejewski P, Wierzba-Bobrowicz T, Majewska MD. Folia Neuropathol. 2010;48(4):258-69.

Thimerosal, an organomercurial added as a preservative to some vaccines, is a suspected iatrogenic factor, possibly contributing to paediatric neurodevelopmental disorders including autism. We examined the effects of early postnatal administration of thimerosal (four i.m. injections, 12 or 240 µg THIM-Hg/kg, on postnatal days 7, 9, 11 and 15) on brain pathology in Wistar rats. Numerous neuropathological changes were observed in young adult rats which were treated postnatally with thimerosal. They included: ischaemic degeneration of neurons and "dark" neurons in the prefrontal and temporal cortex, the hippocampus and the cerebellum, pathological changes of the blood vessels in the temporal cortex, diminished synaptophysin reaction in the hippocampus, atrophy of astroglia in the hippocampus and cerebellum, and positive caspase-3 reaction in Bergmann astroglia. These findings document neurotoxic effects of thimerosal, at doses equivalent to those used in infant vaccines or higher, in developing rat brain, suggesting likely involvement of this mercurial in neurodevelopmental disorders

PERSISTENT BEHAVIORAL IMPAIRMENTS AND ALTERATIONS OF BRAIN DOPAMINE SYSTEM AFTER EARLY POSTNATAL ADMINISTRATION OF THIMEROSAL IN RATS. (OLCZAK 2011)

Olczak M, Duszczyk M, Mierzejewski P, Meyza K, Majewska MD. Behav Brain Res. 2011 Sep 30;223(1):107-18. doi: 10.1016/j.bbr.2011.04.026. Epub 2011 Apr 28.

The neurotoxic organomercurial thimerosal (THIM), used for decades as vaccine preservative, is a suspected factor in the pathogenesis of some neurodevelopmental disorders. Previously we showed that neonatal administration of THIM at doses equivalent to those used in infant vaccines or higher, causes lasting alterations in the brain opioid system in rats. Here we investigated neonatal treatment with THIM (at doses 12, 240, 1440 and 3000 µg Hg/kg) on behaviors, which are characteristically altered in autism, such as locomotor activity, anxiety, social interactions, spatial learning, and on the brain dopaminergic system in Wistar rats of both sexes. Adult male and female rats, which were exposed to the entire range of THIM doses during the early postnatal life, manifested impairments of locomotor activity and increased anxiety/neophobia in the open field test. In animals of both sexes treated with the highest THIM dose, the frequency of prosocial interactions was reduced, while the frequency of asocial/antisocial interactions was increased in males, but decreased in females. Neonatal THIM

treatment did not significantly affect spatial learning and memory. THIM-exposed rats also manifested reduced haloperidol-induced catalepsy, accompanied by a marked decline in the density of striatal D_2 receptors, measured by immunohistochemical staining, suggesting alterations to the brain dopaminergic system. Males were more sensitive than females to some neurodisruptive/neurotoxic actions of THIM. These data document that early postnatal THIM administration causes lasting neurobehavioral impairments and neurochemical alterations in the brain, dependent on dose and sex. If similar changes occur in THIM/mercurial-exposed children, they could contribute do neurodevelopmental disorders.

MERCURY DISPOSITION IN SUCKLING RATS: COMPARATIVE ASSESSMENT FOLLOWING PARENTERAL EXPOSURE TO THIOMERSAL AND MERCURIC CHLORIDE. (BLANUSA 2012)

Blanuša M, Orct T, Vihnanek Lazarus M, Sekovanić A, Piasek M. J Biomed Biotechnol. 2012;2012:256965. doi: 10.1155/2012/256965. Epub 2012 Jul 26.

Due to the facts that thiomersal-containing vaccine is still in use in many developing countries, and all forms of mercury have recognised neurotoxic, nephrotoxic, and other toxic effects, studies on disposition of ethylmercury and other mercury forms are still justified, especially at young age. Our investigation aimed at comparing mercury distribution and rate of excretion in the early period of life following exposure to either thiomersal (TM) or mercuric chloride ($HgCl_2$) in suckling rats. Three experimental groups were studied: control, TM, and $HgCl_2$, with 12 to18 pups in each. Both forms of mercury were administered subcutaneously in equimolar quantities (0.81 µmol/kg b.w.) three times during the suckling period (on the days of birth 7, 9, and 11) to mimic the vaccination regimen in infants. After the last administration of TM or $HgCl_2$, total mercury retention and excretion was assessed during following six days. In TM-exposed group mercury retention was higher in the brain, enteral excretion was similar, and urinary excretion was much lower compared to $HgCl_2$-exposed sucklings. More research is still needed to elucidate all aspects of toxicokinetics and most harmful neurotoxic potential of various forms of mercury, especially in the earliest period of life.

SEX-DEPENDENT CHANGES IN CEREBELLAR THYROID HORMONE-DEPENDENT GENE EXPRESSION FOLLOWING PERINATAL EXPOSURE TO THIMEROSAL IN RATS. (KHAN 2012)

Khan A, Sulkowski ZL, Chen T, Zavacki AM, Sajdel-Sulkowska EM. J Physiol Pharmacol. 2012 Jun;63(3):277-83.

Mammalian brain development is regulated by the action of thyroid hormone (TH) on target genes. We have previously shown that the perinatal exposure to thimerosal (TM, metabolized to ethylmercury) exerts neurotoxic effects on the developing cerebellum and is associated with a decrease in cerebellar D2 activity, which could result in local brain T3 deficiency. We have also begun to examine TM effect on gene expression. The objective of this study was to expand on

our initial observation of altered cerebellar gene expression following perinatal TM exposure and to examine additional genes that include both TH-dependent as well as other genes critical for cerebellar development in male and female neonates exposed perinatally (G10-G15 and P5 to P10) to TM. We report here for the first time that expression of suppressor-of-white-apricot-1 (SWAP-1), a gene negatively regulated by T3, was increased in TM-exposed males (61.1% increase), but not in females; ($p<0.05$). Positively regulated T3-target genes, Purkinje cell protein 2 (Pcp2; $p=0.07$) and Forkhead box protein P4 (FoxP4; $p=0.08$), showed a trend towards decreased expression in TM-exposed males. The expression of deiodinase 2 (DIO2) showed a trend towards an increase in TM-exposed females, while deiodinase 3 (DIO3), transthyretin (TTR), brain derived neurotrophic factor (BDNF) and reelin (RELN) was not significantly altered in either sex. Since regulation of gene splicing is vital to neuronal proliferation and differentiation, altered expression of SWAP-1 may exert wide ranging effects on multiple genes involved in the regulation of cerebellar development. We have previously identified activation of another TH-dependent gene, outer dense fiber of sperm tails 4, in the TM exposed male pups. Together, these results also show sex-dependent differences between the toxic impacts of TM in males and females. Interestingly, the genes that were activated by TM are negatively regulated by TH, supporting our hypothesis of local brain hypothyroidism being induced by TM and suggesting a novel mechanism of action TM in the developing brain.

PRENATAL EXPOSURE TO ORGANOMERCURY, THIMEROSAL, PERSISTENTLY IMPAIRS THE SEROTONERGIC AND DOPAMINERGIC SYSTEMS IN THE RAT BRAIN: IMPLICATIONS FOR ASSOCIATION WITH DEVELOPMENTAL DISORDERS. (IDA-ETO 2012)

Ida-Eto M, Oyabu A, Ohkawara T, Tashiro Y, Narita N, Narita M. Brain Dev. 2012 May 31. [Epub ahead of print]

Thimerosal, an organomercury compound, has been widely used as a preservative. Therefore, concerns have been raised about its neurotoxicity. We recently demonstrated perturbation of early serotonergic development by prenatal exposure to thimerosal (Ida-Eto et al. (2011) [11]). Here, we investigated whether prenatal thimerosal exposure causes persistent impairment after birth. Analysis on postnatal day 50 showed significant increase in hippocampal serotonin following thimerosal administration on embryonic day 9. Furthermore, not only serotonin, striatal dopamine was significantly increased. These results indicate that embryonic exposure to thimerosal produces lasting impairment of brain monoaminergic system, and thus every effort should be made to avoid the use of thimerosal.

ADMINISTRATION OF THIMEROSAL TO INFANT RATS INCREASES OVERFLOW OF GLUTAMATE AND ASPARTATE IN THE PREFRONTAL CORTEX: PROTECTIVE ROLE OF DEHYDROEPIANDROSTERONE SULFATE. (DUSZCZYK-BUDHATHOKI 2012)

Duszczyk-Budhathoki M, Olczak M, Lehner M, Majewska MD. Neurochem Res. 2012 Feb;37(2):436-47. doi: 10.1007/s11064-011-0630-z. Epub 2011 Oct 21.

Thimerosal, a mercury-containing vaccine preservative, is a suspected factor in the etiology of neurodevelopmental disorders. We previously showed that its administration to infant rats causes behavioral, neurochemical and neuropathological abnormalities similar to those present in autism. Here we examined, using microdialysis, the effect of thimerosal on extracellular levels of neuroactive amino acids in the rat prefrontal cortex (PFC). Thimerosal administration (4 injections, i.m., 240 µg Hg/kg on postnatal days 7, 9, 11, 15) induced lasting changes in amino acid overflow: an increase of glutamate and aspartate accompanied by a decrease of glycine and alanine; measured 10-14 weeks after the injections. Four injections of thimerosal at a dose of 12.5 µg Hg/kg did not alter glutamate and aspartate concentrations at microdialysis time (but based on thimerosal pharmacokinetics, could have been effective soon after its injection). Application of thimerosal to the PFC in perfusion fluid evoked a rapid increase of glutamate overflow. Coadministration of the neurosteroid, dehydroepiandrosterone sulfate (DHEAS; 80 mg/kg; i.p.) prevented the thimerosal effect on glutamate and aspartate; the steroid alone had no influence on these amino acids. Coapplication of DHEAS with thimerosal in perfusion fluid also blocked the acute action of thimerosal on glutamate. In contrast, DHEAS alone reduced overflow of glycine and alanine, somewhat potentiating the thimerosal effect on these amino acids. Since excessive accumulation of extracellular glutamate is linked with excitotoxicity, our data imply that neonatal exposure to thimerosal-containing vaccines might induce excitotoxic brain injuries, leading to neurodevelopmental disorders. DHEAS may partially protect against mercurials-induced neurotoxicity.

MATERNAL THIMEROSAL EXPOSURE RESULTS IN ABERRANT CEREBELLAR OXIDATIVE STRESS, THYROID HORMONE METABOLISM, AND MOTOR BEHAVIOR IN RAT PUPS; SEX- AND STRAIN-DEPENDENT EFFECTS. (SULKOWSKI ZL 2012)

Sulkowski ZL, Chen T, Midha S, Zavacki AM, Sajdel-Sulkowska EM. Cerebellum. 2012 Jun;11(2):575-86. doi: 10.1007/s12311-011-0319-5.

Methylmercury (Met-Hg) and ethylmercury (Et-Hg) are powerful toxicants with a range of harmful neurological effects in humans and animals. While Met-Hg is a recognized trigger of oxidative stress and an endocrine disruptor impacting neurodevelopment, the developmental neurotoxicity of Et-Hg, a metabolite of thimerosal (TM), has not been explored. We hypothesized that TM exposure during the perinatal period impairs central nervous system development, and specifically the cerebellum, by the mechanism involving oxidative stress. To

test this, spontaneously hypertensive rats (SHR) or Sprague-Dawley (SD) rat dams were exposed to TM (200 µg/kg body weight) during pregnancy (G10-G15) and lactation (P5-P10). Male and female neonates were evaluated for auditory and motor function; cerebella were analyzed for oxidative stress and thyroid metabolism. TM exposure resulted in a delayed startle response in SD neonates and decreased motor learning in SHR male (22.6%), in SD male (29.8%), and in SD female (55.0%) neonates. TM exposure also resulted in a significant increase in cerebellar levels of the oxidative stress marker 3-nitrotyrosine in SHR female (35.1%) and SD male (14.0%) neonates. The activity of cerebellar type 2 deiodinase, responsible for local intra-brain conversion of thyroxine to the active hormone, 3',3,5-triiodothyronine (T3), was significantly decreased in TM-exposed SHR male (60.9%) pups. This coincided with an increased (47.0%) expression of a gene negatively regulated by T3, Odf4 suggesting local intracerebellar T3 deficiency. Our data thus demonstrate a negative neurodevelopmental impact of perinatal TM exposure which appears to be both strain- and sex-dependent.

Cellular Research

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.

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.

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.

ACTIVATION OF METHIONINE SYNTHASE BY INSULIN-LIKE GROWTH FACTOR-1 AND DOPAMINE: A TARGET FOR NEURODEVELOPMENTAL 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. Mol Psychiatry. 2004 Apr;9(4):358-70.

Methylation events play a critical role in the ability of growth factors to promote normal development. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might exert adverse effects on methylation. We found that insulin-like growth factor-1 (IGF-1)- and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells, via a PI3-kinase- and MAP-kinase-dependent mechanism. The stimulation of this pathway increased DNA methylation, while its inhibition increased methylation-sensitive gene expression. Ethanol potently interfered with IGF-1 activation of MS and blocked its effect on DNA methylation, whereas it did not inhibit the effects of dopamine. Metal ions potently affected IGF-1 and dopamine-stimulated MS activity, as well as folate-dependent phospholipid methylation: Cu(2+) promoted enzyme activity and methylation, while Cu(+), Pb(2+), Hg(2+) and Al(3+) were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC(50) of 1 nM and eliminated MS activity. Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. The potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins.

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 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.

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.

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)

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.

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.

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.

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.

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.

SENSITIZATION EFFECT OF THIMEROSAL IS MEDIATED IN VITRO VIA REACTIVE OXYGEN SPECIES AND CALCIUM SIGNALING. (MIGDAL 2010)

Migdal C, Foggia L, Tailhardat M, Courtellemont P, Haftek M, Serres M. Toxicology. 2010 Jul-Aug;274(1-3):1-9. Epub 2010 May 10.

Thimerosal, a mercury derivative composed of ethyl mercury chloride (EtHgCl) and thiosalicylic acid (TSA), is widely used as a preservative in vaccines and cosmetic products and causes cutaneous reactions. Since dendritic cells (DCs) play an essential role in the immune response, the sensitization potency of chemicals was studied in vitro using U937, a human promyelomonocytic cell line that is used as a surrogate of monocytic differentiation and activation. Currently, this cell line is under ECVAM (European Center for the Validation of Alternative Methods) validation as an alternative method for discriminating chemicals. Thimerosal and mercury derivatives induced in U937 an overexpression of CD86 and interleukin (IL)-8 secretion similarly to 1-chloro-2,4-dinitrobenzene (DNCB), a sensitizer used as a positive control for DC activation. Non-sensitizers, dichloronitrobenzene (DCNB), TSA and sodium dodecyl sulfate (SDS), an irritant, had no effect. U937 activation was prevented by cell pretreatment with N-acetyl-L-cysteine (NAC) but not with thiol-independent antioxidants except vitamin E which affected CD86 expression by preventing lipid peroxidation of cell membranes. Thimerosal, EtHgCl and DNCB induced glutathione (GSH) depletion and reactive oxygen species (ROS) within 15 min; another peak was detected after 2h for mercury compounds only. MitoSOX, a specific mitochondrial fluorescent probe, confirmed that ROS were essentially produced by mitochondria in correlation with its membrane depolarization. Changes in mitochondrial membrane permeability induced by mercury were reversed by NAC but not by thiol-independent antioxidants. Thimerosal and EtHgCl also induced a calcium (Ca2+) influx with a peak at 3h, suggesting that Ca2+ influx is a secondary event following ROS induction as Ca2+ influx was suppressed after pretreatment with NAC but not with thiol-independent antioxidants. Ca2+ influx was also suppressed when culture medium was deprived of Ca2+ confirming the specificity of the measure. In conclusion, these data suggest that thimerosal induced U937 activation via oxidative stress from mitochondrial stores and mitochondrial membrane depolarization with a primordial effect of thiol groups. A cross-talk between ROS and Ca2+ influx was demonstrated.

EVALUATION OF CYTOTOXICITY ATTRIBUTED TO THIMEROSAL ON MURINE AND HUMAN KIDNEY CELLS. (PARK 2007)

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.

THE RELATIVE TOXICITY OF COMPOUNDS USED AS PRESERVATIVES IN VACCINES AND BIOLOGICS. (GEIER 2010)

Geier DA, Jordan SK, Geier MR. Med Sci Monit. 2010 Apr 28;16(5):SR21-7.

BACKGROUND: In vaccines/biologics, preservatives are used to prevent microbial growth. MATERIAL/METHODS: The present study examined: (1) the comparative toxicities of commonly used preservatives in US licensed vaccines to human neurons; and (2) the relative toxicity index of these compounds to human neurons in comparison to bacterial cells. RESULTS: Using human neuroblastoma cells, the relative cytotoxicity of the levels of the compounds commonly used as preservative in US licensed vaccines was found to be phenol <2-phenoxyethanol < benzethonium chloride < Thimerosal. The observed relative toxicity indices (human neuroblastoma cells/bacterial cells) were 2-phenoxyethanol (4.6-fold) < phenol (12.2-fold) < Thimerosal (>330-fold). In addition, for the compounds tested, except for 2-phenoxyethanol, the concentrations necessary to induce significant killing of bacterial cells were significantly higher than those routinely present in US licensed vaccine/biological preparations. CONCLUSIONS: None of the compounds commonly used as preservatives in US licensed vaccine/biological preparations can be considered an ideal preservative, and their ability to fully comply with the requirements of the US Code of Federal Regulations (CFR) for preservatives is in doubt. Future formulations of US licensed vaccines/biologics should be produced in aseptic manufacturing plants as single dose preparations, eliminating the need for preservatives and an unnecessary risk to patients.

LOW MOLECULAR WEIGHT THIOLS REDUCE THIMEROSAL NEUROTOXICITY IN VITRO: MODULATION BY PROTEINS. (ZIEMINSKA 2010)

Zieminska E, Toczylowska B, Stafiej A, Lazarewicz JW. Toxicology. 2010 Aug 7. [Epub ahead of print]

Thimerosal (TH), an ethylmercury complex of thiosalicylic acid has been used as preservative in vaccines. In vitro neurotoxicity of TH at high nM concentrations has been reported. Although a number of toxicological experiments demonstrated high affinity of mercury to thiol groups of the extracellular amino acids and proteins that may decrease concentration of free TH in the organism, less is known about the role of interactions between proteins and amino acids in protection against TH neurotoxicity. In the present study we examined whether the presence of

serum proteins and of l-cysteine (Cys), d,l-homocysteine (Hcy), N-acetyl cysteine (NAC), l-methionine (Met) and glutathione (GSH) in the incubation medium affects the TH-induced changes in the viability, the intracellular levels of calcium and zinc and mitochondrial membrane potential in primary cultures of rat cerebellar granule cells. The cells were exposed to 500nM TH for 48h or to 15-25muM TH for 10min. Our results demonstrated a decrease in the cells viability evoked by TH, which could be prevented partially by serum proteins, albumin or in a dose-dependent manner by 60, 120 or 600muM Cys, Hcy, NAC and GSH, but not by Met. This neuroprotection was less pronounced in the presence of proteins. Incubation of neurons with TH also induced the rise in the intracellular calcium and zinc concentration and decrease in mitochondrial membrane potential, and these effects were abolished by all the sulfur containing compounds studied and administered at 600muM concentration, except Met. The loss of the ethylmercury moiety from TH as a result of interaction with thiols studied was monitored by (1)H NMR spectroscopy. This extracellular process may be responsible for the neuroprotection seen in the cerebellar cell cultures, but also provides a molecular pathway for redistribution of TH-derived toxic ethylmercury in the organism. In conclusion, these results confirmed that proteins and sulfur-containing amino acids applied separately reduce TH neurotoxicity, while their combination modulates in more complex way neuronal survival in the presence of TH.

RESPONSIVENESS OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS TO THIMEROSAL AND MERCURY DERIVATIVES. (MIGDAL 2010)

Migdal C, Tailhardat M, Courtellemont P, Haftek M, Serres M. Toxicol Appl Pharmacol. 2010 Jul;246(1-2):66-73. Epub 2010 Apr 22.

Several cases of skin sensitization have been reported following the application of thimerosal, which is composed of ethyl mercury and thiosalicylic acid (TSA). However, few in vitro studies have been carried out on human dendritic cells (DCs) which play an essential role in the initiation of allergic contact dermatitis. The aim of the present study was to identify the effect of thimerosal and other mercury compounds on human DCs. To address this purpose, DCs derived from monocytes (mono-DCs) were used. Data show that thimerosal and mercury derivatives induced DC activation, as monitored by CD86 and HLA-DR overexpression associated with the secretion of tumor necrosis factor alpha and interleukin 8, similarly to lipopolysaccharide and the sensitizers, 1-chloro-2,4-dinitrobenzene (DNCB) and nickel sulfate, which were used as positive controls. In contrast, TSA, the non-mercury part of thimerosal, as well as dichloronitrobenzene, a DNCB negative control, and the irritant, sodium dodecyl sulfate, had no effect. Moreover, oxidative stress, monitored by ROS induction and depolarization of the mitochondrial membrane potential, was induced by thimerosal and mercury compounds, as well as DNCB, in comparison with hydrogen peroxide, used as a positive control. The role of

thiol oxidation in the initiation of mono-DC activation was confirmed by a pre-treatment with N-acetyl-l-cysteine which strongly decreased chemical-induced CD86 overexpression. These data are in agreement with several clinical observations of the high relevance of thimerosal in patch-test reactions and prove that human mono-DCs are useful in vitro tools for determining the allergenic potency of chemicals.

MERCURY INDUCES AN UNOPPOSED INFLAMMATORY RESPONSE IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO. (GARDNER 2009)

Gardner RM, Nyland JF, Evans SL, Wang SB, Doyle KM, Crainiceanu CM, Silbergeld EK. Environ Health Perspect. 2009 Dec;117(12):1932-8. Epub 2009 Aug 19.

BACKGROUND: The human immune response to mercury is not well characterized despite the body of evidence that suggests that Hg can modulate immune responses, including the induction of autoimmune disease in some mouse models. Dysregulation of cytokine signaling appears to play an important role in the etiology of Hg-induced autoimmunity in animal models. OBJECTIVES: In this study, we systematically investigated the human immune response to Hg in vitro in terms of cytokine release. METHODS: Human peripheral blood mononuclear cells (PBMCs) were isolated from 20 volunteers who donated blood six separate times. PBMCs were cultured with lipopolysaccharide and concentrations of mercuric chloride (HgCl(2)) up to 200 nM. Seven cytokines representing important pathways in physiologic and pathologic immune responses were measured in supernatants. We used multilevel models to account for the intrinsic clustering in the cytokine data due to experimental design. RESULTS: We found a consistent increase in the release of the proinflammatory cytokines interleukin-1beta (IL-1beta) and tumor necrosis factor-alpha, and concurrent decrease in release of the antiinflammatory cytokines interleukin 1-receptor antagonist (IL-1Ra) and IL-10 in human PBMCs treated with subcytotoxic concentrations of HgCl(2). IL-4, IL-17, and interferon-gamma increased in a concentration-response manner. These results were replicated in a second, independently recruited population of 20 different volunteers. CONCLUSIONS: Low concentrations of HgCl(2) affect immune function in human cells by dysregulation of cytokine signaling pathways, with the potential to influence diverse health outcomes such as susceptibility to infectious disease or risk of autoimmunity.

LUTEOLIN AND THIOSALICYLATE INHIBIT HGCL(2) AND THIMEROSAL-INDUCED VEGF RELEASE FROM HUMAN MAST CELLS. (ASADI 2010)

Asadi S, Zhang B, Weng Z, Angelidou A, Kempuraj D, Alysandratos KD, Theoharides TC. Int J Immunopathol Pharmacol. 2010 Oct-Dec;23(4):1015-20.

HgCl2 is a known environemental neurotoxin, but is also used as preservative in vaccines as thimerosal containing ethyl mercury covalently linked to thiosalicylate. We recently reported

that mercury choloride (HgCl(2)) can stimulate human mast cells to release vascular endothelial growth factor (VEGF), which is also vasoactive and pro-inflammatory. Here we show that thimerosal induces significant VEGF release from human leukemic cultured LAD2 mast cells (at 1 microM 326 ± 12 pg/106 cells and 335.5 ± 12 pg/106 cells at 10 microM) compared to control cells (242 ± 21 pg/106 cells, n=5, p less than 0.05); this effect is weaker than that induced by HgCl2 at 10 microM (448 ± 14 pg/106 cells) (n=3, p less than 0.05). In view of this finding, we hypothesize that the thiosalicylate component of thimerosal may have an inhibitory effect on VEGF release. Thimerosal (10 microM) added together with the peptide Substance P (SP) at 2 microM, used as a positive control, reduced VEGF release by 90 percent. Methyl thiosalicylate (1 or 10 microM) added with either SP or HgCl2 (10 microM) inhibited VEGF release by 100 percent, while sodium salicylate or ibuprofen had no effect. Pretreatment for 10 min with the flavonoid luteolin (0.1 mM) before HgCl2 or thimerosal compeletly blocked their effect. Luteolin and methyl thiosalicylate may be useful in preventing mercury-induced toxicity.

INTERMINGLED MODULATORY AND NEUROTOXIC EFFECTS OF THIMEROSAL AND MERCURIC IONS ON ELECTROPHYSIOLOGICAL RESPONSES TO GABA AND NMDA IN HIPPOCAMPAL NEURONS. (WYREMBEK 2010)

Wyrembek P, Szczuraszek K, Majewska MD, Mozrzymas JW. J Physiol Pharmacol. 2010 Dec;61(6):753-8.

The organomercurial, thimerosal, is at the center of medical controversy as a suspected factor contributing to neurodevelopmental disorders in children. Many neurotoxic effects of thimerosal have been described, but its interaction with principal excitatory and inhibitory neurotransmiter systems is not known. We examined, using electrophysiological recordings, thimerosal effects on GABA and NMDA-evoked currents in cultured hippocampal neurons. After brief (3 to 10 min) exposure to thimerosal at concentrations up to 100 µM, there was no significant effect on GABA or NMDA-evoked currents. However, following exposure for 60-90 min to 1 or 10 µM thimerosal, there was a significant decrease in NMDA-induced currents ($p<0.05$) and GABAergic currents ($p<0.05$). Thimerosal was also neurotoxic, damaging a significant proportion of neurons after 60-90 min exposure; recordings were always conducted in the healthiest looking neurons. Mercuric chloride, at concentrations 1 µM and above, was even more toxic, killing a large proportion of cells after just a few minutes of exposure. Recordings from a few sturdy cells revealed that micromolar mercuric chloride markedly potentiated the GABAergic currents ($p<0.05$), but reduced NMDA-evoked currents ($p<0.05$). The results reveal complex interactions of thimerosal and mercuric ions with the GABA(A) and NMDA receptors. Mercuric chloride act rapidly, decreasing electrophysiological responses to NMDA but enhancing responses to GABA, while thimerosal works slowly, reducing both NMDA and GABA responses. The neurotoxic effects of both mercurials are interwoven with their

modulatory actions on GABA(A) and NMDA receptors, which most likely involve binding to these macromolecules.

THIMEROSAL-DERIVED ETHYLMERCURY IS A MITOCHONDRIAL TOXIN IN HUMAN ASTROCYTES: POSSIBLE ROLE OF FENTON CHEMISTRY IN THE OXIDATION AND BREAKAGE OF MTDNA. (SHARPE 2012)

Sharpe MA, Livingston AD, Baskin DS. J Toxicol. 2012;2012:373678. doi: 10.1155/2012/373678. Epub 2012 Jun 28.

Thimerosal generates ethylmercury in aqueous solution and is widely used as preservative. We have investigated the toxicology of Thimerosal in normal human astrocytes, paying particular attention to mitochondrial function and the generation of specific oxidants. We find that ethylmercury not only inhibits mitochondrial respiration leading to a drop in the steady state membrane potential, but also concurrent with these phenomena increases the formation of superoxide, hydrogen peroxide, and Fenton/Haber-Weiss generated hydroxyl radical. These oxidants increase the levels of cellular aldehyde/ketones. Additionally, we find a five-fold increase in the levels of oxidant damaged mitochondrial DNA bases and increases in the levels of mtDNA nicks and blunt-ended breaks. Highly damaged mitochondria are characterized by having very low membrane potentials, increased superoxide/hydrogen peroxide production, and extensively damaged mtDNA and proteins. These mitochondria appear to have undergone a permeability transition, an observation supported by the five-fold increase in Caspase-3 activity observed after Thimerosal treatment.

CYTOPROTECTIVE EFFECT OF HYALURONIC ACID AND HYDROXYPROPYL METHYLCELLULOSE AGAINST DNA DAMAGE INDUCED BY THIMEROSAL IN CHANG CONJUNCTIVAL CELLS. (YE 2012)

Ye J, Zhang H, Wu H, Wang C, Shi X, Xie J, He J, Yang J. Graefes Arch Clin Exp Ophthalmol. 2012 Oct;250(10):1459-66. doi: 10.1007/s00417-012-2087-4. Epub 2012 Jun 24.

BACKGROUND: To investigate genotoxicity of the preservative thimerosal (Thi), and the cytoprotective and antioxidant effects of hyaluronic Acid (HA) and hydroxypropyl methylcellulose (HPMC) on Chang conjunctival cells.
METHOD: Cells were divided into three groups. One group was exposed to Thi at various concentrations (0.00001 %~0.001 %) for 30 min; the other two groups were pretreated with 0.3 % HA or 0.3 % HPMC for 30 min before the Thi exposure. After cell viability was evaluated, alkaline comet assay and detection of the phosphorylated form of the histone variant H2AX (γH2AX) foci were used to determine DNA damage. Reactive oxygen species (ROS) production was assessed by the fluorescent probe, 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA).
RESULTS: A significant change of cell viability was observed after exposure to 0.001 % Thi for 30

min. DNA single- and double-strand breaks were significantly increased in a dose-dependent manner with Thi exposure. In addition, intracellular ROS induced by Thi was dose-dependent, except at 0.001 % less ROS was induced than at 0.0005 %. However, cells pretreated with 0.3 % HA or 0.3 % HPMC showed significantly increased cell survival, decreased DNA damage, and decreased ROS production compared to cells exposed to Thi alone. Pretreatment with 0.3 % HA was found to be even more protective than 0.3 % HPMC. CONCLUSION: Thi can induce DNA damage in human conjunctival epithelial cells, probably due to oxidative stress. HA and HPMC are protective agents that have antioxidant properties and can decrease DNA damage induced by Thi. Pretreatment of 0.3 % HA may be more protective of the ocular surface than 0.3 % HPMC.

ITPKC SUSCEPTIBILITY IN KAWASAKI SYNDROME AS A SENSITIZING FACTOR FOR AUTOIMMUNITY AND CORONARY ARTERIAL WALL RELAXATION INDUCED BY THIMEROSAL'S EFFECTS ON CALCIUM SIGNALING VIA IP3. (YETER 2012)

Yeter D, Deth R. Autoimmun Rev. 2012 Oct;11(12):903-8. doi: 10.1016/j.autrev.2012.03.006. Epub 2012 Apr 1.

Recently, a single nucleotide polymorphism (SNP) of the inositol 1,4,5-triphosphate kinase C (ITPKC), rs28493229, was found to passively confer susceptibility for Kawasaki syndrome (KS) and subsequent coronary arterial lesions. This association is believed to be the result of defective phosphorylation of inositol 1,4,5-triphosphate (IP3), which releases calcium from intracellular stores, resulting from reduced genetic expression of ITPKC in carriers of the SNP. Reduced ITPKC activity would increase IP3 levels, and thus, increase calcium release. We hypothesized that an environmental agent which influences IP3-mediated calcium release is potentiated by the ITPKC SNP. This led us to an attractive candidate, thimerosal, an organomercurial medical preservative still used in several pediatric vaccines. Thimerosal is well-known to sensitize IP3 receptors via its induction of oxidative stress, resulting in enhanced release of intracellular calcium with distinctive consequences for various cell types. Dysregulated calcium signaling in T cells and other immune cells can result in autoimmunity, while hyperpolarization of vascular smooth muscle cells secondary to the stimulation of calcium-activated potassium channels can result in increased vascular permeability and arterial relaxation. We propose that ITPKC susceptibility in KS is related to its synergy with environmental triggers, such as thimerosal, which alter calcium homeostasis and promote oxidative stress. Therefore, carriers of the ITPKC SNP are more susceptible to thimerosal-induced autoimmunity and coronary arterial lesions observed in KS. This would explain why only a susceptible subset of children develops KS although pediatric thimerosal exposure is nearly universal due to vaccination. As was experienced with the infantile acrodynia epidemic, only 1 in 500 children developed the disease although pediatric mercury exposure was nearly

ubiquitous due to the use calomel teething powders. This hypothesis also mirrors the current leading theory for KS in which a widespread infection only induces the disease in susceptible children. We conclude that KS may be the acute febrile form of acrodynia.

THIMEROSAL-INDUCED APOPTOSIS IN MOUSE C2C12 MYOBLAST CELLS OCCURS THROUGH SUPPRESSION OF THE PI3K/AKT/SURVIVIN PATHWAY (LI 2012)

Li WX, Chen SF, Chen LP, Yang GY, Li JT, Liu HZ, Zhu W. PLoS One. 2012;7(11):e49064. doi: 10.1371/journal.pone.0049064. Epub 2012 Nov 7.

BACKGROUND: Thimerosal, a mercury-containing preservative, is one of the most widely used preservatives and found in a variety of biological products. Concerns over its possible toxicity have reemerged recently due to its use in vaccines. Thimerosal has also been reported to be markedly cytotoxic to neural tissue. However, little is known regarding thimerosal-induced toxicity in muscle tissue. Therefore, we investigated the cytotoxic effect of thimerosal and its possible mechanisms on mouse C2C12 myoblast cells. METHODOLOGY/PRINCIPAL FINDINGS: The study showed that C2C12 myoblast cells underwent inhibition of proliferation and apoptosis after exposure to thimerosal (125-500 nM) for 24, 48 and 72 h. Thimerosal caused S phase arrest and induced apoptosis as assessed by flow cytometric analysis, Hoechst staining and immunoblotting. The data revealed that thimerosal could trigger the leakage of cytochrome c from mitochondria, followed by cleavage of caspase-9 and caspase-3, and that an inhibitor of caspase could suppress thimerosal-induced apoptosis. Thimerosal inhibited the phosphorylation of Akt(ser473) and survivin expression. Wortmannin, a PI3K inhibitor, inhibited Akt activity and decreased survivin expression, resulting in increased thimerosal-induced apoptosis in C2C12 cells, while the activation of PI3K/Akt pathway by mIGF-I (50 ng/ml) increased the expression of survivin and attenuated apoptosis. Furthermore, the inhibition of survivin expression by siRNA enhanced thimerosal-induced cell apoptosis, while overexpression of survivin prevented thimerosal-induced apoptosis. Taken together, the data show that the PI3K/Akt/survivin pathway plays an important role in the thimerosal-induced apoptosis in C2C12 cells. CONCLUSIONS/SIGNIFICANCE: Our results suggest that in C2C12 myoblast cells, thimerosal induces S phase arrest and finally causes apoptosis via inhibition of PI3K/Akt/survivin signaling followed by activation of the mitochondrial apoptotic pathway