

**United Nations Environmental Program (UNEP)-
convened Intergovernmental Negotiating
Committee Meeting 4 (INC4), 3 to 4 April 2012,
Salle D, WHO headquarters, Geneva, Switzerland**

Michael E. Pichichero, MD

Director, Research Institute, Rochester General Hospital

**Research Professor, College of Health Sciences and
Technology, Rochester Institute of Technology**

Rochester, NY, USA

Conflicts of Interest

- I received an honorarium to prepare this report from the WHO.
- I donated half of my time to WHO for this project.
- All of my work on thimerosal in vaccines was supported by the US NIH NIAID.
- I have never received any payment from any vaccine or pharmaceutical company relating to thimerosal in vaccines or any product.
- I have received honoraria/consultant fees and my institution has received research grants from several vaccine and pharmaceutical companies for new vaccine and product development. None of these payments had any direct or indirect relationship to the evaluation of thimerosal in vaccines.

Literature Search Strategy

- A literature search was performed using Medline, without language restriction, using the search terms: Thimerosal, Vaccines, 2008-2012 and Autistic Disorders.
- Thimerosal received 1,050 hits. Vaccines received 152,869 hits. Combining Thimerosal and Vaccines received 296 hits. Of the 296 limiting the search to 2008-2012 produced 79 hits – all papers were reviewed. A secondary search was then done on Autistic Disorders which produced 13,929 results and when limited to 2008-2012, 26 hits were produced – all papers were reviewed.
- Bibliography review of articles searched added 5 more papers that were included, yielding a total for this report of 31 papers.

Literature Search Strategy

- Medline was selected as the search tool because it has the broadest journal reach and is the standard for literature searches and can be used without language restriction.
- Search terms: **Thimerosal** and **Vaccines** were selected as primary terms. **Autistic Disorders** was added as a secondary term to avoid missing literature published that studied a putative link between thimerosal and autism.
- Search years 2008 to March 2012 were selected because the WHO last evaluated the question of thimerosal in vaccines in June 2008, a meeting where I and others presented findings regarding this issue.

Current state of knowledge in June, 2008

- Between 1989 and 1998, as more vaccines with earlier administration times were added to the recommended childhood immunization schedule, average cumulative exposure to ethylmercury from vaccines containing thimerosal subsequently rose.
- Calculations showed that some infants could receive, during their first year of life, doses of ethylmercury from childhood vaccines that exceeded limits set for methylmercury exposure established by some public health and environmental agencies.
- No evidence for harm from thimerosal was found.
- Foundational to the decision of the USFDA and American Academy of Pediatrics was the presumption of identical pharmacokinetics of ethylmercury and methylmercury.
- At the June 2008 WHO meeting pharmacokinetic data in human infants, including premature and low birth weight infants, established that the half-life of ethylmercury was 3-5 days, that ethylmercury was excreted in the stools and that there was no accumulation in blood, since levels returned to baseline within < 30 days of vaccination.

Current state of knowledge in June, 2008

- Thus, by June 2008, the foundational component of the decision of the USFDA and American Academy of Pediatrics that pharmacokinetics of ethylmercury and methylmercury were identical was **disproved**.
- At the June 2008 meeting other evidence was reviewed that included the accumulated literature up to that time.
- No evidence for harm from thimerosal was found and the lack of knowledge about alternative preservatives was noted.

New Evidence: Measurements of Blood and Hair Mercury in Children

- [1] Dorea JG, Bezerra VL, Fajon V, Horvat M. Speciation of methyl- and ethyl-mercury in hair of breastfed infants acutely exposed to thimerosal-containing vaccines. *Clin.Chim.Acta* 2011;412(17-18):1563-6.
- [2] Hertz-Picciotto I, Green PG, Delwiche L, Hansen R, Walker C, Pessah IN. Blood mercury concentrations in CHARGE Study children with and without autism. *Environ.Health Perspect.* 2010;118(1):161-6.
- [3] Pichichero ME, Gentile A, Giglio N, et al. Mercury levels in newborns and infants after receipt of thimerosal-containing vaccines. *Pediatrics* 2008;121(2):e208-e214
- [4] Pichichero ME, Gentile A, Giglio N, et al. Mercury levels in premature and low birth weight newborn infants after receipt of thimerosal-containing vaccines. *J.Pediatr.* 2009;155(4):495-9.
- [5] Zhou J, Yan-feng L, Xin-huan Z, Yue-zhi C, Yan X. Analysis of blood mercury levels in premature infants before and after vaccination of hepatitis B. *Clinical Pediatrics* 2007;25(6):478-9.

New Evidence: Epidemiologic Studies of Thimerosal Exposure and Neurodevelopmental Problems in Children

- [6] Adams JB, Baral M, Geis E, et al. The severity of autism is associated with toxic metal body burden and red blood cell glutathione levels. J.Toxicol. 2009;2009:532640
- [7] Croen LA, Matevia M, Yoshida CK, Grether JK. Maternal Rh D status, anti-D immune globulin exposure during pregnancy, and risk of autism spectrum disorders. Am.J.Obstet.Gynecol. 2008;199(3):234-6.
- [8] Geier DA, Mumper E, Gladfelter B, Coleman L, Geier MR. Neurodevelopmental disorders, maternal Rh-negativity, and Rho(D) immune globulins: a multi-center assessment. Neuro.Endocrinol.Lett. 2008;29(2):272-80.
- [9] Geier DA, Young HA, Geier MR. Thimerosal exposure & increasing trends of premature puberty in the vaccine safety datalink. Indian J.Med.Res. 2010;131:500-7.
- [10] Marques RC, Dorea JG, Bernardi JV, Bastos WR, Malm O. Prenatal and postnatal mercury exposure, breastfeeding and neurodevelopment during the first 5 years. Cogn Behav.Neurol. 2009;22(2):134-41.

New Evidence: Epidemiologic Studies of Thimerosal Exposure and Neurodevelopmental Problems in Children

- [11] Marques RC, Dorea JG, Bernardi JV. Thimerosal exposure (from tetanus-diphtheria vaccine) during pregnancy and neurodevelopment of breastfed infants at 6 months. *Acta Paediatr.* 2010;99(6):934-9.
- [12] Mrozek-Budzyn D, Majewska R, Kieltyka A, Augustyniak M. [Lack of association between thimerosal-containing vaccines and autism]. *Przegl.Epidemiol.* 2011;65(3):491-5.
- [13] Price CS, Thompson WW, Goodson B, et al. Prenatal and infant exposure to thimerosal from vaccines and immunoglobulins and risk of autism. *Pediatrics* 2010;126(4):656-64.
- [14] Schechter R, Grether JK. Continuing increases in autism reported to California's developmental services system: mercury in retrograde. *Arch.Gen.Psychiatry* 2008;65(1):19-24.
- [15] Tozzi AE, Bisiacchi P, Tarantino V, et al. Neuropsychological performance 10 years after immunization in infancy with thimerosal-containing vaccines. *Pediatrics* 2009;123(2):475-82.
- [16] Young HA, Geier DA, Geier MR. Thimerosal exposure in infants and neurodevelopmental disorders: an assessment of computerized medical records in the Vaccine Safety Datalink. *J.Neurol.Sci.* 2008;271(1-2):110-8.

New Evidence: Gender (Male) as a Risk Factor

- [17] Gallagher CM, Godman M. Hepatitis B triple series vaccine and developmental disability in US children aged 1-9 years. *Toxicology and Environmental Chemistry* 2008;90(5):997-1008.
- [18] Gallagher CM, Goodman MS. Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997-2002. *J.Toxicol.EnvIRON.Health A* 2010;73(24):1665-77.

New Evidence: Animal Studies

- [19] Berman RF, Pessah IN, Mouton PR, Mav D, Harry J. Low-level neonatal thimerosal exposure: further evaluation of altered neurotoxic potential in SJL mice. *Toxicol.Sci.* 2008;101(2):294-309.
- [20] Branch DR. Gender-selective toxicity of thimerosal. *Exp.Toxicol.Pathol.* 2009;61(2):133-6.
- [21] Hewitson L, Houser LA, Stott C, et al. Delayed acquisition of neonatal reflexes in newborn primates receiving a thimerosal-containing hepatitis B vaccine: influence of gestational age and birth weight. *J.Toxicol.Environ.Health A* 2010;73(19):1298-313.
- [22] Minami T, Miyata E, Sakamoto Y, Yamazaki H, Ichida S. Induction of metallothionein in mouse cerebellum and cerebrum with low-dose thimerosal injection. *Cell Biol.Toxicol.* 2010;26(2):143-52.
- [23] Olczak M, Duszczyk M, Mierzejewski P, Majewska MD. Neonatal administration of a vaccine preservative, thimerosal, produces lasting impairment of nociception and apparent activation of opioid system in rats. *Brain Res.* 2009;1301:143-51.
- [24] Duszczyk-Budhathoki M, Olczak M, Lehner M, Majewska MD. Administration of thimerosal to infant rats increases overflow of glutamate and aspartate in the prefrontal cortex: protective role of dehydroepiandrosterone sulfate. *Neurochem.Res.* 2012;37(2):436-47.
- [25] Olczak M, Duszczyk M, Mierzejewski P, Wierzba-Bobrowicz T, Majewska MD. Lasting neuropathological changes in rat brain after intermittent neonatal administration of thimerosal. *Folia Neuropathol.* 2010;48(4):258-69.
- [26] Olczak M, Duszczyk M, Mierzejewski P, Meyza K, Majewska MD. Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats. *Behav.Brain Res.* 2011;223(1):107-18.

New Evidence: Miscellaneous Publications

- [27] Dorea JG, Marques RC, Brandao KG. Neonate exposure to thimerosal mercury from hepatitis B vaccines. *Am.J.Perinatol.* 2009;26(7):523-7.
- [28] Marques RC, Bernardi JV, Dorea JG, Bastos WR, Malm O. Principal component analysis and discrimination of variables associated with pre- and post-natal exposure to mercury. *Int.J.Hyg.Environ.Health* 2008;211(5-6):606-14.
- [29] Wu X, Liang H, O'Hara KA, Yalowich JC, Hasinoff BB. Thiol-modulated mechanisms of the cytotoxicity of thimerosal and inhibition of DNA topoisomerase II alpha. *Chem.Res.Toxicol.* 2008;21(2):483-93.

New Evidence: Alternative preservatives in vaccines: 2-phenoxyethanol (2-PE)

- [30] Gloriani NG, Srinivasa K, Bock HL, Hoet B. Immunogenicity of HBV vaccine during stated shelf-life. Southeast Asian J.Trop.Med.Public Health 2010;41(4):876-82.
- [31] Khandke L, Yang C, Krylova K, Jansen KU, Rashidbaigi A. Preservative of choice for Prev(e)nar 13 in a multi-dose formulation. Vaccine 2011;29(41):7144-53.

Zhou J, Yan-feng L, Xin-huan Z et al. Analysis of blood mercury levels in premature infants before and after vaccination of hepatitis B. *J Clin Pediatr (Chinese)* 2007; 25(6):478-479.

- 22 premature infants weighing 1500 - 2400 grams and 21 term infants weighing >3000 grams who received a HepB vaccine containing 25 µg ethylmercury/dose. Levels = 1.47 - 3.33 µg/L in premature infants and 1.84 - 3.07 µg/L in full term infants.
- Author's conclusions: The blood mercury level was under safe level 5 days after vaccination in premature infants weighing 1,500-2,400 grams who received hepatitis B vaccine at birth.
- Reviewer's conclusions: The results are consistent with those of: Pichichero ME, et al. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. *Lancet* 2002;360:1737-1741. Pichichero M et al. mercury levels in newborns and infants after receipt of thimerosal-containing vaccines. *Pediatrics* 2008;121:e208-e214. Pichichero M, et al. Mercury levels in premature and low birth weight newborn infants after receipt of thimerosal-containing vaccines. *J. Pediatr* 2009; 155:495-499.

Hertz-Picciotto I, Green PG, Delwiche L, Hansen R, Walker C and Pessah IN. Blood Mercury Concentrations in CHARGE Study Children with and without Autism. Environmental Health Perspectives 2010; 118(1):161-166.

- The Childhood Autism Risk from Genetics and the Environment (CHARGE) study enrolled children 2-5 years of age. After adjustment for fish and other Hg sources, blood Hg levels in AU/ASD children were similar to those of TD children ($p= 0.75$); this was also true among non-fish eaters ($p = 0.73$).
- Author's conclusions: After accounting for dietary and other differences in Hg exposures, total Hg in blood was neither elevated nor reduced in CHARGE Study preschoolers with AU/ASD (Autism/Autism Spectrum Disorder) compared with unaffected controls, and resembled those of nationally representative samples.
- Reviewer's conclusions: By 2 to 5 years of age the contribution of thimerosal ethylmercury to total mercury exposure in infants and children is multi-fold lower than the contribution from methylmercury in fish.

Pichichero M, Gentile A, Giglio N et al. Mercury Levels in Newborns and Infants After Receipt of Thimerosal-Containing Vaccines. Pediatrics 2008;121(2):e208-e214

- 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). For groups 1, 2, and 3, respectively, maximal mean \pm 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; The blood mercury half-life was calculated to be 3.7 days and returned to prevaccination levels by day 30.
- Author's 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. 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.
- Reviewer's conclusions: Reviewer was the lead author.

Pichichero ME, Gentile A, Giglio N, et al. Mercury levels in premature and low birth weight newborn infants after receipt of thimerosal-containing vaccines. *J. Pediatr* 2009; 155(4):495-499.

- Blood, stool, and urine samples were obtained pre-vaccination and 12 hours to 30 days post-vaccination from 72 premature newborns. Maximal mean \pm SD blood mercury levels was 3.6 ± 2.1 ng/mL occurring at 1 day after vaccination. The blood mercury half-life was calculated to be 6.3 (95% CI:3.85-8.77) days and mercury levels returned to pre-vaccination levels by day 30.
- Author's conclusions: The blood half-life of intramuscular ethyl mercury from thimerosal in vaccines given to premature/low birth weight infants is substantially shorter than that of oral methyl mercury in adults. Because of the differing pharmacokinetics, exposure guidelines based on oral methyl mercury in adults may not be accurate for premature/low birth weight infants who receive thimerosal-containing vaccines.
- Reviewer's conclusions: Reviewer was the lead author.

Dorea JG, Lucia VA, Fajon V and Horvat M. Speciation of methyl- and ethyl-mercury in hair of breastfed infants acutely exposed to thimerosal-containing vaccines. *Chimica Chimica Acta* 412 2011; 412:1563-1566.

- In 20 samples of infants' hair, all but two samples showed variable amounts of MeHg (10.3 to 6668ng/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 ($r = -0.5572$; $p = 0.0384$) was found between hair-EtHg concentrations and the time elapsed after the last thimerosal containing vaccine.
- Author's conclusions: The analytical method proved sensitive enough to quantify EtHg in babies' hair after acute exposure to thimerosal in vaccine shots.
- Reviewer's conclusions: By one year of age the contribution of thimerosal ethylmercury to total mercury exposure in infants and children is multi-fold lower than the contribution from methylmercury in fish in Amazonia.

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

Neurodevelopmental disorders, maternal Rh-negativity, and Rho(D) immune globulins: a multi-center assessment. *Neuro Endocrinol Lett.* 2008; 29(2): 272-280

- There were significant increases in neurodevelopmental disorders (NDs) among children born to mothers with Rh-negativity (24.2%, n=47) compared to controls (13.9%, n=142), $p < 0.001$. Children with NDs born post-2001 had maternal Rh-negativity frequency (13.6%) similar to controls.
- Author's conclusions: This study associates Thimerosal-containing Rho(D) immune globulin exposure with some (NDs) in children.
- Reviewer's conclusions: There is no information on how the subjects were selected for inclusion in the study. The data from one of the clinic populations appears to be the same as that described in an earlier publication (Geier and Geier, 2007), this time with the maternal Rh-negativity status of 87 autism spectrum children compared to the Rh-negativity status of 1021 pregnant women who presented to the same clinic between 1980 and 1989. No explanation is given as to how the comparison group was selected or why it was appropriate.

Schechter R and Grether JK. Continuing Increases in Autism Reported to California's Developmental Services System (DSS). Mercury in Retrograde. Arch Gen Psychiatry 2008; 65(1):19-24.

- The estimated prevalence of DSS clients aged 3 to 5 years with autism increased for each quarter from January 1995 through March 2007. Since 2004, the absolute increase and the rate of increase in DSS clients aged 3 to 5 years with autism were higher than those in DSS clients of the same ages with any eligible condition including autism.
- Author's conclusions: The Department of Developmental Services data do not show any recent decrease in autism in California despite the exclusion of more than trace levels of thimerosal from nearly all childhood vaccines. The DDS data do not support the hypothesis that exposure to thimerosal during childhood is a primary cause of autism.
- Reviewer's conclusions: Despite removal of thimerosal from most vaccines used in the U.S. (except injectable multidose influenza vaccines) rates of diagnosed and reported autism continue to escalate, strongly arguing against a causal association.

Young HA, Geier DA and Geier MR. Thimerosal exposure in infants and neurodevelopmental disorders: An assessment of computerized medical records in the Vaccine Safety Datalink. *Journal of Neurological Sciences* 2008; 271:110-118.

- Hg exposure from thimerosal-containing vaccines was significantly associated with an increase in diagnosis of neurodevelopmental disorders (NDs). By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs.
- Author's conclusions: Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be conducted to further evaluate the relationship between Hg exposure and NDs.
- Reviewer's conclusions: This was a retrospective ecological study of a possible association between thimerosal exposure from vaccines and NDs. The control disorders selected (pneumonia, congenital anomalies and failure to thrive) have not shown secular trends similar to those seen for NDs and an increase in use of vaccines containing thimerosal in the U.S.

Croen LA, Matevia M, Yoshida CK and Grether JK. Maternal Rh D status, anti-D immune globulin exposure during pregnancy, and risk of autism spectrum disorders. Am J Obstet Gynecol 2008; 199:234.e1-234.e6.

- No case-control differences were observed for maternal Rh negative status (11.5% vs. 10.0%, $P = .5$) or prenatal anti-D immune globulin exposure (10.0% vs. 9.3%, $P = .7$). Risk of autism remained unassociated with maternal Rh status or prenatal exposure to anti-D immune globulins after adjustment for covariates.
- Author's conclusions: These data support previous findings that prenatal exposure to thimerosal-containing anti-D immune globulins does not increase the risk of autism.
- Reviewer's conclusions: Same as the authors.

Tozzi AE, Bisiacchi P, Tarantino V, De Mei B, D'Elia L, Chiarotti F and Salmaso S. Neuropsychological Performance 10 Years After Immunization in Infancy With Thimerosal-Containing Vaccines. *Pediatrics* 2009; 123:475-482.

- Among the 24 neuropsychological outcomes that were evaluated, only 2 were significantly associated with thimerosal exposure.
- Author's conclusions: Given the large number of statistical comparisons performed, the few associations found between thimerosal exposure and neuropsychological development might be attributable to chance. The associations found, although statistically significant, were based on small differences in mean test scores, and their clinical relevance remains to be determined.
- Reviewer's conclusions: Given that the expected number of false rejections of the null hypothesis for 72 tests is four, the authors correctly concluded that the two significant findings may have been attributable to chance.

Adams JB, Baral M, Geis E, Mitchell_J, Ingram J, Hensley A, Zappia I, Newmark S, Gehn E, Rubin A, Mitchell K, Bradstreet J and El-Dahr JM. The Severity of Autism Is Associated with Toxic Metal Body Burden and Red Blood Cell Glutathione Levels. J Toxicol 2009; 2009: 532640. Epub 2009 Aug 26.

- Multiple positive correlations were found between the severity of autism and the urinary excretion of toxic metals. Variations in the severity of autism measurements could be explained, in part, by regression analyses of urinary excretion of toxic metals before and after DMSA and the level of RBC glutathione (adjusted R2 of 0.22-0.45, $P < .005$ in all cases).
- Author's conclusions: This study demonstrates a significant positive association between the severity of autism and the relative body burden of toxic metals.
- Reviewer's conclusions: Chelation of the blood of children with a diagnosis of autism resulted in a 638% increase in urine excretion of lead but an insignificant change in mercury levels of 63 children.

Marques RC, Dorea JG, Bernardi J, Bastoa WR and Malm O. Prenatal and Postnatal Mercury Exposure, Breastfeeding and Neurodevelopment During the First 5 Years. *Cog Behav Neurol* 2009; 22:134-141

- Length of lactation and hair-Hg were each significantly correlated with Gesell Scores (GS), but in opposite ways: length of lactation was positive and significantly correlated with all GS at 60 months; hair-Hg concentrations were negative and significantly correlated with GS at 6 months ($r = -0.333$; $P = 0.002$) and 60 months ($r = -0.803$; $P = 0.010$), but not at 36 months.
- Author's conclusions: Until there is more refined approach to recognize children sensitive to Hg exposure, and in situations of uncertainty (EtHg exposure), the neurodevelopment benefit of breastfeeding should be recommended.
- Reviewer's conclusions: The neurodevelopment benefit of breastfeeding encourages that it should be recommended.

Marques RC, Dorea JG and Bernardi JVE. Thimerosal exposure (from tetanus-diphtheria vaccine) during pregnancy and neurodevelopment of breastfed infants at 6 months. *Acta Paediatrica* 2010;99:934-939.

- Compared with the group of infants not exposed to ethylmercury in utero, the infants of exposed mothers showed no significant difference in neurodevelopment delays. Although there was a significant correlation between Hair-Hg of mothers and hair-Hg of neonates (Spearman $r = 0.353$; $p = 0.001$), there was no significant correlation between the level of in utero exposure to ethylmercury in Td vaccines and neonate's hair-Hg concentrations (Spearman $r = 0.06$; $p = 0.59$).
- Author's conclusions: Early neurodevelopment of exclusively breastfed infants is sensitive to in utero exposure to mercury, but maternal thimerosal exposure in tetanus-diphtheria vaccines per se cannot portend clinical neurodevelopment delays measured by GDS at 6 months.
- Reviewer's conclusions: Methylmercury exposure among fish-eating Amazonian women may be associated with neurodevelopmental delays. A dose of tetanus-diphtheria vaccine has no measurable additive association effect. 63 women received tetanus-diphtheria vaccine and 19 women who did not receive the vaccine served as controls.

Geier DA, Young HA and Geier MR. Thimerosal exposure & increasing trends of premature puberty in the vaccine safety datalink. Indian J Med Res 2010; 131:500-507.

- Significantly increased ($P < 0.0001$) rate ratios were observed for premature puberty for $100\mu\text{g}$ difference in Hg exposure from thimerosal containing vaccines (TCVs) in the birth-7 months (rate ratio = 5.58) and birth-13 months (rate ratio = 6.45) of age exposure windows. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs.
- Author's conclusions: Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be done to evaluate the relationship between Hg exposure and premature puberty.
- Reviewer's conclusions: A retrospective ecological study of a possible association between thimerosal exposure from vaccines and premature puberty diagnoses. The control disorders selected (pneumonia, congenital anomalies and failure to thrive) have not shown secular trends similar to those seen for premature puberty diagnoses and TCVs.

Price C, Thompson WW, Goodson B et al. Prenatal and Infant Exposure to Thimerosal From Vaccines and Immunoglobulins and Risk of Autism. *Pediatrics* 2010;126:656-664.

- There were no findings of increased risk for any of the 3 autism spectrum disorder (ASD) outcomes with thimerosal exposure. The adjusted odds ratios (95% confidence intervals) for ASD associated with a 2-SD increase in ethylmercury exposure were 1.12 (0.83-1.51) for prenatal exposure, 0.88 (0.62-1.26) for exposure from birth to 1 month, 0.60 (0.36-0.99) from exposure from birth to 7 months, and 0.60 (0.32-0.97) for exposure from birth to 20 months.
- Author's conclusions: In the study of Managed Care Organizations (MCO) MCO members, prenatal and early-life exposure to ethylmercury from thimerosal-containing vaccines and immunoglobulin preparations was not related to increased risk of ASDs.
- Reviewer's conclusions: In the study of MCO members, prenatal and early-life exposure to ethylmercury from thimerosal-containing vaccines and immunoglobulin preparations was not related to increased risk of ASDs.

Mrozek-Budzyn D, Majewska R, Kieltyka A, Augustyniak M. Lack of Association between Thimerosal-Containing Vaccines and Autism. *Przegl Epidemiol* 2011; 65:491-495.

- Study population included 96 cases diagnosed with childhood or atypical autism and 192 controls matched individually by year of birth, gender, and physician's practice. No significant association was found between thimerosal-containing vaccine (TCV) exposure and autism.
- Author's conclusions: No evidence of an association between thimerosal containing vaccines (TCVs) and autism.
- Reviewer's conclusions: The study was under-powered.

Gallagher C and Goodman M. Hepatitis B triple series vaccine and developmental disability in US children aged 1-9 years. *Toxicological and Environmental Chemistry* 2008; 90(5):997-1008 .

- Vaccination with 3 doses of Hepatitis B was determined by parental report. Developmental disability was proxied by early intervention or special education services (abbreviated as EIS). The odds of receiving EIS were approximately nine times as great for vaccinated boys (n= 46) as for unvaccinated boys (n=7), after adjustment for confounders.
- Author's conclusions: 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.
- Reviewer's conclusions: Detailed descriptions of potential biases and pitfalls that could arise from attempting to use VAERS, VSD, NHANES or other similar databases to make causal inferences have been described by the Institute of Medicine (U.S.) and by a report by Parker et al (2004). Therefore the identified associations between multiple variables including male gender and EIS cannot provide evidence of causality.

Gallagher CM and Goodman MS. Hepatitis B Vaccination of Male Neonates and Autism Diagnosis, NHIS 1997-2002. J of Toxicology and Environmental Health. 2010; Part A, 73:1665-1677.

- Boys vaccinated as neonates had threefold greater odd for autism diagnosis compared to boys never vaccinated or vaccinated after the first month of life. Non-Hispanic white boys were 64% less likely to have autism diagnosis relative to nonwhite boys.
- Author's conclusions: The association between hepatitis B vaccination of male neonates and parental report of autism diagnosis was determined.
- Reviewer's conclusions: Detailed descriptions of potential biases and pitfalls that could arise from attempting to use VAERS, VSD, NHANES, National Health Interview Surveys or other similar databases to make causal inferences have been described by the Institute of Medicine (U.S.) and by a report by Parker et al (2004). Therefore the identified associations between multiple variables including male gender and EIS cannot provide evidence of causality.

Berman RF, Pessah IN, Mouton PR, Mav D and Harry J. Low-level neonatal thimerosal exposure: further evaluation of altered neurotoxic potential in SJL mice. *Toxicol Sci.* 2008; 101(2): 294-309.

- In an open-field test the majority of behaviors tested were unaffected by thimerosal injection, although thimerosal-injected female mice showed increased time in the margin of an open field at 4 weeks of age.
- Author's conclusions: Considered together the present results do not indicate pervasive developmental neurotoxicity following vaccine-level thimerosal injections in SJL mice, and provide little if any support for the hypothesis that thimerosal exposure contributes to the etiology of neurodevelopmental disorders.
- Reviewer's conclusions: Using experimental procedures that closely followed the report by Hornig et al (*Mol Psychiatry* 2004; 9: 833), this investigation failed to find evidence that exposure to thimerosal caused abnormal growth or development in mice.

Branch D. Gender-selective toxicity of thimerosal. Exp. Toxicol. Pathol. 2009;61(2): 133-136

- . At doses of 38.4-76.8 mg/kg using 10% DMSO as diluent, seven of seven male mice compared to zero of seven female mice tested succumbed to thimerosal.
- Author's conclusions: While not directly addressing the controversy of thimerosal and autism, this is 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.
- Reviewer's conclusions: The doses are not relevant to those included in vaccines containing thimerosal.

Minami T, Miyata E, Sakamoto Y, Yamazaki H and Ichida S. Induction of metallothionein in mouse cerebellum and cerebrum with low-dose thimerosal injection. *Cell Biol Toxicol* 2010; 26:143-152.

- The induction of metallothionein (MT) messenger RNA (mRNA) and protein was observed in the cerebellum and cerebrum of mice after thimerosal injection. MT-1 mRNA expression in the cerebellum was three times higher than that in the cerebrum after the injection of 12µg/kg thimerosal.
- Author's conclusions: 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.
- Reviewer's conclusions: The aim of this study was to determine whether the cerebellum of mice was “damaged” by low-dose (12 microgram/Kg) subcutaneous injection in 5 week old ddY strain mice. In a previous study (Minami T, Oda K, Gina N, Yamazaki H. *Environ Toxicol Pharmacol* 2007; 34: 316-320) the content of mercury in the cerebrum of ddY mice did not increase significantly following injection of 12 microgram/Kg of thimerosal. While MT-1 and MT-3 levels increased in the cerebellum of the mice there was no study of the brain tissue for “damage”. The relevance to the injection of thimerosal in vaccines in humans is unknown.

Olczak M, Duszyk M, Mierzeiowski P, Majewska MD. Neonatal administration of a vaccine preservative, thimerosal, produces lasting impairment of nociception and apparent activation of opioid systems in rats. *Brain Res.* 2009; 1301:143-151.

- Thimerosal solutions were injected into Wistar and Lewis rats in a vaccination-like mode on postnatal days 7, 9, 11 and 15 in four equal doses of 12, 48, 240, 720, 1440, 2160, 3000 $\mu\text{g Hg/kg}$ (Wistar rats) and 54, 216, 540 and 1080 $\mu\text{g Hg/kg}$ (Lewis rats). Pharmacokinetic analysis revealed that Hg 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. Thimerosal-treated rats of both strains and sexes manifested statistically significantly elevated pain threshold on a hot plate (56 °C).
- Author's conclusions: Thimerosal administration to suckling or adult rats impairs sensitivity to pain apparently due to activation of the endogenous opioid system.
- Reviewer's conclusions: The doses are not relevant to those included in vaccines containing thimerosal. The investigators gave the suckling rats a dose 4-fold higher than human infant exposures and escalated the dose further. There was a significant difference between the rat strains.

Hewitson L, Houser LA, Stott C, Sackett G, Tomko JL, Atwood D, Blue L, White ER. Delayed acquisition of neonatal reflexes in newborn primates receiving a thimerosal-containing hepatitis B vaccine: influence of gestational age and birth weight. *J Toxicol Environ Health* 2010; 73(19):1298-1313.

- A single dose of hepatitis B vaccine containing the preservative thimerosal (Th) was administered to male macaques within 24 h of birth (n= 13). Unexposed animals received saline placebo (n = 4) or no injection (n = 3). In exposed newborn rhesus macaques there was a significant delay in the acquisition of *root*, *snout*, and *suck* reflexes, compared with unexposed animals.
- Author's conclusions: 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 gestational age (GA) or birth weight (BW). The mechanisms underlying these effects and the requirements for Th requires further study.
- Reviewer's conclusions: The investigators acknowledged that the results were preliminary and a larger study was needed to verify the observations. The investigators noted that animal allocation was semi-random in order to complete peer groups of 4 animals for later social testing and unbalanced in sample size.

Olczak M, Duszczyk M, Mierzejewski P, Wierzba-Bobrowicz T and Majewska M. Lasting neuropathological changes in rat brain after intermittent neonatal administration of thimerosal. Folia Neuropathol 2010; 48(4):258-269.

- 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 was studied. Numerous neuropathological changes were observed in young adult rats.
- Author's conclusions: 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.
- Reviewer's conclusions: The applicability of the Wistar rat experiments to humans is unknown. The use of 12 microgram/kg/dose of thimerosal is biologically relevant; higher doses are not. The close dose spacing of 4 doses would allow accumulation of EtHg unlike the human infant.

Olczak M, Duszczyk M, Mierzejewski P and Meyza K. Persistent behavioral impairments and alterations of brain dopamine system after early postnatal administration of thimerosal in rats. Behavioural Brain Research 2011; 223:107-118.

- Neonatal treatment with thimerosal (at doses 12, 240, 1440 and 3000 µg Hg/kg) on behaviors, which are characteristically altered in autism, were studied 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.
- Author's conclusions: 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 to neurodevelopmental disorders.
- Reviewer's conclusions: The applicability of the Wistar rat experiments to humans is unknown. The use of 12 microgram/kg/dose of thimerosal is biologically relevant; higher doses are not. The close dose spacing of 4 doses would allow accumulation of EtHg unlike the human infant. Only one experiment used an EtHg dose of 12 microgram/kg for four doses; all other experiments used 20-fold, 120-fold and 250-fold higher doses.

Duszczyk-Budhathoki M, Olczak M, Lehner M and Majewska M. Administration of Thimerosal to Infant Rats Increases Overflow of Glutamate and Aspartate in the Prefrontal Cortex: Protective Role of Dehydroepiandrosterone Sulfate. Neurochem Res 2012; 37:436-447.

- 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.
- Author's conclusions: 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.
- Reviewer's conclusions: The applicability of the Wistar rat experiments to humans is unknown. The use of 12.5 microgram/kg/dose of thimerosal is biologically relevant; higher doses are not. The close dose spacing of 4 doses would allow accumulation of EtHg unlike the human infant. Only one experiment (Figure 3) showed a chronic effect of 12.5 microgram/kg dose given at infant rat age 7, 9, 11 and 15 days.

Dorea JG, Marques RC and Brandao KG. Neonate Exposure to Thimerosal Mercury from Hepatitis B Vaccines. Am J Perinatol 2009; 26(7):523-527.

- Over the 5 years (2001 to 2005), 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 not being vaccinated within the first 24 hours. Range of mercury exposure spread from 4.2 to 21.1 μ g mercury/kg body weight for those receiving vaccines with the highest thimerosal concentration.
- Author's conclusions: This study reveals critical issues that can modulate toxicokinetics and toxicodynamics of organomercurials in neonates.
- Reviewer's conclusions: Not unexpectedly, the dose of ethylmercury in thimerosal varies when calculated according to the weight of the child, with lower birth weight infants receiving a higher dose/body weight.

Wu X, Liang H, O' Hara KA, Yalowich JC and Hasinoff BB. Thiol-Modulated Mechanisms of the Cytotoxicity of Thimerosal and Inhibition of DNA Topoisomerase II α . Chem. Res Toxicol 2008; 21:483-493.

- The results indicated that thimerosal would be quickly metabolized in vivo because of its reactions with protein and nonprotein thiols. Thimerosal also potently inhibited the decatenation activity of DNA topoisomerase II α , likely through reaction with critical free cysteine thiol groups.
- Author' s conclusions: These studies have elucidated some of the chemistry and biological activities of the interaction of thimerosal with topoisomerase II α and protein and nonprotein thiols and with DNA.
- Reviewer' s conclusions: The study employed an in vitro model using high doses of ethylmercury (50-100 micromolar) that do not mimic those included as thimerosal in pediatric vaccines.

Marques RC, Bernardi J, Dorea JG, Bastos WR and Malm O. Principal component analysis (PCA) and discrimination of variables associated with pre- and post-natal exposure to mercury. Int. J. Hyg. Environ. Health 2008; 211:606-614.

Methods and results from the abstract:

- The variables associated with neurodevelopment at 180 days were examined in 82 exclusively breastfed infants using principal component analysis (PCA). The PCA yielded a two-factor solution: the first component represented birth weight and vaccine (first doses of Hepatitis B and DTP) variability and explained 57% of variance; the second component represented a gradient of neurodevelopment (Gesell scores) and explained 35% of variance.
- Author's Conclusions: Infant neurodevelopmental (ND) disorders linked to Thimerosal-Hg stands in need of proof, but PCA points to the possibility of identifying exposure risk variables associated with ND schedules.
- Reviewer's conclusions: The main findings were that low birth weight and dose of thimerosal/birth weight were colinear among Brazilian infants born in Amazonia and birth weight or thimerosal dose/ birth weight was associated with lower neurodevelopmental scores .

Gloriani NG, Srinivasa K, Bock HL and Hoet B. Immunogenicity of HBV vaccine during stated shelf-life. *Southeast Asian J Trop Med Public Health* 2010; 41(4):876-882.

- 420 subjects aged ≥ 18 years were randomized to receive three doses (0, 1, 6 months) of 2-PE preserved hepatitis B vaccine kept on the shelf <12 months (2PE New group), 2PE preserved hepatitis B vaccine kept on the shelf >18 months (2PE Old group), or thiomersal preserved hepatitis B vaccine (Thio group). There was no difference detected between the 2PE New and 2PE Old groups in anti-HBs seroprotection rates and geometric mean concentrations one month after dose 3. However, both 2PE groups had significantly lower seroprotection rates than the Thio group and the number of non-responders was higher in the 2PE groups than in the Thio group.
- Author's conclusions: The shelf-life of the vaccines had no impact on immunogenicity or reactogenicity and 2PE preserved hepatitis B vaccine can be considered stable over time.
- Reviewer's conclusions: Thimerosal is a superior preservative at 50 micrograms/dose compared to 2-phenoxyethanol at 5 mg/dose in Hepatitis B vaccines when assessed from the perspective of preserved immunogenicity.

Khandke L, Yang C, Krylova K, Jansen KU and Rashidbaigi A. Preservative of choice for Prev(e)nar 13 in a multi-dose formulation. *Vaccine* 2011; 29(41):7144-7153.

- A Prev(e)nar 13 formulation containing 2-Phenoxyethanol (2-PE) at a concentration of 5.0 mg/dose was stable and met EP recommended criteria for antimicrobial effectiveness tests when the formulation was kept over a 30 month period. A recommended dose of thimerosal, as a comparator, did not meet EP antimicrobial effectiveness acceptance criteria. The rate of growth inhibition of thimerosal compared to 2-PE on *S. aureus*, a resilient organism in these tests, was significantly slower in single and multi-challenge studies.
- Author's conclusions: Results indicate that 2-PE provides a superior antimicrobial effectiveness over thimerosal for this vaccine formulation.
- Reviewer's conclusions: Neither 2-PE (5 mg/dose) nor thimerosal (25 or 50 µg/dose) met all criteria by all regulatory authorities for all acceptance criteria. The studies suggested that ingredients in PCV13 (sugars) facilitate microbial growth. Antibacterial efficacy for 2-PE at 5 mg/dose was equivalent or higher than that seen with thimerosal at 25 µg/dose and 50 µg/dose. However, 2-PE was less effective than either dose of thimerosal in controlling growth of *C. albicans* or *A. niger*.

**United Nations Environmental Program (UNEP)-
convened Intergovernmental Negotiating
Committee Meeting 4 (INC4), 3 to 4 April 2012,
Salle D, WHO headquarters, Geneva, Switzerland**

Summary

- No new evidence could be found in the published literature that brings into question the decision by WHO to endorse the continued use of thimerosal as a safe preservative in multi-dose vaccines.