

How safe or effective are vaccines? Not very.

As the Centers for Disease Control continues to ratchet its public relations campaign to promote the H1NI flu vaccine, two basic assumptions have been made: the vaccine is proven to be safe and effective. However, a careful examination of the scientific literature raises serious questions about both assumptions, not just for the flu vaccine, but for all vaccines now licensed and approved.

Based upon a careful review of the scientific literature, we have determined that all vaccines contain ingredients that have been shown to have serious, even fatal side effects. The medical authorities would like us to believe that if a adverse effect occurs, it is a rare incident and that the most that can be expected is irritation or redness at the injection site and temporary low grade fever. This manipulation represents fear mongering at its worse.

The information gathered together below comes from peer-reviewed, highly respected journals and publications. Our review is not definitive but represents a random sampling. After you realize how many adverse effects might occur from any single vaccine, as well as from multiple vaccines in combination, then the question is why the American public hasn't been given an honest, objective insight about vaccine safety. In point of fact, all of the major experts in the nation's vaccine programs are fully aware of all of these side effects, since they automatically receive the information and studies for their review. It is not for us to advocate for or against vaccines, but whether or not vaccine safety is being accurately and honestly reported to American citizens so that they can make well-informed choices on whether to be vaccinated or not.

Studies examining the Safety and Efficacy of Vaccines:

MMR (and Pertussis)

- A study printed in the Journal of Pediatric Endocrinology and Metabolism concluded that clusters of cases of type 1 diabetes mellitus (T1DM) are potentially linked to the hemophilus vaccine. Furthermore, the study also found that there are clusters of cases of T1DM that occur 2 to 4 years after the administration of the Pertussis, MMR, and BCG vaccine.

MMR

- A preliminary study conducted by scientists at University of California San Diego, and San Diego State University, found that acetaminophen use after individuals had been administered a measles-mumps-rubella vaccination (MMR) is associated with autistic disorder.
- The increasing number of recommended vaccinations in the United States is paralleled by growing concerns about the safety of those vaccinations. The variety of substances used in vaccines sometimes causes the development of negative skin reactions in susceptible adults and children.
- The Kitasato Institute conducted a study that examined the serious adverse side effects/events that can sometimes occur in the aftermath of vaccinations. These included two main groups: allergic reactions and severe systemic illnesses.

NEUROLOGIC DISORDERS AFTER MEASLES-MUMPS-RUBELLA VACCINATION

- According to a study published in Pediatrics, findings of neurologic disorders after the administration of the MMR vaccine included encephalitis, aseptic meningitis, and autistic disorders. In those cases, some of the diseases developed within 3 months of the child receiving the vaccination.

TETANUS VACCINE:

- Tests showed a significant though temporary drop in T-helper lymphocytes (a class of white blood cells which helps govern the immune system) after adults, in a controlled study, were given a tetanus vaccination.

POLIO VACCINE:

- According to studies, intramuscular injections (such as the polio virus vaccine) may increase the likelihood of skeletal muscle injury which is known to predispose individuals to neurological complications that are concurrent with polio virus infections, as well as paralytic poliomyelitis.

MEASLES:

- From 1963 through 1971, eighty-four cases of neurologic disorders with onset less than 30 days after live measles-virus vaccination were reported in the United States.
- A 10-year follow-up study revealed that the incidence of clinical measles was 11.5% among those inoculated with live vaccines in combination with killed vaccines. So despite people receiving the vaccine, they were still also getting measles.

PERTUSSIS:

- Adverse effects of the pertussis vaccine include: infantile spasms; hypsarrhythmia; aseptic meningitis; encephalopathy (including acute encephalopathy and chronic neurologic damage); deaths classified as sudden infant death syndrome (SIDS); anaphylaxis; autism; erythema multiforme or other rashes; Guillain-Barré syndrome (polyneuropathy); peripheral mononeuropathy; hemolytic anemia; juvenile diabetes; learning disabilities and hyperactivity; protracted inconsolable crying or screaming; Reye syndrome; shock and "unusual shock-like state" with hypotonicity, hyporesponsiveness, and short-lived convulsions (usually febrile); and thrombocytopenia—and *3 adverse events for rubella vaccine*—arthritis (acute and chronic); radiculoneuritis and other neuropathies; and thrombocytopenic purpura.

ADJUVANTS:

THE DANGERS OF SQUALENE:

- Gulf War Syndrome
- chronic arthritis
- characteristic pathological changes both in the central and peripheral nervous system

ALUMINUM:

- chronic fatigue and associated manifestations known as the Gulf war syndrome

DTP (or Tetanus)

- “DTP or tetanus vaccination appears to increase the risk of allergies and related respiratory symptoms in children and adolescents.”

MERCURY/THIMEROSAL

- contributing to some regressive ASDs
- immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining/associated with ASDs
- Kawasaki's Disease

INFLUENZA

- Aside from containing dangerous adjuvants, according to several some studies (see studies below), mass influenza vaccination campaigns are ineffective in preventing influenza

MMR and Pertussis

Clustering of cases of type 1 diabetes mellitus occurring 2-4 years after vaccination is consistent with clustering after infections and progression to type 1 diabetes mellitus in autoantibody positive individuals.

Classen JB, Classen DC. Clustering of cases of type 1 diabetes mellitus occurring 2-4 years after vaccination is consistent with clustering after infections and progression to type 1 diabetes mellitus in autoantibody positive individuals. J Pediatr Endocrinol Metab. 2003 Apr-May;16(4):495-508.

“We previously analyzed data from a hemophilus vaccine trial and identified clusters of extra cases of type 1 diabetes mellitus (T1DM) caused by the vaccine that occurred between 36 and 48 months after immunization. Published reports indicate clustering of cases of T1DM occurring approximately 2-4 years after mumps infection. Others have reported a 2-4 year delay between the onset of autoantibodies and the development of T1DM. We attempted to determine whether similar clustering of cases of T1DM occurred after immunization with vaccines other than hemophilus. METHODS: We searched MEDLINE and reviewed references from published papers to find databases on the incidence of T1DM and then searched MEDLINE to determine whether changes in immunization occurred in these regions during the times the incidence of DM was being recorded. RESULTS: Distinct rises in the incidence of T1DM occurred 2-4 years following the introduction of the MMR and pertussis vaccines. A drop in the incidence of T1DM was detected between 3-4 years following discontinuation of pertussis and BCG vaccines. CONCLUSION: The identification of clusters of cases of T1DM occurring in consistent temporal time periods allowed a link between the hemophilus vaccine and T1DM to be established. The current findings indicate there are also clusters of cases of T1DM occurring 2-4 years post-immunization with the pertussis, MMR, and BCG vaccine. The data are consistent with the occurrence of

clusters following mumps infection and the progression to T1DM in patients with antipancreatic autoantibodies.”

MMR

Acetaminophen (paracetamol) use, measles-mumps-rubella vaccination, and autistic disorder

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The present study was performed to determine whether acetaminophen (paracetamol) use after the measles-mumps-rubella vaccination could be associated with autistic disorder. This case-control study used the results of an online parental survey conducted from 16 July 2005 to 30 January 2006, consisting of 83 children with autistic disorder and 80 control children. Acetaminophen use after measles-mumps-rubella vaccination was significantly associated with autistic disorder when considering children 5 years of age or less (OR 6.11, 95% CI 1.42—26.3), after limiting cases to children with regression in development (OR 3.97, 95% CI 1.11—14.3), and when considering only children who had post-vaccination sequelae (OR 8.23, 95% CI 1.56—43.3), adjusting for age, gender, mother's ethnicity, and the presence of illness concurrent with measles-mumps-rubella vaccination. Ibuprofen use after measles-mumps-rubella vaccination was not associated with autistic disorder. This preliminary study found that acetaminophen use after measles-mumps-rubella vaccination was associated with autistic disorder.

Autism, Vol. 12, No. 3, 293-307 (2008)

Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism.

Singh V, Lin S, Newell E, Nelson C. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. Journal Of Biomedical Science [serial online]. July 2002;9(4):359-364.

“Autoimmunity to the central nervous system (CNS), especially to myelin basic protein (MBP), may play a causal role in autism, a neurodevelopmental disorder. Because many autistic children harbor elevated levels of measles antibodies, we conducted a serological study of measles-mumps-rubella (MMR) and MBP

autoantibodies. Using serum samples of 125 autistic children and 92 control children, antibodies were assayed by ELISA or immunoblotting methods. ELISA analysis showed a significant increase in the level of MMR antibodies in autistic children. Immunoblotting analysis revealed the presence of an unusual MMR antibody in 75 of 125 (60%) autistic sera but not in control sera. This antibody specifically detected a protein of 73-75 kD of MMR. This protein band, as analyzed with monoclonal antibodies, was immunopositive for measles hemagglutinin (HA) protein but not for measles nucleoprotein and rubella or mumps viral proteins. Thus the MMR antibody in autistic sera detected measles HA protein, which is unique to the measles subunit of the vaccine. Furthermore, over 90% of MMR antibody-positive autistic sera were also positive for MBP autoantibodies, suggesting a strong association between MMR and CNS autoimmunity in autism. Stemming from this evidence, we suggest that an inappropriate antibody response to MMR, specifically the measles component thereof, might be related to pathogenesis of autism.”

Hypersensitivity reactions to vaccine components.

Heidary N, Cohen D. Hypersensitivity reactions to vaccine components. *Dermatitis: Contact, Atopic, Occupational, Drug: Official Journal Of The American Contact Dermatitis Society, North American Contact Dermatitis Group* [serial online]. September 2005;16(3):115-120.

“Vaccines are responsible for the control of many infectious diseases that were once common in the United States, including polio, measles, diphtheria, pertussis (whooping cough), rubella (German measles), mumps, tetanus, and Haemophilus influenzae type b. National efforts to generate collaboration between federal, state, and local governments and public and private health care providers have resulted in record high levels of vaccination coverage in the United States. The high rate of US vaccinations is paralleled by growing concerns about the safety of their delivery. The variety of substances used in vaccines sometimes causes the development of cutaneous reactions in susceptible adults and children. This article will review adverse cutaneous events consistent with hypersensitivity reactions to the following ingredients in vaccines: aluminum, thimerosal, 2-phenoxyethanol, formaldehyde, and neomycin.”

Vaccine adverse events reported in post-marketing study of the Kitasato Institute from 1994 to 2004.

Nakayama T, Onoda K. Vaccine adverse events reported in post-marketing study of the Kitasato Institute from 1994 to 2004. *Vaccine* [serial online]. January 5, 2007;25(3):570-576.

“General physicians, pediatricians and parents realize that serious adverse events occur with an extremely rare incidence, but have no information on the incidences of vaccine-associated adverse events. A proper understanding of vaccine adverse events would be helpful in promoting an immunization strategy. Causal association can rarely be determined in adverse events through laboratory examinations. We examined the cases reported in the post-marketing surveillance of the Kitasato Institute, categorizing them into two groups: allergic reactions and severe systemic illnesses. Anaphylactic patients with gelatin allergy after immunization with live measles, rubella and mumps monovalent vaccines have been reported since 1993, but the number of reported cases with anaphylaxis dramatically decreased after 1999 when gelatin was removed from all brands of DPT. The incidence of anaphylactic reaction was estimated to be 0.63 per million for Japanese encephalitis virus (JEV) vaccine, 0.95 for DPT and 0.68 for Influenza vaccine, but the causative component has not yet been specified. Among 67.2 million immunization practices, 6 cases with encephalitis or encephalopathy, 7 with acute disseminated encephalomyelitis (ADEM), 10 with Guillain-Barré syndrome and 12 with idiopathic thrombocytopenic purpura (ITP) were reported. The wild-type measles virus genome was detected in a patient with encephalitis and in two of four bone marrow aspirates obtained from ITP after measles vaccination. Enterovirus infection was identified in two patients after mumps vaccination (one each with

encephalitis and ADEM), one patient with encephalitis after immunization with JEV vaccine, and one with aseptic meningitis after immunization with influenza vaccine. The total estimated incidence of serious neurological illness after vaccination was 0.1-0.2 per million immunization practices. We found that enterovirus or wild-type measles virus infection was coincidentally associated with vaccination in several cases suspected of being vaccine adverse events.”

Neurologic Disorders After Measles-Mumps-Rubella Vaccination

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Neurologic Disorders After Measles-Mumps-Rubella Vaccination PEDIATRICS Vol. 110 No. 5 November 2002, pp. 957-963.

“The possibility of adverse neurologic events has fueled much concern about the safety of measles-mumps-rubella (MMR) vaccinations. The available evidence concerning several of the postulated complications is controversial. The aim of this study was to assess whether an association prevails between MMR vaccination and encephalitis, aseptic meningitis, and autism. conducted among 535 544 1- to 7-year-old children who were vaccinated between November 1982 and June 1986 in Finland. For encephalitis and aseptic meningitis, the numbers of events observed within a 3-month risk interval after vaccination were compared with the expected numbers estimated on the basis of occurrence of encephalitis and aseptic meningitis during the subsequent 3-month intervals.... *Results.* Of the 535 544 children who were vaccinated, 199 were hospitalized for encephalitis, 161 for aseptic meningitis, and 352 for autistic disorders. In 9 children with encephalitis and 10 with meningitis, the disease developed within 3 months of vaccination”

TETANUS VACCINE:

Abnormal T-lymphocyte subpopulations in healthy subjects after tetanus booster immunization.

Buttram, Harold E. MD, Abnormal T-lymphocyte subpopulations in healthy subjects after tetanus booster immunization. N Engl J Med. 1984 Jan 19;310(3):198-9. No abstract available. PMID: 6228737 [PubMed - indexed for MEDLINE]

"As reported in a letter to the New England Journal of Medicine in 1984, tests of T-lymphocyte subpopulations were done on 11 healthy adults before-and-after routine tetanus booster immunizations. Tests showed a significant though temporary drop in T-helper lymphocytes (a class of white blood cells which helps govern the immune system) in all of the subjects. Special concern rests in the fact that in 4 of the subjects the T-helper cells fell to levels found in active AIDS patients. (2) If this was the result of a single vaccine in healthy adults, it is sobering to think of the consequences of the multiple vaccines (twenty-one at last count) routinely given to infants with their immature systems during the first six months of life. However, we can only speculate as to the consequences, as this test has never been repeated."

POLIO VACCINE:

Mechanism of Injury-Provoked Poliomyelitis

Gromeier M, Wimmer E. Mechanism of injury-provoked poliomyelitis. *Journal Of Virology* [serial online]. June 1998;72(6):5056-5060.

“Skeletal muscle injury is known to predispose its sufferers to neurological complications of concurrent poliovirus infections. This phenomenon, labeled “provocation poliomyelitis,” continues to cause numerous cases of childhood paralysis due to the administration of unnecessary injections to children in areas where poliovirus is endemic. Recently, it has been reported that intramuscular injections may also increase the likelihood of vaccine-associated paralytic poliomyelitis in recipients of live attenuated poliovirus vaccines. We have studied this important risk factor for paralytic polio in an animal system for poliomyelitis and have determined the pathogenic mechanism linking intramuscular injections and provocation poliomyelitis. Skeletal muscle injury induces retrograde axonal transport of poliovirus and thereby facilitates viral invasion of the central nervous system and the progression of spinal cord damage. The pathogenic mechanism of provocation poliomyelitis may differ from that of polio acquired in the absence of predisposing factors.”

Evidence for a high risk of vaccine-associated disease and reintroduction of wild-virus infection.

Strebel P, Aubert-Combescu A, Ion-Nedelcu N, et al. Paralytic poliomyelitis in Romania, 1984-1992. Evidence for a high risk of vaccine-associated disease and reintroduction of wild-virus infection. *American Journal Of Epidemiology* [serial online]. December 15, 1994;140(12):1111-1124.

“Although poliomyelitis due to *wild-virus infection* has virtually disappeared from Romania, with no cases having been documented between 1984 and 1989, *vaccine-associated* paralytic poliomyelitis has been reported at very *high rates* for over two decades. In November 1990, to decrease the *risk of vaccine-associated* paralytic poliomyelitis, oral poliovirus *vaccine* produced in Romania was replaced by imported oral *vaccine* made by a Western European manufacturer. To better quantify the *risk of vaccine-associated* paralytic poliomyelitis and the impact of the change in *vaccine* manufacturer, the authors reviewed clinical, epidemiologic, and laboratory data on poliomyelitis cases that occurred in Romania from 1984 to 1992. Poliovirus isolates were characterized at the US Centers for Disease Control and Prevention. During the period 1984-1992, 132 confirmed cases of paralytic poliomyelitis were reported in Romania, of which 13 were classified as *wild-virus-associated*, 93 as *vaccine-associated*, and 26 as “of unknown origin.” *Wild* type 1 poliovirus was isolated during 1990-1992 from nine of 13 (69%) cases in an outbreak that occurred primarily among undervaccinated gypsy children. *Vaccine-associated* cases were epidemiologically and virologically distinct from *wild-virus* cases. Of the 93 *vaccine-associated* cases, 45 children were recipients and 48 were contacts. The overall *risk of vaccine-associated* paralytic poliomyelitis in Romania (1 case per 183,000 doses of oral poliovirus *vaccine* distributed) was 14-fold higher than the *risk* in the United States. The risks of recipient *vaccine-associated* paralytic poliomyelitis related to the first dose of oral *vaccine* were similar for Romanian and imported *vaccine* (1 case per 95,000 doses and 1

case per 65,000 doses, respectively), as were the total risks of *vaccine-associated* paralytic poliomyelitis. These findings definitively demonstrate a substantially elevated risk of *vaccine-associated* paralytic poliomyelitis in Romania which was not affected by a change in oral poliovirus vaccine manufacturer.”

Paralytic poliomyelitis in vaccinated children.

Pamecha R. Paralytic poliomyelitis in vaccinated children. *Indian Journal Of Pediatrics* [serial online]. May 1987;54(3):431-435.

“Although the oral poliovaccine has been effective in the control of poliomyelitis, there are many observations of its failure. To ascertain clinical picture of the disease in vaccinated children and probable causative factors, this study was undertaken. Out of 125 children with Ac. poliomyelitis presented over a period of two years, 16 had received three or more doses of Trivalent-oral poliovaccine. Parents of these children were interviewed to find out possible cause of vaccine failure. All 16 children were below five years, male-female ratio was 9:7. Twelve received three doses of polio vaccine and four had more doses. Intervals between the last dose of vaccine received and the onset of the disease in all were less than two years. Two had less than one week. Muscle powers in all children at the time of admission were in the range of grade 0/6 to grade 3/6. Poor maintenance of cold chain is probably the only identifiable cause of vaccine failure in 11 children. DPT inoculation along with poliovaccine might be the precipitating factor for paralysis in two children. To overcome these problems there is an urgent need of community involvement in health programmes, reorientation of health workers, a close supervision and steps to improve cold chain. The possibility of using killed poliovaccine should also be explored.”

MORE ON POLIO:

Long-Term Circulation of Vaccine-Derived Poliovirus That Causes Paralytic Disease

Cherkasova E, Korotkova E, Yakovenko M, et al. Long-term circulation of vaccine-derived poliovirus that causes paralytic disease. *Journal Of Virology* [serial online]. July 2002;76(13):6791-6799

“Here, we report the characterization of a highly evolved derivative of the Sabin vaccine strain isolated in a case of paralytic poliomyelitis from a 7-month-old immunocompetent baby in an apparently adequately immunized population. Analysis of the genome of this isolate showed that it is a double (type 1-type 2-type 1) vaccine-derived recombinant. The number of mutations accumulated in both the type 1-derived and type 2-derived portions of the recombinant genome suggests that both had diverged from their vaccine predecessors 2 years before the onset of the illness. This fact, along with other recent observations, points to the possibility of long-term circulation of Sabin vaccine strain derivatives associated with an increase in their neurovirulence. Comparison of genomic sequences of this and other evolved vaccine-derived isolates reveals some general features of natural poliovirus evolution. They include a very high preponderance and nonrandom distribution of synonymous substitutions, conservation of secondary structures of important *cis*-acting elements of the genome, and an apparently adaptive character of most of the amino acid mutations, with only a few of them occurring in the antigenic determinants. Another interesting feature is a frequent occurrence of tripartite intertypic recombinants with either type 1 or type 3 homotypic genomic ends.”

MEASELS:

Neurologic Disorders Following Live Measles-Virus Vaccination

Philip J. Landrigan, MD; John J. Witte, MD Neurologic Disorders Following Live Measles-Virus Vaccination. *JAMA*. 1973;223(13):1459-1462.

“From 1963 through 1971, eighty-four cases of neurologic disorders with onset less than 30 days after live measles-virus vaccination were reported in the United States. Thirteen could be adequately accounted for by causes other than vaccine, and another 11 were uncomplicated febrile convulsions probably related to vaccination. One case met diagnostic criteria for subacute sclerosing panencephalitis. The remaining 59 showed clinical features of encephalitis or encephalopathy. Causes of these cases could not be established, but 45 (76%) had onset between 6 and 15 days after vaccination; this clustering suggests that some may have been caused by vaccine. From 1963 through 1971, 50.9 million doses of measles vaccine were distributed, and, therefore, incidence of the reported neurologic disorders was 1.16 per million doses. Risk of encephalitis following measles infection is one per thousand cases.”

Measles, mumps, rubella vaccine induced subacute sclerosing panencephalitis.

Belgamwar R, Prasad S, Appaya P. Measles, mumps, rubella vaccine induced subacute sclerosing panencephalitis. *Journal Of The Indian Medical Association* [serial online]. November 1997;95(11):594.

“The incidence of subacute sclerosing panencephalitis (SSPE), a progressive and fatal neurodegenerative disease caused by the measles virus, has declined with widespread use of measles vaccine. The risk of SSPE after measles vaccination has been estimated at 0.7/million doses. This paper reports the case of a 15-year-old girl from India who developed SSPE presumably as a result of a delayed effect of measles, mumps, and rubella (MMR) vaccine. She presented with a 2-month history of behavioral disturbances, a deterioration in school performance, forgetfulness, silly smiling, handwriting changes, social withdrawal, and ataxia. The girl had received MMR vaccine at 9 months of age and had no past history of measles. Her measles antibody titre was 1:625 in both serum and cerebrospinal fluid.”

Measles vaccines used in Japan

Hirayama, M. "Measles vaccines used in Japan." *Reviews of Infectious Diseases* 1983; 5:495-503.

“The history of the research and development of measles vaccines is described and the efficacy of and adverse reactions to the Japanese licensed vaccines are discussed. The 10-year follow-up studies revealed that the incidence of clinical measles was 11.5% among those inoculated with live vaccines in combination with killed vaccines, whereas it was only 1.9% among those given live vaccines attenuated to the level of the Schwarz vaccine. Use of the Schwarz and Biken-CAM vaccines resulted in satisfactory antibody responses in greater than or equal to 97% of vaccinees. However, these vaccines caused a febrile reaction of greater than or equal to 37.5 C in 50% of vaccinees and one of greater than or equal to 39 C in 15% of vaccinees. On the other hand, a febrile reaction was observed in 20% and 5%, respectively, of children immunized with the AIK-C vaccine or the further-attenuated Schwarz vaccine, both of which were developed in Japan. The worldwide use of further-attenuated vaccines is strongly recommended. The system of fixed surveillance stations, which was started in 1981 by the Japanese government with the voluntary cooperation of pediatricians and

ophthalmologists, is described. This system proved effective in obtaining information about the prevalence of communicable diseases, including measles, in childhood and about the efficacy of vaccines.”

PERTUSSIS:

Adverse Events Associated with Childhood Vaccines: Evidence Bearing on Casuality

Kathleen R. Stratton, Cynthia J. Howe, and Richard B. Johnston, Jr., *Adverse Events Associated with Childhood Vaccines: Evidence Bearing on Casuality*. Institute of Medicine (IOM). 1994.

“During the 20 months of the study, the committee reviewed altogether *17 adverse events for pertussis vaccine*—infantile spasms; hypsarrhythmia; aseptic meningitis; encephalopathy (including acute encephalopathy and chronic neurologic damage); deaths classified as sudden infant death syndrome (SIDS); anaphylaxis; autism; erythema multiforme or other rashes; Guillain-Barré syndrome (polyneuropathy); peripheral mononeuropathy; hemolytic anemia; juvenile diabetes; learning disabilities and hyperactivity; protracted inconsolable crying or screaming; Reye syndrome; shock and "unusual shock-like state" with hypotonicity, hyporesponsiveness, and short-lived convulsions (usually febrile); and thrombocytopenia—and *3 adverse events for rubella vaccine*—arthritis (acute and chronic); radiculoneuritis and other neuropathies; and thrombocytopenic purpura. Although the committee was not asked expressly to examine febrile seizures, afebrile seizures, or epilepsy in relation to diphtheria-pertussis-tetanus (DPT) vaccine, it did so because these conditions may also be serious and are considered by some to be components of encephalopathy. Conclusions regarding these conditions are given. The committee's conclusions on acute encephalopathy, also presented, refer only to conditions diagnosed as encephalopathy, encephalitis, or encephalomyelitis. (For additional information on the committee's charge and the events leading to the enactment of Public Law 99-660, see the Preface and Appendix B, *Pertussis and Rubella Vaccines: A Brief Chronology*.)”

Antibodies to Squalene in Gulf War Syndrome

Asa P, Cao Y, Garry R. Antibodies to squalene in Gulf War syndrome. *Experimental And Molecular Pathology* [serial online]. February 2000;68(1):55-64.

“Gulf War Syndrome (GWS) is a multisystemic illness afflicting many Gulf War-era veterans. The molecular pathological basis for GWS has not been established. We sought to determine whether the presence of antibodies to squalene correlates with the presence of signs and symptoms of GWS. Participants in this blinded cohort study were individuals immunized for service in Desert Shield/Desert Storm during 1990–1991. They included 144 Gulf War-era veterans or military employees (58 in the blinded study), 48 blood donors, 40 systemic lupus erythematosus patients, 34 silicone breast implant recipients, and 30 chronic fatigue syndrome patients. Serum antibodies to squalene were measured. In our small cohort, the substantial majority (95%) of overtly ill deployed GWS patients had antibodies to squalene. All (100%) GWS patients immunized for service

in Desert Shield/Desert Storm who did not deploy, but had the same signs and symptoms as those who did deploy, had antibodies to squalene. In contrast, none (0%) of the deployed Persian Gulf veterans not showing signs and symptoms of GWS have antibodies to squalene. Neither patients with idiopathic autoimmune disease nor healthy controls had detectable serum antibodies to squalene. The majority of symptomatic GWS patients had serum antibodies to squalene.”

The Endogenous Adjuvant Squalene Can Induce a Chronic T-Cell-Mediated Arthritis in Rats.

Carlson B, Jansson A, Larsson A, Bucht A, Lorentzen J. The endogenous adjuvant squalene can induce a chronic T-cell-mediated arthritis in rats. *The American Journal Of Pathology* [serial online]. June 2000;156(6):2057-2065.

“Squalene is a cholesterol precursor, which stimulates the immune system nonspecifically. We demonstrate that one intradermal injection of this adjuvant lipid can induce joint-specific inflammation in arthritis-prone DA rats. Histopathological and immunohistochemical analyses revealed erosion of bone and cartilage, and that development of polyarthritis coincided with infiltration of alphabeta(+) T cells. Depletion of these cells with anti-alphabeta TcR monoclonal antibody (R73) resulted in complete recovery, whereas anti-CD8 and anti-gammadelta TcR injections were ineffective. The apparent dependence on CD4(+) T cells suggested a role for genes within the major histocompatibility complex (MHC), and this was concluded from comparative studies of MHC congenic rat strains, in which DA.1H rats were less susceptible than DA rats. Furthermore, LEW.1AV1 and PVG.1AV1 rats with MHC identical to DA rats were arthritis-resistant, demonstrating that non-MHC genes also determine susceptibility. Some of these genetic influences could be linked to previously described arthritis susceptibility loci in an F2 intercross between DA and LEW.1AV1 rats (ie, Cia3, Oia2 and Cia5). Interestingly, some F2 hybrid rats developed chronic arthritis, a phenotype not apparent in the parental inbred strains. Our demonstration that an autoadjuvant can trigger chronic, immune-mediated joint-specific inflammation may give clues to the pathogenesis of rheumatoid arthritis, and it raises new questions concerning the role of endogenous molecules with adjuvant properties in chronic inflammatory diseases.”

Identification of arthritogenic adjuvants of self and foreign origin.

Lorentzen J. Identification of arthritogenic adjuvants of self and foreign origin. *Scandinavian Journal Of Immunology* [serial online]. January 1999;49(1):45-50.

“The lack of defined triggers for human inflammatory joint diseases warrants efforts to identify candidate molecules. For this task, it may be an important lead that nonspecific activation of the immune system can precipitate arthritis in rats. Consequently, arthritis-prone rat strains were used to search for disease-triggering factors among molecules which initially induce innate defence reactions rather than specific immune responses. A variety of immunological adjuvants were investigated by intradermal injection into DA and LEW.1AV1 rats and monitoring of clinical signs for 30 days. Several arthritogenic cell-wall structures from yeast and bacteria were identified, such as beta-glucan, lipopolysaccharide and trehalosedimycolate. The test procedures also revealed arthritogens of chemical origin, such as dioctadecyldiammoniumbromide (DDA = C38H80NBr) and heptadecane (C17H36). Furthermore, it allowed the precise definition of arthritogenic determinants of lipids, since C16H34 induced arthritis, whereas the closely related linear hydrocarbons C16H32, C16H33Br and C15H32 did not. The observed pathogenicity of organic lipids raised the question

of whether endogenous lipids can also precipitate arthritis. Indeed, this was true for the cholesterol precursor squalene (C₃₀H₅₀). In conclusion, this article describes the rational use of arthritis-prone rat strains to identify arthritogenic factors of both foreign and self origin. Although structurally unrelated, the pathogenic molecules defined here share the feature of being nonspecific triggers of the immune system. This consolidates a general principle for the induction of adjuvant arthritis which may provide clues to the aetiology of human arthritides, including rheumatoid arthritis.”

The arthritogenic adjuvant squalene does not accumulate in joints, but gives rise to pathogenic cells in both draining and non-draining lymph nodes

Holm B, Svelander L, Bucht A, Lorentzen J. The arthritogenic adjuvant squalene does not accumulate in joints, but gives rise to pathogenic cells in both draining and non-draining lymph nodes. *Clinical And Experimental Immunology* [serial online]. March 2002;127(3):430-435.

“A single intradermal injection of the adjuvant-oil squalene induces T cell-mediated arthritis in DA rats. The chain of events leading from non-specific provocation of the immune system to arthritis, with clinical similarities to rheumatoid arthritis, is largely undetermined. Here, we combined *in vivo* tracking of tritium-labelled squalene with lymph node (LN) cell transfer experiments to determine where critical activation events may take place. The majority of squalene remained at the injection site (79%). The amounts recovered in peripheral joints (<1%) were equal to that recovered in other organs that can be targets in autoimmune diseases. This argues that arthritis does not develop as a consequence of adjuvant accumulation in joints. In contrast, substantial amounts of squalene were recovered in hyperplastic LN draining the injection site (1–13%). The adjuvant was deposited to a larger extent in cells than in extracellular matrix. The draining LN cells could transfer arthritis to naïve irradiated DA rats following *in vitro* stimulation with conA. Interestingly, non-draining LN were also hyperplastic and harboured arthritogenic cells, although they contained low amounts of squalene (<1%). Consequently, the amount of arthritogenic adjuvant in a particular LN is not closely linked to the development of pathogenic cells. The distribution pattern of squalene was similar in MHC-identical but arthritis-resistant PVG.1AV1 and LEW.1AV1 rats, and it was unaffected by T cell depletion with a monoclonal antibody (R73). Thus, T cells and non-MHC genes do not regulate dissemination of squalene, but rather determine arthritis development at the level of adjuvant response.”

Distinctive patterns of autoimmune response induced by different types of mineral oil

Kuroda Y, Akaogi J, Nacionales D, et al. Distinctive patterns of autoimmune response induced by different types of mineral oil. *Toxicological Sciences: An Official Journal Of The Society Of Toxicology* [serial online]. April 12, 2004;78(2):222-228.

“Although mineral oils are generally considered nontoxic and have a long history of use in humans, the mineral oil Bayol F (incomplete Freund's adjuvant, IFA) and certain mineral oil components (squalene and n-hexadecane) induce lupus-related anti-nRNP/Sm or -Su autoantibodies in nonautoimmune mice. In the present study, we investigated whether medicinal mineral oils can induce other types of autoantibodies and whether structural features of hydrocarbons influence autoantibody specificity. Female 3-month-old BALB/c (16-45/group) mice each received an i.p. injection of

pristane (C19), squalene (C30), IFA, three medicinal mineral oils (MO-F, MO-HT, MO-S), or PBS. Sera were tested for autoantibodies and immunoglobulin levels. Hydrocarbons were analyzed by gas chromatography/mass spectrometry. IFA contained mainly C15-C25 hydrocarbons, whereas MO-HT and MO-S contained C20-C40, and MO-F contained C15-C40. Pristane and n-hexadecane were found in IFA (0.17% and 0.10% w/v, respectively) and MOs (0.0026-0.027%). At 3 months, pristane and IFA induced mainly IgG2a, squalene IgG1, and MOs IgG3 and IgM in sera. Anti-cytoplasmic antibodies were common in mice treated with MO-F, as well as those treated with pristane, squalene, and IFA. Anti-ssDNA and -chromatin antibodies were higher in MO-F and MO-S than in untreated/PBS, squalene-, or IFA-treated mice, suggesting that there is variability in the induction of anti-nRNP/Sm versus -chromatin/DNA antibodies. The preferential induction of anti-chromatin/ssDNA antibodies without anti-nRNP/Sm/Su by MO-S and MO-F is consistent with the idea that different types of autoantibodies are regulated differently. Induction of autoantibodies by mineral oils considered nontoxic also may have pathogenetic implications in human autoimmune diseases.”

The experimental squalene encephaloneuropathy in the rat

Gajkowska B, Smialek M, Ostrowski R, Piotrowski P, Frontczak-Baniewicz M. The experimental squalene encephaloneuropathy in the rat. *Experimental And Toxicologic Pathology: Official Journal Of The Gesellschaft Für Toxikologische Pathologie* [serial online]. January 1999;51(1):75-80.

“Accumulation of squalene in the CNS is observed after administration of tellurium and squalene has been proposed to be a mediator of tellurium encephaloneuropathy. The aim of this study was to investigate the effects of squalene on the central and peripheral nervous systems in rat at the ultrastructural level. Squalene was administered at a dose of 20 g/kg body weight, once daily for 4 days, and the animals were sacrificed 7 days and 30 days after the initiation of the experiment. After 7 days a mild swelling of mitochondria and dilation of the Golgi complex cisterns in few neurons in the cerebral cortex and hippocampus were observed. The swelling of astrocytes and their processes was also seen. Some myelin sheaths in the cerebral white matter were disintegrated. In the peripheral nervous system (the sciatic nerve), a damage of the Schwann cells, a destruction of the myelin sheaths, and lipid-like deposits between myelin lamellae causing a secondary compression of axons were present. Squalene administration caused a stimulation of fibroblast to synthesize collagen and an activation of macrophages in the perineurium. After 30 days, the lipid-like material was present in some neurons as well as in the myelin sheaths in the central nervous system. Endothelial cells were hypertrophic and a few demonstrated features of apoptosis. Endothelial cell hypertrophy caused a narrowing of vessel lumen associated with an aggregation of blood morphological elements. Disturbances in myelination and swelling of astrocytic processes persisted in the central nervous system. In the peripheral nervous system, lipid-like deposits were localized in some fibroblasts and extracellularly between the collagen fibers in the perineurium. In conclusion, our electron microscopic studies indicate that squalene produces characteristic pathological changes both in the central and peripheral nervous systems. However, these alterations differ in some aspects (changes in endothelia, accumulation of lipid-like material) from the known features of tellurium encephaloneuropathy.”

The endogenous adjuvant squalene can induce a chronic T-cell-mediated arthritis in rats.

Carlson B, Jansson A, Larsson A, Bucht A, Lorentzen J. The endogenous adjuvant squalene can induce a chronic T-cell-mediated arthritis in rats. *The American Journal Of Pathology* [serial online]. June 2000;156(6):2057-2065.

“Squalene is a cholesterol precursor, which stimulates the immune system nonspecifically. We demonstrate that one intradermal injection of this adjuvant lipid can induce joint-specific inflammation in arthritis-prone DA rats. Histopathological and immunohistochemical analyses revealed erosion of bone and cartilage, and that development of polyarthritis coincided with infiltration of β^+ T cells. Depletion of these cells with anti- β TcR monoclonal antibody (R73) resulted in complete recovery, whereas anti-CD8 and anti- TcR injections were ineffective. The apparent dependence on CD4⁺ T cells suggested a role for genes within the major histocompatibility complex (MHC), and this was concluded from comparative studies of MHC congenic rat strains, in which DA.1H rats were less susceptible than DA rats. Furthermore, LEW.1AV1 and PVG.1AV1 rats with MHC identical to DA rats were arthritis-resistant, demonstrating that non-MHC genes also determine susceptibility. Some of these genetic influences could be linked to previously described arthritis susceptibility loci in an F2 intercross between DA and LEW.1AV1 rats (ie, *Cia3*, *Oia2* and *Cia5*). Interestingly, some F2 hybrid rats developed chronic arthritis, a phenotype not apparent in the parental inbred strains. Our demonstration that an autoadjuvant can trigger chronic, immune-mediated joint-specific inflammation may give clues to the pathogenesis of rheumatoid arthritis, and it raises new questions concerning the role of endogenous molecules with adjuvant properties in chronic inflammatory diseases.”

The arthritogenic adjuvant squalene does not accumulate in joints, but gives rise to pathogenic cells in both draining and non-draining lymph nodes.

Holm BC, Svelander L, Bucht A, Lorentzen]C [Department of Medicine, Unit of Rheumatology, Karolinska Institutet, Stockholm and Department of Medical Countermeasures, Division of NBC Defense, Defense Research Agency, Umea, Sweden], ‘The arthritogenic adjuvant squalene does not accumulate in joints, but gives rise to pathogenic cells in both draining and non-draining lymph nodes,’ *Clinical and Experimental Immunology*, (2002) Mar;127(3):430-5.

“A single intradermal injection of the adjuvant-oil squalene induces T cell-mediated arthritis in DA rats. The chain of events leading from non-specific provocation of the immune system to arthritis, with clinical similarities to rheumatoid arthritis, is largely undetermined. Here, we combined in vivo tracking of tritium-labelled squalene with lymph node (LN) cell transfer experiments to determine where critical activation events may take place. The majority of squalene remained at the injection site (79%). The amounts recovered in peripheral joints (<1%) were equal to that recovered in other organs that can be targets in autoimmune diseases. This argues that arthritis does not develop as a consequence of adjuvant accumulation in joints. In contrast, substantial amounts of squalene were recovered in hyperplastic LN draining the injection site (1–13%). The adjuvant was deposited to a larger extent in cells than in extracellular matrix. The draining LN cells could transfer arthritis to naïve irradiated DA rats following in vitro stimulation with conA. Interestingly, non-draining LN were also hyperplastic and harboured arthritogenic cells, although they contained low amounts of squalene (<1%). Consequently, the amount of arthritogenic adjuvant in a particular LN is not closely linked to the development of pathogenic cells. The distribution pattern of squalene was similar in MHC-identical but arthritis-resistant PVG.1AV1 and LEW.1AV1 rats, and it was unaffected by T cell depletion with a monoclonal antibody (R73).

Thus, T cells and non-MHC genes do not regulate dissemination of squalene, but rather determine arthritis development at the level of adjuvant response.”

Lessons from macrophagic myofasciitis: towards definition of a vaccine adjuvant-related syndrome.

Gherardi R. [Lessons from macrophagic myofasciitis: towards definition of a vaccine adjuvant-related syndrome].

Revue Neurologique [serial online]. February 2003;159(2):162-164.

“Macrophagic myofasciitis is a condition first reported in 1998, which cause remained obscure until 2001. Over 200 definite cases have been identified in France, and isolated cases have been recorded in other countries. The condition manifests by diffuse myalgias and chronic fatigue, forming a syndrome that meets both Center for Disease Control and Oxford criteria for the so-called chronic fatigue syndrome in about half of patients. One third of patients develop an autoimmune disease, such as multiple sclerosis. Even in the absence of overt autoimmune disease they commonly show subtle signs of chronic immune stimulation, and most of them are of the HLADRB1*01 group, a phenotype at risk to develop polymyalgia rheumatica and rheumatoid arthritis. Macrophagic myofasciitis is characterized by a stereotyped and immunologically active lesion at deltoid muscle biopsy. Electron microscopy, microanalytical studies, experimental procedures, and an epidemiological study recently demonstrated that the lesion is due to persistence for years at site of injection of an aluminum adjuvant used in vaccines against hepatitis B virus, hepatitis A virus, and tetanus toxoid. Aluminum hydroxide is known to potently stimulate the immune system and to shift immune responses towards a Th-2 profile. It is plausible that persistent systemic immune activation that fails to switch off represents the pathophysiologic basis of chronic fatigue syndrome associated with macrophagic myofasciitis, similarly to what happens in patients with post-infectious chronic fatigue and possibly idiopathic chronic fatigue syndrome. Therefore, the WHO recommended an epidemiological survey, currently conducted by the French agency AFSSAPS, aimed at substantiating the possible link between the focal macrophagic myofasciitis lesion (or previous immunization with aluminium-containing vaccines) and systemic symptoms. Interestingly, special emphasis has been put on Th-2 biased immune responses as a possible explanation of chronic fatigue and associated manifestations known as the Gulf war syndrome. Results concerning macrophagic myofasciitis may well open new avenues for etiologic investigation of this syndrome. Indeed, both type and structure of symptoms are strikingly similar in Gulf war veterans and patients with macrophagic myofasciitis. Multiple vaccinations performed over a short period of time in the Persian gulf area have been recognized as the main risk factor for Gulf War syndrome. Moreover, the war vaccine against anthrax, which is administered in a 6-shot regimen and seems to be crucially involved, is adjuvanted by aluminium hydroxide and, possibly, squalene, another Th-2 adjuvant. If safety concerns about long-term effects of aluminium hydroxide are confirmed it will become mandatory to propose novel and alternative vaccine adjuvants to rescue vaccine-based strategies and the enormous benefit for public health they provide worldwide.”

Induction of lupus autoantibodies by adjuvants.

Satoh M, Kuroda Y, Yoshida H, Behney KM, Mizutani A, Akaogi], Nacionales DC, Lorenson TD, Rosenbauer R], Reeves WH [Division of Rheumatology and Clinical Immunology, Department of Medicine, University of Florida, Gainesville], "Induction of lupus autoantibodies by adjuvants" *Journal of Autoimmunity*, (2003) Aug;21(1):1-9.

"Exposure to the hydrocarbon oil pristane induces lupus specific autoantibodies in non-autoimmune mice. We investigated whether the capacity to induce lupus-like autoimmunity is a unique property of pristane or is shared by other adjuvant oils. Seven groups of 3-month-old female BALB/cJ mice received a single intraperitoneal injection of pristane, squalene (used in the adjuvant MF59), incomplete Freund's adjuvant (IFA), three different medicinal mineral oils, or saline, respectively. Serum autoantibodies and peritoneal cytokine production were measured. In addition to pristane, the mineral oil Bayol F (IFA) and the endogenous hydrocarbon squalene both induced anti-nRNP/Sm and -Su autoantibodies (20% and 25% of mice, respectively). All of these hydrocarbons had prolonged effects on cytokine production by peritoneal APCs. However, high levels of IL-6, IL-12, and TNF α production 2–3 months after intraperitoneal injection appeared to be associated with the ability to induce lupus autoantibodies. The ability to induce lupus autoantibodies is shared by several hydrocarbons and is not unique to pristane. It correlates with stimulation of the production of IL-12 and other cytokines, suggesting a relationship with a hydrocarbon's adjuvanticity. The potential to induce autoimmunity may complicate the use of oil adjuvants in human and veterinary vaccines."

Distinctive Patterns of Autoimmune Response Induced by Different Types of Mineral Oil

Kuroda Y, Akaogi], Nacionales DC, Wasdo SC, Szabo N], Reeves WH, Satoh M [Division of Rheumatology and Clinical Immunology, Department of Medicine, University of Florida, Gainesville], "Distinctive Patterns of Autoimmune Response Induced by Different Types of Mineral Oil," *Toxicological Sciences*, (2004) Apr;78(2):222-8.

"Although mineral oils are generally considered nontoxic and have a long history of use in humans, the mineral oil Bayol (incomplete Freund's adjuvant, IFA) and certain mineral oil components (squalene and n-hexadecane) induce lupus-related anti-nRNP/Sm or -Su autoantibodies in nonautoimmune mice. In the present study, we investigated whether medicinal mineral oils can induce other types of autoantibodies and whether structural features of hydrocarbons influence autoantibody specificity. Female 3-month-old BALB/c (16–45/group) mice each received an i.p. injection of pristane (C19), squalene (C30), IFA, three medicinal mineral oils (MO-F, MO-HT, MO-S), or PBS. Sera were tested for autoantibodies and immunoglobulin levels. Hydrocarbons were analyzed by gas chromatography/mass spectrometry. IFA contained mainly C15–C25 hydrocarbons, whereas MO-HT and MO-S contained C20–C40, and MO-F contained C15–C40. Pristane and n-hexadecane were found in IFA (0.17% and 0.10% w/v, respectively) and MOs (0.0026–0.027%). At 3 months, pristane and IFA induced mainly IgG2a, squalene IgG1, and MOs IgG3 and IgM in sera. Anti-cytoplasmic antibodies were common in mice treated with MO-F, as well as those treated with pristane, squalene, and IFA. Anti-ssDNA and -chromatin antibodies were higher in MO-F and MO-S than in untreated/PBS, squalene-, or IFA-treated mice, suggesting that there is variability in the induction of anti-nRNP/Sm versus -chromatin/DNA antibodies. The preferential induction of anti-chromatin/ssDNA antibodies without anti-nRNP/Sm/Su by MO-S and MO-F is consistent with the idea that different types of autoantibodies are regulated differently. Induction of autoantibodies by mineral oils considered nontoxic also may have pathogenetic implications in human autoimmune diseases."

Autoimmunity induced by adjuvant hydrocarbon oil components of vaccine.

Kuroda Y, Nacionales D, Akaogi J, Reeves W, Satoh M. Autoimmunity induced by adjuvant hydrocarbon oil components of vaccine. *Biomedicine & Pharmacotherapy = Biomédecine & Pharmacothérapie* [serial online]. June 2004;58(5):325-337.

“Adjuvant oils such as Bayol F (Incomplete Freund’s adjuvant: IFA) and squalene (MF59) have been used in human and veterinary vaccines despite poor understanding of their mechanisms of action. Several reports suggest an association of vaccination and various autoimmune diseases, however, few were confirmed epidemiologically and the risk of vaccination for autoimmune diseases has been considered minimal. Microbial components, not the adjuvant components, are considered to be of primary importance for adverse effects of vaccines. We have reported that a single intraperitoneal injection of the adjuvant oils pristane, IFA or squalene induces lupus-related autoantibodies to nRNP/Sm and -Su in non-autoimmune BALB/c mice. Induction of these autoantibodies appeared to be associated with the hydrocarbon’s ability to induce IL-12, IL-6, and TNF- α , suggesting a relationship with hydrocarbon’s adjuvanticity. Whether this is relevant in human vaccination is a difficult issue due to the complex effects of vaccines and the fact that immunotoxicological effects vary depending on species, route, dose, and duration of administration. Nevertheless, the potential of adjuvant hydrocarbon oils to induce autoimmunity has implications in the use of oil adjuvants in human and veterinary vaccines as well as basic research.”

Adjuvant oil induces waves of arthritogenic lymph node cells prior to arthritis onset

Holm B, Lorentzen J, Bucht A. Adjuvant oil induces waves of arthritogenic lymph node cells prior to arthritis onset. *Clinical And Experimental Immunology* [serial online]. July 2004;137(1):59-64

“A single intradermal injection of the adjuvant-oil squalene induces T cell mediated arthritis in DA rats. The chain of events leading from nonspecific provocation of the immune system to arthritis is largely unknown. Previous studies have demonstrated that lymph node (LN) cells are of pathogenic importance, i.e. cells from LNs draining the injection site can transfer arthritis to naïve DA rats. Recently we have demonstrated cellular uptake of adjuvant oil in draining lymph nodes but also that nondraining LNs become hyperplastic and harbour arthritogenic cells. Here, we aimed to determine from which time-point prior to arthritis onset arthritogenic cells appear in draining inguinal and nondraining axillary/brachial LNs, respectively. We demonstrated that the ability to transfer arthritis was strongly dependent on the time-point after adjuvant-injection with clear-cut differences between draining and nondraining LN cells. Cells harvested at day 5 postinjection (p.i) were not able to transfer arthritis, while at day 8 p.i, a first wave of arthritogenic cells appeared in draining LNs. The ability to transfer arthritis was associated with a pro-inflammatory cytokine profile as indicated by the IL-1 β and IFN γ expression in cells from draining LNs. Subsequently, at day 11 p.i., just before arthritis onset, arthritogenic cells appeared also in nondraining LNs. These results shed new light on the induction of arthritic diseases, implicating a two step mechanism for the development of pathogenic cells. Firstly, a pro-inflammatory burst in responding lymphoid organs leading to a local pool of arthritogenic cells and, secondly, a transmission of arthritogenicity to other LNs and precipitation of disease in peripheral joints.”

Pilot evaluation of influenza virus vaccine (IV) combined with adjuvant

Keitel W, Couch R, Bond N, Adair S, Van Nest G, Dekker C. Pilot evaluation of influenza virus vaccine (IVV) combined with adjuvant. *Vaccine* [serial online]. 1993;11(9):909-913.

“The safety of licensed influenza virus vaccine (IVV) combined with a novel adjuvant containing muramyl tripeptide (MTP) conjugated to phosphatidylethanolamine (PE) was evaluated in a randomized pilot study. Ten healthy 23–30-year-old men were given a single intramuscular dose of IVV combined with saline ($n = 5$) or with 100 μg of MTP PE in the MF59 adjuvant emulsion (MF59-100) ($n = 5$). Evaluations were performed on days 0, 1, 2, 4, 7 and 28 after inoculation. IVV alone was well tolerated. All volunteers immunized with IVV/MF59-100 experienced moderate to severe local and systemic reactions which interfered with usual activities. Discomfort at the injection site was first noted at 2–6 h; induration (5/5), erythema (3/5), and regional adenopathy (3/5) persisted for up to 4 days. Systemic symptoms including chills (5/5), fever (3/5), nausea (3/5) and/or dizziness (2/5) developed within 12 h of inoculation and resolved by 48 h. Elevated white blood cell count (days 1 and 2), erythrocyte sedimentation rate and serum fibrinogen were transiently observed. Although peak serum neutralizing antibody titres versus influenza A/H3N2 and influenza B antigens were higher in the group given IVV with MF59-100, these unexpected reactions indicate that this dose of adjuvant is unsuitable for use in combination with this IVV.”

DTP (or Tetanus)

Effects of diphtheria-tetanus-pertussis or tetanus vaccination on allergies and allergy related respiratory symptoms among children and adolescents in the US

Hurwitz E, Morgenstern H. Effects of diphtheria-tetanus-pertussis or tetanus vaccination on allergies and allergy-related respiratory symptoms among children and adolescents in the United States. *Journal Of Manipulative And Physiological Therapeutics* [serial online]. February 2000;23(2):81-90.

“Findings from animal and human studies confirm that diphtheria and tetanus toxoids and pertussis (DTP) and tetanus vaccinations induce allergic responses; associations between childhood vaccinations and subsequent allergies have been reported recently. Objective: The association of DTP or tetanus vaccination with allergies and allergy-related respiratory symptoms among children and adolescents in the United States was assessed. Methods: Data were used from the Third National Health and Nutrition Examination Survey on infants aged 2 months through adolescents aged 16 years. DTP or tetanus vaccination, lifetime allergy history, and allergy symptoms in the past 12 months were based on parental or guardian recall. Logistic regression modeling was performed to estimate the effects of DTP or tetanus vaccination on each allergy. Results: The odds of having a history of asthma was twice as great among vaccinated subjects than among unvaccinated subjects (adjusted odds ratio). 2.00; 95% confidence interval, 0.59 to 6.74). The odds of having had any allergy-related respiratory symptom in the past 12 months was 63% greater among vaccinated subjects than unvaccinated subjects (adjusted odds ratio. 1.63; 95% confidence interval 1.05 to 2.54). The associations between vaccination and subsequent allergies and symptoms were greatest among children aged 5 through 10 years. Conclusions: DTP or tetanus vaccination appears to increase the risk of allergies and related respiratory symptoms in children and adolescents. Although it is unlikely that these results are entirely because of any sources of bias, the small number of unvaccinated subjects and the study design limit our ability to make firm causal inferences about the true magnitude of effect.”

An evaluation of the effects of thimerosal on neurodevelopmental disorders reported following DTP and Hib vaccines in comparison to DTPH vaccine in the United States.

Geier D, Geier M. An evaluation of the effects of thimerosal on neurodevelopmental disorders reported following DTP and Hib vaccines in comparison to DTPH vaccine in the United States. *Journal Of Toxicology And Environmental Health. Part A* [serial online]. August 2006;69(15):1481-1495.

“Thimerosal is an ethylmercury (49.55% mercury by weight) preservative historically added to some vaccines. Toxicokinetic studies showed children in the United States received doses of mercury from Thimerosal-containing vaccines (TCVs) in excess of safety guidelines. In the United States during the 1990s, diphtheria-tetanus-pertussis (DTP) and Haemophilus influenzae type b (Hib) vaccines (maximally, 50 mug mercury per joint administration) and diphtheria-tetanus-pertussis-Haemophilus influenzae type b (DTPH) vaccines (25 mug mercury per administration) were given to children in the same childhood vaccination schedule at 2, 4, 6, and 15-18 mo, so that children receiving DTP and Hib vaccines may have maximally received an additional 100 mug more mercury exposure from TCVs than children administered DTPH vaccines. A case-control epidemiological study of neurodevelopmental disorders (NDs) reported to the Vaccine Adverse Event Reporting System (VAERS) (online public access version; updated 31 August 2004) following administration of DTP vaccines in comparison DTPH vaccines manufactured by Lederle Laboratories (Pearl River, NY) from 1994 through 1998 was undertaken. Significantly increased odds ratios for autism, speech disorders, mental retardation, infantile spasms, and thinking abnormalities reported to VAERS were found following DTP vaccines in comparison to DTPH vaccines with minimal bias or systematic error. Additional ND research should be undertaken in the context of evaluating mercury-associated exposures, especially since in 2005 the Institute of Medicine issued a report calling into question handling of vaccine safety data by the National Immunization Program of the Centers for Disease Control and Prevention.”

A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States.

Geier D, Geier M. A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States. *Neuro Endocrinology Letters* [serial online]. August 2006;27(4):401-413.

“Thimerosal is an ethylmercury-containing compound (49.6% mercury by weight) used as at the preservative level in vaccines (0.005% to 0.01%). METHODS: Statistical modeling in a meta-analysis epidemiological assessment of the Vaccine Adverse Event Reporting System (VAERS) for neurodevelopment disorders (NDs) reported following Diphtheria-Tetanus-whole-cell-Pertussis (DTP) vaccines in comparison to Diphtheria-Tetanus-whole-cell-Pertussis-Haemophilus Influenzae Type b (DTPH) vaccines (administered: 1994-1997) and following Thimerosal-containing Diphtheria-Tetanus-acellular-Pertussis (DTaP), vaccines in comparison to Thimerosal-free DTaP vaccines (administered: 1997-2000), was undertaken. RESULTS: Significantly increased adjusted (sex, age, vaccine type, vaccine manufacturer) risks of autism, speech disorders, mental retardation, personality disorders, thinking abnormalities, ataxia, and NDs in general, with minimal systematic error or confounding, were associated with TCV exposure. CONCLUSION: It is clear from the results of the present epidemiological study and other recently published data associating mercury exposure with childhood NDs, additional ND research should be undertaken in the context of evaluating mercury-associated exposures, especially from Thimerosal-containing vaccines.”

Lung pathology and immediate hypersensitivity in a mouse model after vaccination with pertussis vaccines and challenge with *Bordetella pertussis*.

Vandebriel R, Gremmer E, Vermeulen J, et al. Lung pathology and immediate hypersensitivity in a mouse model after vaccination with pertussis vaccines and challenge with *Bordetella pertussis*. *Vaccine* [serial online]. March 8, 2007;25(12):2346-2360.

“While evaluating vaccine efficacy against clinical *Bordetella pertussis* isolates in mice, after challenge vaccinated mice showed increased lung pathology with eosinophilia, compared to challenged, non-vaccinated animals. This led us to study bacterial clearance, lung pathology, lung TNF-alpha expression, and parameters of immediate hypersensitivity (IH), being serum IgE levels, eosinophil numbers in the bronchoalveolar lavage fluid, and ex vivo IL-4, IL-5, IL-10, IL-13, and IFN-gamma production by the bronchial lymph node cells. BALB/c mice received a combined Diphtheria (D), Tetanus (T), Poliomyelitis, and whole-cell Pertussis vaccine (WCV), a combined D, T, and three-component acellular Pertussis vaccine (ACV), aluminium hydroxide adjuvant, or PBS, 28 and 14 days before *B. pertussis* infection. Similarly treated non-infected mice were taken as a control. Infection induced pathology; this induction was stronger after (especially WCV) vaccination. WCV but not ACV vaccination induced TNF-alpha expression after challenge. After challenge, IH parameters were strongly increased by (especially ACV) vaccination. Vaccinated IL-4 KO mice showed similar clearance and pathology, in the absence of IgE and with reduced numbers of eosinophils. Vaccinated (Th1-deficient) T-bet KO mice showed reduced clearance and similar pathology. In summary, after challenge vaccination increased lung pathology, TNF-alpha expression (only WCV), and IH parameters. Th1 cells were critical for clearance.”

Thimerosal induces DNA breaks, caspase-3 activation, membrane damage, and cell death in cultured human neurons and fibroblasts

David Baskin, M.D. et al. Thimerosal induces DNA breaks, caspase-3 activation, membrane damage, and cell death in cultured human neurons and fibroblasts. *Toxicol Sci*. 2003 Aug;74(2):361-8. Epub 2003 May 28. PMID: 12773768

“Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. Little is known about the reactions of human neuronal and skin cells to its micro- and nanomolar concentrations, which can occur after using thimerosal-containing products. A useful combination of fluorescent techniques for the assessment of thimerosal toxicity is introduced. Short-term thimerosal toxicity was investigated in cultured human cerebral cortical neurons and in normal human fibroblasts. Cells were incubated with 125-nM to 250-microM concentrations of thimerosal for 45 min to 24 h. A 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) dye exclusion test was used to identify nonviable cells and terminal transferase-based nick-end labeling (TUNEL) to label DNA damage. Detection of active caspase-3 was performed in live cell cultures using a cell-permeable fluorescent caspase inhibitor. The morphology of fluorescently labeled nuclei was analyzed. After 6 h of incubation, the thimerosal toxicity was observed at 2 microM based on the manual detection of the fluorescent attached cells and at a 1-microM level with the more sensitive GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence. The lower limit did not change after 24 h of incubation. Cortical neurons demonstrated higher sensitivity to thimerosal compared to fibroblasts. The first sign of toxicity was an increase in membrane permeability to DAPI after 2 h of incubation with 250 microM thimerosal. A 6-h incubation resulted in failure to exclude DAPI, generation of DNA breaks, caspase-3 activation, and development of morphological signs of apoptosis. We demonstrate that thimerosal in micromolar concentrations rapidly induce membrane and DNA damage and initiate caspase-3-dependent apoptosis in human neurons and fibroblasts. We conclude that a proposed combination of fluorescent techniques can be useful in analyzing the toxicity of thimerosal.”

Elevated levels of measles antibodies in children with autism.

Singh VK, Jensen RL. Elevated levels of measles antibodies in children with autism. *Pediatr Neurol.* 2003 Apr;28(4):292-4. PMID: 12849883

“Virus-induced autoimmunity may play a causal role in autism. To examine the etiologic link of viruses in this brain disorder, we conducted a serologic study of measles virus, mumps virus, and rubella virus. Viral antibodies were measured by enzyme-linked immunosorbent assay in the serum of autistic children, normal children, and siblings of autistic children. The level of measles antibody, but not mumps or rubella antibodies, was significantly higher in autistic children as compared with normal children ($P = 0.003$) or siblings of autistic children ($P \leq 0.0001$). Furthermore, immunoblotting of measles vaccine virus revealed that the antibody was directed against a protein of approximately 74 kd molecular weight. The antibody to this antigen was found in 83% of autistic children but not in normal children or siblings of autistic children. Thus autistic children have a hyperimmune response to measles virus, which in the absence of a wild type of measles infection might be a sign of an abnormal immune reaction to the vaccine strain or virus reactivation.”

Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism.

Singh VK et al. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. *J Biomed Sci.* 2002 Jul-Aug;9(4):359-64. PMID 12145534

“Autoimmunity to the central nervous system (CNS), especially to myelin basic protein (MBP), may play a causal role in autism, a neurodevelopmental disorder. Because many autistic children harbor elevated levels of measles antibodies, we conducted a serological study of measles-mumps-rubella (MMR) and MBP autoantibodies. Using serum samples of 125 autistic children and 92 control children, antibodies were assayed by ELISA or immunoblotting methods. ELISA analysis showed a significant increase in the level of MMR antibodies in autistic children. Immunoblotting analysis revealed the presence of an unusual MMR antibody in 75 of 125 (60%) autistic sera but not in control sera. This antibody specifically detected a protein of 73–75 kD of MMR. This protein band, as analyzed with monoclonal antibodies, was immunopositive for measles hemagglutinin (HA) protein but not for measles nucleoprotein and rubella or mumps viral proteins. Thus the MMR antibody in autistic sera detected measles HA protein, which is unique to the measles subunit of the vaccine. Furthermore, over 90% of MMR antibody-positive autistic sera were also positive for MBP autoantibodies, suggesting a strong association between MMR and CNS autoimmunity in autism. Stemming from this evidence, we suggest that an inappropriate antibody response to MMR, specifically the measles component thereof, might be related to pathogenesis of autism.”

Autism: a Novel Form of Mercury Poisoning

Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a novel form of mercury poisoning. *Medical Hypotheses* [serial online]. April 2001;56(4):462-471.

“Autism is a syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movements, and sensory dysfunction. Recent epidemiological studies suggest that autism may affect 1 in 150 U. S. children. Exposure to mercury can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autism, and the similarities extend to neuroanatomy, neurotransmitters, and biochemistry. Thimerosal, a preservative added to many vaccines, has become a major source of mercury in children who, within their first two years, may have received a quantity of mercury that exceeds safety guidelines. A review of medical literature and U.S. government data suggests that (i) many cases of idiopathic autism are induced by early mercury exposure from thimerosal; (ii) this type of

autism represents an unrecognized mercurial syndrome; and (iii) genetic and non-genetic factors establish a predisposition whereby thimerosal's adverse effects occur only in some children.”

A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorders

Geier D, Geier M. A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorders. *Journal of Toxicology & Environmental Health: Part A* [serial online]. May 15, 2007;70(10):837-851.

“Impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns characterize autism spectrum disorders (ASDs). It is clear that while genetic factors are important to the pathogenesis of ASDs, mercury exposure can induce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASDs. The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, U.S. Department of Health and Human Services, IRB number IRB00005375) approved the present study. A case series of nine patients who presented to the Genetic Centers of America for a genetic/developmental evaluation are discussed. Eight of nine patients (one patient was found to have an ASD due to Rett's syndrome) (a) had regressive ASDs; (b) had elevated levels of androgens; (c) excreted significant amounts of mercury post chelation challenge; (d) had biochemical evidence of decreased function in their glutathione pathways; (e) had no known significant mercury exposure except from Thimerosal-containing vaccines/Rho(D)-immune globulin preparations; and (f) had alternate causes for their regressive ASDs ruled out. There was a significant dose-response relationship between the severity of the regressive ASDs observed and the total mercury dose children received from Thimerosal-containing vaccines/Rho (D)-immune globulin preparations. Based upon differential diagnoses, 8 of 9 patients examined were exposed to significant mercury from Thimerosal-containing biologic/vaccine preparations during their fetal/infant developmental periods, and subsequently, between 12 and 24 mo of age, these previously normally developing children suffered mercury toxic encephalopathies that manifested with clinical symptoms consistent with regressive ASDs. Evidence for mercury intoxication should be considered in the differential diagnosis as contributing to some regressive ASDs.”

A Comprehensive Review of Mercury Provoked Autism.

Geier DA, King PG, Sykes LK, Geier MR. A comprehensive review of mercury provoked autism. *Indian J Med Res* 2008;128:383-411.

"Emerging evidence supports the theory that some autism spectrum disorders (ASDs) may result from a combination of genetic/biochemical susceptibility, specifically a reduced ability to excrete mercury (Hg), and exposure to Hg at critical developmental periods. Elemental/inorganic Hg is released into the air/water where it becomes methylated and accumulates in animal tissues. The US population is primarily exposed to methyl-Hg by fish consumption. In addition, many pharmaceuticals have been, and some continue to be, a ubiquitous source of danger because they contain mercurials. Mercurials may be found in drugs for the eye, ear, nose, throat, and skin; in bleaching creams; as preservatives in cosmetics, tooth pastes, lens solutions, vaccines, allergy test and immunotherapy solutions; in antiseptics, disinfectants, and contraceptives; in fungicides and herbicides; in dental fillings and thermometers; and many other products. Hg has been found to cause immune, sensory, neurological, motor, and behavioural dysfunctions similar to traits defining/associated with ASDs, and that these similarities extend to neuroanatomy, neurotransmitters, and biochemistry. Furthermore, a review of molecular mechanisms indicates that Hg exposure can induce death, disorganization and/or damage to selected neurons in the brain similar to that seen in recent ASD brain pathology studies, and this alteration may likely produce the symptoms by which ASDs are diagnosed. Finally, a review of treatments suggests that ASD patients who undergo protocols to reduce Hg and/or its effects show significant clinical improvements in some

cases. In conclusion, the overwhelming preponderance of the evidence favours acceptance that Hg exposure is capable of causing some ASDs."

Mercury and Autism: Accelerating Evidence?

Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? Neuro Endocrinol Lett 2005;26:439-46.

"The causes of autism and neurodevelopmental disorders are unknown. Genetic and environmental risk factors seem to be involved. Because of an observed increase in autism in the last decades, which parallels cumulative mercury exposure, it was proposed that autism may be in part caused by mercury. We review the evidence for this proposal. Several epidemiological studies failed to find a correlation between mercury exposure through thimerosal, a preservative used in vaccines, and the risk of autism. Recently, it was found that autistic children had a higher mercury exposure during pregnancy due to maternal dental amalgam and thimerosal-containing immunoglobulin shots. It was hypothesized that children with autism have a decreased detoxification capacity due to genetic polymorphism. In vitro, mercury and thimerosal in levels found several days after vaccination inhibit methionine synthetase (MS) by 50%. Normal function of MS is crucial in biochemical steps necessary for brain development, attention and production of glutathione, an important antioxidative and detoxifying agent. Repetitive doses of thimerosal leads to neurobehavioral deteriorations in autoimmune susceptible mice, increased oxidative stress and decreased intracellular levels of glutathione in vitro. Subsequently, autistic children have significantly decreased level of reduced glutathione. Promising treatments of autism involve detoxification of mercury, and supplementation of deficient metabolites."

Cultured Lymphocytes from Autistic Children and Non-Autistic Siblings Up-Regulate Heat Shock Protein.

Walker SJ, Segal J, Aschner M. Cultured lymphocytes from autistic children and non-autistic siblings up-regulate heat shock protein RNA in response to Thimerosal challenge. Neurotoxicology. 2006 Sep;27(5):685-92.

"There are reports suggesting that some autistic children are unable to mount an adequate response following exposure to environmental toxins. This potential deficit, coupled with the similarity in clinical presentations of autism and some heavy metal toxicities, has led to the suggestion that heavy metal poisoning might play a role in the etiology of autism in uniquely susceptible individuals. Thimerosal, an anti-microbial preservative previously added routinely to childhood multi-dose vaccines, is composed of 49.6% ethyl mercury. Based on the levels of this toxin that children receive through routine immunization schedules in the first years of life, it has been postulated that thimerosal may be a potential triggering mechanism contributing to autism in susceptible individuals. One potential risk factor in these individuals may be an inability to adequately up-regulate metallothionein (MT) biosynthesis in response to presentation of a heavy metal challenge. To investigate this hypothesis, cultured lymphocytes (obtained from the Autism Genetic Resource Exchange, AGRE) from autistic children and non-autistic siblings were challenged with either 10 microM ethyl mercury, 150 microM zinc, or fresh media (control). Following the challenge, total RNA was extracted and used to query "whole genome" DNA microarrays. Cultured lymphocytes challenged with zinc responded with an impressive up-regulation of MT transcripts (at least nine different MTs were over-expressed) while cells challenged with thimerosal responded by up-regulating numerous heat shock protein transcripts, but not MTs."

Cellular and Mitochondrial Glutathione Redox Imbalance in Lymphoblastoid Cells Derived from Children with Autism.

James SJ, Rose S, Melnyk S, Jernigan S, Blossom S, Pavliv O, Gaylor DW. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. FASEB J. 2009 Aug;23(8):2374-83.

"Research into the metabolic phenotype of autism has been relatively unexplored despite the fact that metabolic abnormalities have been implicated in the pathophysiology of several other neurobehavioral disorders. Plasma biomarkers of oxidative stress have been reported in autistic children; however, intracellular redox status has not yet been evaluated. Lymphoblastoid cells (LCLs) derived from autistic children and unaffected controls were used to assess relative concentrations of reduced glutathione (GSH) and oxidized disulfide glutathione (GSSG) in cell extracts and isolated mitochondria as a measure of intracellular redox capacity. The results indicated that the GSH/GSSG redox ratio was decreased and percentage oxidized glutathione increased in both cytosol and mitochondria in the autism LCLs. Exposure to oxidative stress *via* the sulfhydryl reagent thimerosal resulted in a greater decrease in the GSH/GSSG ratio and increase in free radical generation in autism compared to control cells...Thimerosal was a commonly used antimicrobial preservative in vaccines and pharmaceuticals for many decades. Questions about the cumulative dose toxicity with multiple infant vaccines in the 1990s resulted in an FDA mandate for its removal in 2001 from all infant vaccines except for the influenza vaccine. Although experimental evidence for the neurotoxicity and immunotoxicity of thimerosal is unequivocal (47, 66, 67), the potential contribution of thimerosal to the increased prevalence of autism in the 1990s is a complex issue, and quantitative ascertainment of incidence from retrospective studies remains controversial. Although cell models provide insights into mechanism, the extrapolation of thimerosal dose/response characteristics from artificial, albeit controlled, cell culture conditions to the complexities of the *in vivo* cell milieu is tenuous at best. Nonetheless, based on previous experimental evidence and the results reported here, it is plausible to hypothesize that exposures to prooxidant environmental toxins, including thimerosal, would have the greatest effect on individuals with a preexisting fragile redox homeostasis or depleted glutathione reserves due to concurrent infection, or who are simultaneously exposed to other prooxidant contaminants that in combination can reach a toxic threshold (68). These potentially vulnerable subpopulations need to be identified and evaluated independently because large population epidemiologic studies do not have the sensitivity to detect minor high-risk subpopulations."

A Case Series of Children with Apparent Mercury Toxic Encephalopathies Manifesting with Clinical Symptoms of Regressive Autistic Disorders.

Geier DA, Geier MR. A Case series of children with apparent mercury toxic encephalopathies manifesting with clinical symptoms of regressive autistic disorders. J Toxicol Environ Health A. 2007 May 15;70(10):837-51.

"Impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movement patterns characterize autism spectrum disorders (ASDs). It is clear that while genetic factors are important to the pathogenesis of ASDs, mercury exposure can induce immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with ASDs. The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, U.S. Department of Health and Human Services, IRB number IRB00005375) approved the present study. A case series of nine patients who presented to the Genetic Centers of America for a genetic/developmental evaluation are discussed. Eight of nine patients (one patient was found to have an ASD due to Rett's syndrome) (a) had regressive ASDs; (b) had elevated levels of androgens; (c) excreted significant amounts of mercury post chelation challenge; (d) had biochemical evidence of decreased function in their glutathione pathways; (e) had no known significant mercury exposure except from Thimerosal-containing vaccines/Rho(D)-immune globulin preparations; and (f) had alternate causes for their regressive ASDs ruled out. There was a significant dose-response relationship between the severity of the regressive ASDs observed and the total mercury dose children received from Thimerosal-containing vaccines/Rho (D)-immune globulin preparations. Based upon differential diagnoses, 8 of 9 patients examined were exposed to significant mercury from Thimerosal-containing biologic/vaccine preparations during their fetal/infant developmental periods, and subsequently, between 12 and 24 mo of age, these previously normally developing children suffered mercury toxic encephalopathies that manifested with clinical symptoms consistent with regressive ASDs. Evidence for mercury intoxication should be considered in the differential diagnosis as contributing to some regressive ASDs."

Induction of Metallothionein in Mouse Cerebellum and Cerebrum with Low-Dose Thimerosal Injection.

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. 2009 Apr 9. [Epub ahead of print]

"Thimerosal, an ethyl mercury compound, is used worldwide as a vaccine preservative. We previously observed that the mercury concentration in mouse brains did not increase with the clinical dose of thimerosal injection, but the concentration increased in the brain after the injection of thimerosal with lipopolysaccharide, even if a low dose of thimerosal was administered. Thimerosal may penetrate the brain, but is undetectable when a clinical dose of thimerosal is injected; therefore, the induction of metallothionein (MT) messenger RNA (mRNA) and protein was observed in the cerebellum and cerebrum of mice after thimerosal injection, as MT is an inducible protein. MT-1 mRNA was expressed at 6 and 9 h in both the cerebrum and cerebellum, but MT-1 mRNA expression in the cerebellum was three times higher than that in the cerebrum after the injection of 12 microg/kg thimerosal. MT-2 mRNA was not expressed until 24 h in both organs. MT-3 mRNA was expressed in the cerebellum from 6 to 15 h after the injection, but not in the cerebrum until 24 h. MT-1 and MT-3 mRNAs were expressed in the cerebellum in a dose-dependent manner. Furthermore, MT-1 protein was detected from 6 to 72 h in the cerebellum after 12 microg/kg of thimerosal was injected and peaked at 10 h. MT-2 was detected in the cerebellum only at 10 h. In the cerebrum, little MT-1 protein was detected at 10 and 24 h, and there were no peaks of MT-2 protein in the cerebrum. In conclusion, MT-1 and MT-3 mRNAs but not MT-2 mRNA are easily expressed in the cerebellum rather than in the cerebrum by the injection of low-dose thimerosal. It is thought that the cerebellum is a sensitive organ against thimerosal. As a result of the present findings, in combination with the brain pathology observed in patients diagnosed with autism, the present study helps to support the possible biological plausibility for how low-dose exposure to mercury from thimerosal-containing vaccines may be associated with autism."

An Epidemiological Analysis of the 'Autism as Mercury Poisoning' Hypothesis.

Austin D. An epidemiological analysis of the 'autism as mercury poisoning' hypothesis. International Journal of Risk and Safety in Medicine 2008;20:135-142.

"Where direct experimental research into a causal hypothesis of a disease is impossible due to ethical and practical considerations, epidemiological inference is the accepted route to establishing cause. Therefore, to examine the *autism as mercury poisoning* hypothesis, this paper reviews the existing scientific literature within the context of established epidemiological criteria and finds that the evidence for a causal relationship is compelling. Exposure to mercury (via vaccines and maternal dental amalgam) *in utero* and during infant years is confirmed; mercury poisoning is known to cause symptoms consistent with autism; animal modeling supports the link and, critically, mercury levels are higher in both the urine and blood of autistic children than in non-autistic peers. Analogous to epidemiological evidence of the smoking–lung cancer relationship, a mercury–autism relationship is confirmed. The *precautionary principle* demands that health professionals not take an action if there is suspicion that the action may cause severe or lifelong health effects: it does not require certainty. Therefore, given the severity, devastating lifelong impact and extremely high prevalence of autism, it would be negligent to continue to expose pregnant and nursing mothers and infant children to any amount of avoidable mercury."

A Comparative Evaluation of the Effects of MMR Immunization and Thimerosal on the Population Prevalence of Autism.

Mark R. Geier, David A. Geier, Med Sci Monit, 2004; 10(3): PI33-39

“Background: The purpose of the study was to evaluate the effects of MMR immunization and mercury from thimerosal-containing childhood vaccines on the prevalence of autism.

Material/Methods: Evaluations of the Biological Surveillance Summaries of the Centers for Disease Control and Prevention (CDC), the U.S. Department of Education datasets, and the CDC’s yearly live birth estimates were undertaken.

Results: It was determined that there was a close correlation between mercury doses from thimerosal-containing childhood vaccines and the prevalence of autism from the late 1980s through the mid-1990s. In contrast, there was a potential correlation between the number of primary pediatric measles-containing vaccines administered and the prevalence of autism during the 1980s. In addition, it was found that there were statistically significant odds ratios for the

development of autism following increasing doses of mercury from thimerosal-containing vaccines (birth cohorts: 1985 and 1990–1995) in comparison to a baseline measurement (birth cohort: 1984). The contribution of thimerosal from childhood vaccines (>50% effect) was greater than MMR vaccine on the prevalence of autism observed in this study.

Conclusions: The results of this study agree with a number of previously published studies. These studies have shown that there is biological plausibility and epidemiological evidence showing a direct relationship between increasing doses of mercury from thimerosal-containing vaccines and neurodevelopmental disorders, and measles-containing vaccines and serious neurological disorders. It is recommended that thimerosal be removed from all vaccines, and additional research be undertaken to produce a MMR vaccine with an improved safety profile.”

A Prospective Study of Thimerosal-Containing Rho(D)-Immune Globulin Administration as a Risk Factor for Autistic Disorders.

Mark R. Geier, David A. Geier, The Journal of Maternal-Fetal and Neonatal Medicine, May 2007; 20(5): 385–390.

“Background. This study evaluated the relationship between prenatal mercury exposure from thimerosal (49.55% mercury by weight)-containing Rho(D)-immune globulins (TCRs) and autism spectrum disorders (ASDs). Methods. The Institutional Review Board of the Institute for Chronic Illnesses approved the present study. A total of 53 consecutive non-Jewish Caucasian patients with ASDs (Diagnostic and statistical manual of mental disorders, fourth ed. – DSM

IV) born between 1987 and 2001 who presented to the Genetic Centers of America for outpatient genetic/developmental evaluations were prospectively collected from June 1, 2005 through March 31, 2006. Imaging and laboratory testing were conducted on each patient to rule out other causal factors for their ASDs. As race-matched

controls, the frequency of Rh negativity was determined from 926 non-Jewish Caucasian pregnant women who had presented for outpatient prenatal genetics care to the Genetic Centers of America between 1980 and 1989.

Results. Children with ASDs (28.30%) were significantly more likely (odds ratio 2.35, 95% confidence interval 1.17–4.52, p=0.01) to have Rh-negative mothers than controls (14.36%). Each ASD patient's mother was determined to have been administered a TCR during her pregnancy.

Conclusion. The results provide insights into the potential role prenatal mercury exposure may play in some children with ASDs."

Kawasaki's Disease, Acrodynia, and Mercury.

Mutter J, Yeter D. Kawasaki's disease, acrodynia, and mercury. *Curr Med Chem* 2008;15:3000-10.

"A superantigen or autoimmunity has been hypothesized to be the main cause of the Kawasaki's Disease but the etiology is unknown. Medical literature, epidemiological findings, and some case reports have suggested that mercury may play a pathogenic role. Several patients with Kawasaki's Disease have presented with elevated urine mercury levels compared to matched controls. Most symptoms and diagnostic criteria which are seen in children with acrodynia, known to be caused by mercury, are similar to those seen in Kawasaki's Disease. Genetic depletion of glutathione S-transferase, a susceptibility marker for Kawasaki's Disease, is known to be also a risk factor for acrodynia and may also increase susceptibility to mercury. Coinciding with the largest increase (1985-1990) of thimerosal (49.6% ethyl mercury) in vaccines, routinely given to infants in the U.S. by 6 months of age (from 75microg to 187.5microg), the rates of Kawasaki's Disease increased ten times, and, later (1985-1997), by 20 times. Since 1990 88 cases of patients developing Kawasaki's Disease some days after vaccination have been reported to the Centers of Disease Control (CDC) including 19% manifesting symptoms the same day. The presented pathogenetic model may lead to new preventive- and therapeutic strategies for Kawasaki's disease."

Neurotoxic Effects of Postnatal Thimerosal are Mouse Strain Dependent.

Hornig M, Chian D, Lipkin WI. Neurotoxic effects of postnatal thimerosal are mouse strain dependent. *Mol Psychiatry*. 2004 Sep;9(9):833-45.

"The developing brain is uniquely susceptible to the neurotoxic hazard posed by mercurials. Host differences in maturation, metabolism, nutrition, sex, and autoimmunity influence outcomes. How population-based variability affects the safety of the ethylmercury-containing vaccine preservative, thimerosal, is unknown. Reported increases in the prevalence of autism, a highly heritable neuropsychiatric condition, are intensifying public focus on environmental exposures such as thimerosal. Immune profiles and family history in autism are frequently consistent with autoimmunity. We hypothesized that autoimmune propensity influences outcomes in mice following thimerosal challenges that mimic routine childhood immunizations. Autoimmune disease-sensitive SJL/J mice showed growth delay; reduced locomotion; exaggerated response to novelty; and densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Strains resistant to autoimmunity, C57BL/6J and BALB/cJ, were not susceptible. These findings implicate genetic influences and provide a model for investigating thimerosal-related neurotoxicity."

Time-Dependent Distribution of Hg-Mercury Compounds in Rat and Monkey as studied by Whole Body Autoradiography.

Takahashi, T, Kimura, T, Sato, Y, Shiraki, H, Ukita, T. Time-Dependent Distribution of Hg-Mercury Compounds in Rat and Monkey as studied by Whole Body Autoradiography. The Journal of Hygienic Chemistry. 1971; 17(2): 93-107.

“The time-dependent autoradiograms of rat that received Hg-ethylmercuric chloride indicated a longer lasting accumulation of radioactivity in almost all the organs than that in mouse. No such difference in the accumulation of radioactivity was observed between the two animals by the administration of Hg-mercuric chloride.

The whole body autoradiograms were prepared from rats and monkeys at different time intervals after both intravenous and intraperitoneal administrations of Hg-mercuric chloride or Hg-ethyl-mercuric chloride. The distribution pattern in the whole body differed greatly between the two compounds. The mode of migration and accumulation of the radioactivity into the central nervous system was more precisely observed in monkeys than in rats which received Hg-ethylmercuric chloride.

A pronounced migration of the radioactivity into the cortices of cerebrum and cerebellum especially into the occipital lobe, was observed in a monkey 8 days after receiving Hg-ethylmercuric chloride. The mercury compound that accumulated in the brain was extracted as dithizonate and identified as ethylmercuric dithizonate. The time-dependent migration of mercury into the brain was much larger in monkey than in rat.

Autoradiogram of the central nervous system of monkey suggested that ethylmercury residue possibly migrated into the central nervous system through blood-brain barrier, since the radioactivity first appeared in the choroid plexi, intracranial and extracerebral arteries, and then distributed into the cerebral and cerebella cortices with lapse of time, and there was no remarkable radioactivity in the areas adjacent to the lateral ventricles in all of the autoradiograms prepared from the monkeys 60 min, 20 hr, and 8 days after the administration of Hg-ethylmercuric chloride.”

Neurotoxic Effects of Thimerosal at Vaccines Doses on the Encephalon and Development in 7 Days-Old Hamsters.

Laurente J, Remuzgo F, Avalos B, Chiquinta J, Ponce B, Avendano R, Maya L. Neurotoxic effects of Thimerosal at vaccines doses on the encephalon and development in 7 days-old hamsters. An Fac Med (Lima) 2006;68:222-37.

Objectives: To determine if thimerosal administration in amounts equivalent to vaccines content produces neurotoxic effects on the encephalon in postnatal hamsters and on experimentation animal's development. **Design:** Experimental, prospective, bioetapic study. **Setting:** San Fernando Faculty of Medicine, Universidad Nacional Mayor de San Marcos. **Biological material:** Seven daysold hamsters. **Material:** We divided 45 postnatal hamsters in three groups: group A (n=15), group B (n=15) and group C (n=15). We administered three intramuscular equivalent doses of sucrose and thimerosal in 20 µL of saline to groups B and C, respectively, on birth-days 7 (0,227 µg), 9 (0,216 µg) and 11 (0,220 µg). Group A received only 20 µL of saline solution. **Main outcome measures:** Body weight, encephalon weight, height (skull-caudal length), and encephalon histopathological alterations. **Results:** Anova and student t tests showed statistical significance in favor of low body weight, low encephalon weight, and smaller stature in group C with respect to groups A and B hamsters ($p < 0,0001$). X2 statistical significance in relation to the presence of hystopathological alterations in group C was also obtained ($p < 0,0001$). We observed greater relative risk of encephalic alterations in group C. **Conclusions:** The administration of thimerosal in equivalent doses to vaccines content was associated with low corporal weight, low encephalon weight, and smaller stature in postnatal hamsters. Neurotoxic effects were also produced at encephalic level: at hippocampus (*regions CA1, CA3 and DG*), cerebral cortex, and cerebellum

(Purkinje cells and granule cells); with decrease in neuronal density, neuronal necrosis, axonal demyelination, and gliosis. In addition, risk increase in developing any of these alterations was high just in the animal group receiving thimerosal."

Thimerosal Neurotoxicity is Associated with Glutathione Depletion: Protection with Glutathione Precursors.

S.J. James¹, William Slikker III, Stepan Melnyk, Elizabeth New, Marta Pogribna, Stefanie Jernigan. Neurotoxicology. Department of Pediatrics, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, Little Rock, AR 72202, USA

"Thimerosal is an antiseptic containing 49.5% ethyl mercury that has been used for years as a preservative in many infant vaccines and in flu vaccines. Environmental methyl mercury has been shown to be highly neurotoxic, especially to the developing brain. Because mercury has a high affinity for thiol (sulfhydryl (-SH)) groups, the thiol-containing antioxidant, glutathione (GSH), provides the major intracellular defense against mercury-induced neurotoxicity. Cultured neuroblastoma cells were found to have lower levels of GSH and increased sensitivity to thimerosal toxicity compared to glioblastoma cells that have higher basal levels of intracellular GSH. Thimerosal-induced cytotoxicity was associated with depletion of intracellular GSH in both cell lines. Pretreatment with 100 mM glutathione ethyl ester or N-acetylcysteine (NAC), but not methionine, resulted in a significant increase in intracellular GSH in both cell types. Further, pretreatment of the cells with glutathione ethyl ester or NAC prevented cytotoxicity with exposure to 15 mM Thimerosal. Although Thimerosal has been recently removed from most children's vaccines, it is still present in flu vaccines given to pregnant women, the elderly, and to children in developing countries. The potential protective effect of GSH or NAC against mercury toxicity warrants further research as possible adjunct therapy to individuals still receiving Thimerosal-containing vaccinations."

Thimerosal Induces Neuronal Cell Apoptosis by Causing Cytochrome C and Apoptosis-Inducing Factor Release from Mitochondria.

Leman Yel, Lorrel E. Brown, Kevin Su, Sastry Gollapudi and Sudhir Gupta. Cellular and Molecular Immunology Laboratories, Division of Basic and Clinical Immunology, Department of Medicine, University of California, Irvine, CA 92697, USA

"There is a worldwide increasing concern over the neurological risks of thimerosal (ethylmercury thiosalicylate) which is an organic mercury compound that is commonly used as an antimicrobial preservative. In this study, we show that thimerosal, at nanomolar concentrations, induces neuronal cell death through the mitochondrial pathway. Thimerosal, in a concentration- and time-dependent manner, decreased cell viability as assessed by calcein-ethidium staining and caused apoptosis detected by Hoechst 33258 dye. Thimerosal-induced apoptosis was associated with depolarization of mitochondrial membrane, generation of reactive oxygen species, and release of cytochrome c and apoptosis-inducing factor (AIF) from mitochondria to cytosol. Although thimerosal did not affect cellular expression of Bax at the protein level, we observed translocation of Bax from cytosol to mitochondria. Finally, caspase-9 and caspase-3 were activated in the absence of caspase-8 activation. Our data suggest that thimerosal causes apoptosis in neuroblastoma cells by changing the mitochondrial microenvironment."

Thimerosal Induces DNA Breaks, Caspase-3 Activation, Membrane Damage, and Cell Death in Cultured Human Neurons and Fibroblasts

David S. Baskin, Hop Ngo, and Vladimir V. Didenko. Toxicological Sciences. 74, 361–368 (2003).

“Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. Little is known about the reactions of human neuronal and skin cells to its micro- and nanomolar concentrations, which can occur after using thimerosal-containing products. A useful combination of fluorescent techniques for the assessment of thimerosal toxicity is introduced.

Short-term thimerosal toxicity was investigated in cultured human cerebral cortical neurons and in normal human fibroblasts. Cells were incubated with 125-nM to 250- M concentrations of thimerosal for 45 min to 24 h. A 4 , 6-diamidino-2-phenylindole dihydrochloride (DAPI) dye exclusion test was used to identify non-viable cells and terminal transferase-based nick-end labeling (TUNEL) to label DNA damage. Detection of active caspase-3 was performed in live cell cultures using a cell-permeable fluorescent caspase inhibitor. The morphology of fluorescently labeled nuclei was analyzed. After 6 h of incubation, the thimerosal toxicity was observed at 2 M based on the manual detection of the fluorescent attached cells and at a 1- M level with the more sensitive GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence. The lower limit did not change after 24 h of incubation. Cortical neurons demonstrated higher sensitivity to thimerosal compared to fibroblasts. The first sign of toxicity was an increase in membrane permeability to DAPI after 2 h of incubation with 250 M thimerosal. A 6-h incubation resulted in failure to exclude DAPI, generation of DNA breaks, caspase-3 activation, and development of morphological signs of apoptosis. We demonstrate that thimerosal in micromolar concentrations rapidly induce membrane and DNA damage and initiate caspase-3–dependent apoptosis in human neurons and fibroblasts. We conclude that a proposed combination of fluorescent techniques can be useful in analyzing the toxicity of thimerosal.”

Thimerosal Induces Apoptosis in a Neuroblastoma Model via the cJun N-Terminal Kinase Pathway

Michelle L. Herdman, Aileen Marcelo, Ying Huang, Richard M. Niles, Sanjit Dhar, and Kinsley Kelley Kinningham. TOXICOLOGICAL SCIENCES 92(1), 246–253 (2006).

“The cJun N-terminal kinase (JNK)-signaling pathway is activated in response to a variety of stimuli, including environmental insults, and has been implicated in neuronal apoptosis. In this study, we investigated the role that the JNK pathway plays in neurotoxicity caused by thimerosal, an ethylmercury-containing preservative. SK-N-SH cells treated with thimerosal (0–10mM) showed an increase in the phosphorylated (active) form of JNK and cJun with 5 and 10mM thimerosal treatment at 2 and 4 h. To examine activator protein-1 (AP-1) transcription, cells were transfected with a pGL2 vector containing four AP-1 consensus sequences and then treated with thimerosal (0–2.5mM) for 24 h. Luciferase studies showed an increase in AP-1 transcriptional activity upon thimerosal administration. To determine the components of the AP-1 complex, cells were transfected with a dominant negative to either cFos (A-Fos) or cJun (TAM67). Reporter analysis showed that TAM67, but not A-Fos, decreased AP-1 transcriptional activity, indicating a role for cJun in this pathway. To assess which components are essential to apoptosis, cells were treated with a cell-permeable JNK inhibitor II (SP600125) or transfected with TAM67, and the downstream effectors of apoptosis were analyzed. Cells pretreated with SP600125 showed decreases in activation of caspases 9 and 3, decreases in degradation of poly(ADP-ribose) polymerase (PARP), and decreased levels of proapoptotic Bim, in comparison to cells treated with thimerosal alone. However, cells transfected with TAM67 showed no changes in those same components. Taken together, these results indicate that thimerosal-induced neurotoxicity occurs through the JNK-signaling pathway, independent of cJun activation, leading ultimately to apoptotic cell death.”

Alterations of Spontaneous Systemic Autoimmunity in Mice by Treatment with Thimerosal (Ethyl Mercury)

Havarinasab S, Hultman P. Alteration of the spontaneous systemic autoimmune disease in (NZB x NZW)F1 mice by treatment with thimerosal (ethyl mercury). Toxicol Appl Pharmacol. 2006 Jul 1;214(1):43-54.

"Inorganic mercury may aggravate murine systemic autoimmune diseases which are either spontaneous (genetically determined) or induced by non-genetic mechanisms. Organic mercury species, the dominating form

of mercury exposure in the human population, have not been examined in this respect. Therefore, ethyl mercury in the form of thimerosal, a preservative recently debated as a possible health hazard when present in vaccines, was administered in a dose of 0.156-5 mg/L drinking water to female (NZB x NZW)F1 (ZBWF1) mice. These mice develop an age-dependent spontaneous systemic autoimmune disease with high mortality primarily due to immune-complex (IC) glomerulonephritis. Five mg thimerosal/L drinking water (295 microg Hg/kg body weight (bw)/day) for 7 weeks induced glomerular, mesangial and systemic vessel wall IC deposits and antinuclear antibodies (ANA) which were not present in the untreated controls. After 22-25 weeks, the higher doses of thimerosal had shifted the localization of the spontaneously developing renal glomerular IC deposits from the capillary wall position seen in controls to the mesangium. The altered localization was associated with less severe histological kidney damage, less proteinuria, and reduced mortality. The effect was dose-dependent, lower doses having no effect compared with the untreated controls. A different effect of thimerosal treatment was induction of renal and splenic vessel walls IC deposits. Renal vessel wall deposits occurred at a dose of 0.313-5 mg thimerosal/L (18-295 microg Hg/kg bw/day), while splenic vessel wall deposits developed also in mice given the lowest dose of thimerosal, 0.156 mg/L (9 microg Hg/kg bw/day). The latter dose is 3- and 15-fold lower than the dose of Hg required to induce vessel wall IC deposits in genetically susceptible H-2s mice by HgCl₂ and thimerosal, respectively. Further studies on the exact conditions needed for induction of systemic IC deposits by low-dose organic mercurials in autoimmune-prone individuals, as well as the potential effect of these deposits on the vessel walls, are warranted...The main recent cause for thimerosal exposure in the human population is however as a preservative in vaccines. The maximum cumulative dose of mercury from thimerosal in vaccines before 1999 in the U.S. was estimated to 200 and 275 µg in a 6-month- and 2-year-old child (Stratton et al., 2001). By using a cautious way of calculation, using an averaging period of only 1 day, a maximum single-day exposure of 10–15 µg Hg/ kg bw is obtained (Stratton et al., 2001). This is clearly within the dose range observed for the accelerating effect on development of systemic vessel wall IC deposits observed in the present study using the autoimmune-prone ZBWF1 hybrid mice. However, such an accelerating effect requires an autoimmune-prone genotype and a protracted exposure time. Anyway, the safety factor of 10 for comparing animal and human exposure (Barnes and Dourson, 1988; Clarkson, 1992) is clearly not attained."

Effects of Thimerosal on NGF Signal Transduction and Cell Death in Neuroblastoma Cells.

Damani K. Parran, Angela Barker, and Marion Ehrich. TOXICOLOGICAL SCIENCES 86(1), 132–140 (2005)

“Signaling through neurotrophic receptors is necessary for differentiation and survival of the developing nervous system. The present study examined the effects of the organic mercury compound thimerosal on nerve growth factor signal transduction and cell death in a human neuroblastoma cell line (SH-SY5Y cells). Following exposure to 100 ng/ml NGF and increasing concentrations of thimerosal (1 nM–10 mM), we measured the activation of TrkA, MAPK, and PKC-d. In controls, the activation of TrkA MAPK and PKC-d peaked after 5 min of exposure to NGF and then decreased but was still detectable at 60 min. Concurrent exposure to increasing concentrations of thimerosal and NGF for 5 min resulted in a concentration-dependent decrease in TrkA and MAPK phosphorylation, which was evident at 50 nM for TrkA and 100 nM for MAPK. Cell viability was assessed by the LDH assay. Following 24-h exposure to increasing concentrations of thimerosal, the EC₅₀ for cell death in the presence or absence of NGF was 596 nM and 38.7 nM, respectively. Following 48-h exposure to increasing concentrations of thimerosal, the EC₅₀ for cell death in the presence and absence of NGF was 105 nM and 4.35 nM, respectively. This suggests that NGF provides protection against thimerosal cytotoxicity. To determine if apoptotic versus necrotic cell death was occurring, oligonucleosomal fragmented DNA was quantified by ELISA. Control levels of fragmented DNA were similar in both the presence and absence of NGF. With and without NGF, thimerosal caused elevated levels of fragmented DNA appearing at 0.01 mM (apoptosis) to decrease at concentrations >1 mM (necrosis). These data demonstrate that thimerosal could alter NGF-induced signaling in neurotrophin-treated cells at concentrations lower than those responsible for cell death.”

Mitochondrial Mediated Thimerosal-Induced Apoptosis in a Human Neuroblastoma Cell Line (SK-N-SH).

Michelle L. Humphrey, Marsha P. Cole, James C. Pendergrass, Kinsley K. Kinningham. NeuroToxicology.

“Signaling through neurotrophic receptors is necessary for differentiation and survival of the developing nervous system. The present study examined the effects of the organic mercury compound thimerosal on nerve growth factor signal transduction and cell death in a human neuroblastoma cell line (SH-SY5Y cells). Following exposure to 100 ng/ml NGF and increasing concentrations of thimerosal (1 nM–10 mM), we measured the activation of TrkA, MAPK, and PKC-d. In controls, the activation of TrkA MAPK and PKC-d peaked after 5 min of exposure to NGF and then decreased but was still detectable at 60 min. Concurrent exposure to increasing concentrations of thimerosal and NGF for 5 min resulted in a concentration-dependent decrease in TrkA and MAPK phosphorylation, which was evident at 50 nM for TrkA and 100 nM for MAPK. Cell viability was assessed by the LDH assay. Following 24-h exposure to increasing concentrations of thimerosal, the EC50 for cell death in the presence or absence of NGF was 596 nM and 38.7 nM, respectively. Following 48-h exposure to increasing concentrations of thimerosal, the EC50 for cell death in the presence and absence of NGF was 105 nM and 4.35 nM, respectively. This suggests that NGF provides protection against thimerosal cytotoxicity. To determine if apoptotic versus necrotic cell death was occurring, oligonucleosomal fragmented DNA was quantified by ELISA. Control levels of fragmented DNA were similar in both the presence and absence of NGF. With and without NGF, thimerosal caused elevated levels of fragmented DNA appearing at 0.01 mM (apoptosis) to decrease at concentrations >1 mM (necrosis). These data demonstrate that thimerosal could alter NGF-induced signaling in neurotrophin-treated cells at concentrations lower than those responsible for cell death.”

Activation of Methionine Synthase by Insulin-Like Growth Factor-1 and Dopamine: A Target for Neurodevelopmental Toxins and Thimerosal.

M Waly, H Olteanu, R Banerjee, S-W Choi, JB Mason, BS Parker, S Sukumar, S Shim, A Sharma, JM Benzecry, V-A Power-Charnitsky and RC Deth. Molecular Psychiatry (2004), 1–13.

“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: Cu²⁺ promoted enzyme activity and methylation, while Cu⁺, Pb²⁺, Hg²⁺ and Al³⁺ were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC50 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.”

Thimerosal Induces Programmed Cell Death of Neuronal Cells Via Changes in the Mitochondrial Environment.

Brown, L. The UCI Undergraduate Research Journal. 2003.

“Thimerosal, a preservative and anti-microbial agent used in vaccines, ophthalmic solutions, and cosmetics, is a mercury-containing compound that has raised public concern due to its potentially harmful effects. While past studies have implicated mercurial compounds in apoptosis, or programmed cell death, in human T-cells and cells of the central nervous system, no studies have examined the specific effect of thimerosal on neuronal cells, despite evidence that mercurial compounds readily cross the blood-brain barrier. This study examines whether thimerosal induces apoptosis in neuronal cells, and, if so, via which mechanism. To this end, neuronal cells were incubated in the absence and presence of thimerosal at various concentrations for various exposure times and then examined for cell viability, specific morphological changes associated with apoptosis, and changes in the mitochondrial environment. Thimerosal decreased neuronal cell viability in time- and dose-dependent trials, with 90% viability at 2 hr, decreasing to 60% viability at 24 hr (1 μ M); at 5 μ M thimerosal, viability decreased below 20% at 24 and 48 hr. Thimerosal caused depolarization of the mitochondrial membrane and enhanced superoxide generation. At 5 μ M thimerosal, cytochrome c was released from mitochondria to the cytosol in 30% of cells at 1 hr and 85% of cells at 3 hr. Apoptosis-Inducing Factor was released in 40% and 90% of cells at 30 min and 1 hr, respectively. The results suggest that thimerosal causes apoptosis via the mitochondrial pathway and warrant continued efforts to find a replacement compound.”

Neonatal Administration of a Vaccine Preservative, Thimerosal, Produces Lasting Impairment of Nociception and Apparent Activation of Opioid System in Rats.

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 Sep 9. [Epub ahead of print]

"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 mug Hg/kg and for Lewis: 54, 216, 540 and 1080 mug 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."

Essential Neuropathy of Alkylmercury Intoxications In Humans from the Acute to the Chronic Stage With Special Reference to Experimental Whole Body Autoradiographic Study Using Labeled Mercury Compounds.

Shiraki, H, Nagashima, K. Essential Neuropathy of Alkylmercury Intoxications In Humans from the Acute to the Chronic Stage With Special Reference to Experimental Whole Body Autoradiographic Study Using Labeled Mercury Compounds. Neurotoxicology. 1977.

“The scientists of the world are more or less acquainted with the epidemioclinical features of Minamata disease. In addition, several important papers on the neuropathology of Minamata disease have already been published by certain Japanese scientists. The present chapter deals particularly with the essential neuropathology not only of Minamata disease but also of other alkylmercury intoxications in humans from the most acute to the most chronic stage through experimental results of time-dependent whole body autoradiography in different animals using labeled alkylmercury and/or inorganic mercury compounds.”

OSC Forwards Public Health Concerns on Vaccines to Congress.

Scott J. Bloch, U.S. Office of Special Counsel. May 20, 2004

"The Office of Special Counsel (OSC) today forwarded to Congress hundreds of disclosures alleging public health and safety concerns about childhood vaccines that include a mercury-based preservative known as thimerosal, and its possible link to neurological disorders, including autism. Notwithstanding a new Institute of Medicine study released yesterday that concludes there is no link between thimerosal and autism, the OSC sent copies of the letters to both Senator Judd Gregg and Rep. Joe Barton, to ensure that the proper Congressional oversight committees are aware of these serious allegations."

Mercury in Medicine - Taking Unnecessary Risks.

Congressional Record, May 20, 2003, Staff Report of the Subcommittee on Human Rights and Wellness, Government Reform Committee, the United States House of Representatives, "Mercury in Medicine - Taking Unnecessary Risks."

Report prepared by the staff of the Subcommittee on Human Rights and Wellness, Committee on Government Reform. This report is the result of a three-year investigation initiated in the Committee on Government Reform.

“Vaccines are the only medicines that American citizens are mandated to receive as a condition for school and day care attendance, and in some instances, employment. Additionally, families who receive federal assistance are also required to show proof that their children have been fully immunized. While the mandate for which vaccines must be administered is a state mandate, it is the Federal Government, through the Centers for Disease Control and Prevention (CDC) and its Advisory Committee for Immunization Practices that make the Universal Immunization Recommendations to which the majority of states defer when determining mandates. Since the early to mid-1990s, Congress has been concerned about the danger posed by mercury in medical applications, and in 1997, directed the Food and Drug Administration (FDA) to evaluate the human exposure to mercury through foods and drugs.”

Compensation for Vaccine-Related Injuries: A Technical Memorandum.

***Compensation for vaccine-related injuries: a technical memorandum.* Congress of the United States, Office of Technology Assessment. 1980.**

“In September 1979, the Office of Technology Assessment published a report A Review of Selected Federal Vaccine and Immunization Policies. That report included a chapter that reviewed issues related to legal liability and compensation for vaccine-related injuries. The report noted that all vaccines, even when properly manufactured and administered, may pose risks to users. Under the existing legal liability system, persons injured as a result of vaccination must go to court and establish fault for their injury in order to receive compensation. To establish fault, the plaintiff (injured person) generally sues one or more of the participants in the vaccination process (e.g., administers the vaccine). The report noted that in three major cases in the past 11 years, plaintiffs have won large judgments against vaccine manufacturers for injuries caused by nondefective and properly administered vaccines. The resulting uncertainty for manufacturers has affected their willingness to produce and supply vaccines.

Because of these problems, OTA suggested that it might be desirable to establish a federally operated program to compensate vaccinees injured as a result of being vaccinated in public immunization programs.

Early in 1980, the House Interstate and Foreign Commerce Committee asked OTA to delineate the specific elements and principles necessary for inclusion in a legislative proposal to implement this option. This memorandum does not analyze the positives and negatives of establishing such a program. It begins with the assumption that establishing a compensation program is desirable, and then discusses the questions that Congress must answer in developing such a program.”

Influenza Vaccine: Review of Effectiveness of the U.S. Immunization Program, and Policy Considerations.

Geier, DA, King, PG, Geier, MR. Influenza Vaccine: Review of Effectiveness of the U.S. Immunization Program, and Policy Considerations. Journal of American Physicians and Surgeons. Fall 2006; 11(3).

“A number of studies have reported that influenza vaccine (IV) administration has been less than optimally effective in certain subpopulations. This study examines yearly influenza death rate, yearly influenza case rate, and yearly rate of hospitalizations with influenza as the first-listed discharge diagnosis. By these measures, the yearly U.S. mass influenza vaccination campaign has been ineffective in preventing influenza in vaccine recipients. The use of antiviral drugs to treat influenza, in light of the potential for an influenza pandemic, needs further consideration.”

Incidence of Influenza in Ontario following the Universal Influenza Immunization Campaign.

Groll, DL, Thomson, DJ. Incidence of Influenza in Ontario following the Universal Influenza Immunization Campaign. Vaccine 24 (2006) 5245-5250.

“The purpose of this study was to determine whether the incidence of influenza in Ontario, Canada has decreased following the introduction of the Universal Influenza Immunization Campaign (UIIC) in 2000. All laboratory-confirmed influenza cases in Ontario, from January 1990 to August 2005 were analyzed using multitaper time series analysis. We found that there has not been a decrease in the mean monthly influenza rate following the introduction of the UIIC (109.5 (S.D. 20) versus 160 (S.D> 50.3) $p>0.1$). Despite increased vaccine distribution and financial resources towards promotion, the incidence of influenza in Ontario has not decreased following the introduction of the UIIC.”

Assessment of the Efficacy and Effectiveness of Influenza Vaccines in Healthy Children: Systematic Review.

Jefferson, T, Smith, S, Demicheli, v, Harnden, A, Rivetti, A, Di Pietrantonj, D. Assessment of the Efficacy and Effectiveness of Influenza Vaccines in Healthy Children: Systematic Review. Lancet. 2005; 365.

“Background: We aimed to assess evidence of efficacy and effectiveness of live attenuated and inactivated influenza vaccines in children up to 16 years of age.

Methods: We searched the Cochrane Library, MEDLINE, EMBASE Biological Abstracts, and Science Citation Index to June, 2004, in any language, and contacted vaccine manufacturers and authors of relevant studies to identify additional data. We included randomised, cohort, and case-control studies comparing efficacy of vaccines against influenza (reduction in laboratory-confirmed cases), effectiveness of vaccines against influenza-like illness (reduction in symptomatic cases), or both, with placebo or no intervention. We analysed the following outcomes: influenza, influenza-like illness, admissions, school absences, complications, and secondary transmission.

Findings: We included 14 randomised controlled trials, eight cohort studies, one case-control study, and one randomised controlled trial of intraepidemic use of the vaccines. Live attenuated influenza vaccines had 79% efficacy and 38% effectiveness in children older than 2 years compared with placebo or no immunisation. Inactivated vaccines had lower efficacy (65%) than live attenuated vaccines, and in children aged 2 years or younger they had similar effects to placebo. Effectiveness of inactivated vaccines was about 28% in children older than 2 years. Vaccines were effective in reducing long school absences (relative risk 0.14 [95% CI 0.07–0.27]). Studies assessing the effects of vaccines against secondary cases, lower-respiratory tract disease suggested no difference with placebo or standard care, but lacked statistical power.

Interpretation: Influenza vaccines (especially two-dose live attenuated vaccines) are efficacious in children older than 2 years. Efficacy and effectiveness of the vaccines differed strikingly. Only two small studies assessed the effects of influenza vaccines on hospital admissions and no studies assessed reductions in mortality, serious complications, and community transmission of influenza. If influenza immunisation in children is to be recommended as public-health policy, large-scale studies assessing such important outcomes and undertaking direct comparisons of vaccines are urgently needed.”

Impact of Influenza Vaccination on Seasonal Mortality in the US Elderly Population.

Simonsen L, Reichert TA, Viboud C, Blackwelder WC, Taylor RJ, Miller MA. Impact of Influenza Vaccination on Seasonal Mortality in the US Elderly Population. Arch Intern Med. 2005; 165:265-272.

Background: Observational studies report that influenza vaccination reduces winter mortality risk from any cause by 50% among the elderly. Influenza vaccination coverage among elderly persons (65 years) in the United States increased from between 15% and 20% before 1980 to 65% in 2001. Unexpectedly, estimates of influenza-related mortality in this age group also increased during this period. We tried to reconcile these conflicting findings by adjusting excess mortality estimates for aging and increased circulation of influenza A(H3N2) viruses.

Methods: We used a cyclical regression model to generate seasonal estimates of national influenza-related mortality (excess mortality) among the elderly in both pneumonia and influenza and all-cause deaths for the 33 seasons from 1968 to 2001. We stratified the data by 5-year age group and separated seasons dominated by A(H3N2) viruses from other seasons.

Results: For people aged 65 to 74 years, excess mortality rates in A(H3N2)-dominated seasons fell between 1968 and the early 1980s but remained approximately constant thereafter. For persons 85 years or older, the mortality rate remained flat throughout. Excess mortality in A(H1N1) and B seasons did not change. All-cause excess mortality for persons 65 years or older never exceeded 10% of all winter deaths.

Conclusions: We attribute the decline in influenza-related mortality among people aged 65 to 74 years in the decade after the 1968 pandemic to the acquisition of immunity to the emerging A(H3N2) virus. We could not correlate increasing vaccination coverage after 1980 with declining mortality rates in any age group. Because fewer than 10% of all winter deaths were attributable to influenza in any season, we conclude that observational studies substantially overestimate vaccination benefit.

A Review of Thimerosal (Merthiolate) and its Ethylmercury Breakdown Product: Specific Historical Considerations Regarding Safety and Effectiveness.

Geier DA, Sykes LK, Geier MR. A review of Thimerosal (Merthiolate) and its ethylmercury breakdown product: specific historical considerations regarding safety and effectiveness. J Toxicol Environ Health B 2007;10:575-96.

"Thimerosal (Merthiolate) is an ethylmercury-containing pharmaceutical compound that is 49.55% mercury and that was developed in 1927. Thimerosal has been marketed as an antimicrobial agent in a range of products, including topical antiseptic solutions and antiseptic ointments for treating cuts, nasal sprays, eye solutions, vaginal spermicides, diaper rash treatments, and perhaps most importantly as a preservative in vaccines and other injectable biological products, including Rho(D)-immune globulin preparations, despite evidence, dating to the early 1930s, indicating Thimerosal to be potentially hazardous to humans and ineffective as an antimicrobial agent. Despite this, Thimerosal was not scrutinized as part of U.S. pharmaceutical products until the 1980s, when the U.S. Food and Drug Administration finally recognized its demonstrated ineffectiveness and toxicity in topical pharmaceutical products, and began to eliminate it from these. Ironically, while Thimerosal was being eliminated from topicals, it was becoming more and more ubiquitous in the recommended immunization schedule for infants and pregnant women. Furthermore, Thimerosal continues to be administered, as part of mandated immunizations and other pharmaceutical products, in the United States and globally. The ubiquitous and largely unchecked place of Thimerosal in pharmaceuticals, therefore, represents a medical crisis."

Influenza Treatment Studies

Prevention and Treatment of Seasonal Influenza.

Glezen, WP. Prevention and Treatment of Seasonal Influenza. N Engl J Med. 2008; 359:2579-85.

"In February, 2007, fever developed in a previously healthy 15-year-old girl, with a peak temperature of 102°F (38.9°C) and mild upper respiratory congestion. The next day she was seen by her primary care physician. A rapid screening test for group A streptococcus was negative, and oseltamivir was prescribed. After two doses, she continued to have fever and also had nausea and emesis, malaise, and restlessness but could not get out of bed. Two days later, she was taken to the local emergency room, where she was found to be hypotensive. Despite intensive resuscitative efforts, she died 12 hours later; the postmortem examination showed necrotizing pneumonia and extensive alveolar hemorrhage. A viral culture confirmed an influenza A (H1N1) infection, and methicillin-resistant *Staphylococcus aureus* was isolated from a tracheal aspirate. Could this death have been prevented?"

Zanamivir Prophylaxis: An Effective Strategy for the Prevention of Influenza Types A and B within Households.

Monto, AS, Pichichero, ME, Blanckenberg, SJ, Ruuskanen, O, Cooper, C, Fleming, DM, Kerr, C. Zanamivir Prophylaxis: An Effective Strategy for the Prevention of Influenza Types A and B within Households. Journal of Infectious Diseases 2002; 186:1582-8.

“A double-blind, randomized study of inhaled zanamivir for the prevention of influenza in families was conducted. Once a person with a suspected case of influenza was identified (index patient), treatment of all other household members (contacts) 5 years old was initiated. Contacts received either 10 mg zanamivir or placebo inhaled once daily for 10 days. Index patients received relief medication only. In total, 487 households (242 placebo and 245 zanamivir) were enrolled, with 1291 contacts randomly assigned to receive prophylaxis. Four percent of zanamivir versus 19% of placebo households ($P < .001$) had at least 1 contact who developed symptomatic, laboratory-confirmed influenza, representing 81% protective efficacy (95% confidence interval, 64%–90%). Protective efficacy was similarly high for individuals (82%) and against both influenza types A and B (78% and 85%, respectively, for households). Zanamivir was well tolerated and was effective in preventing influenza types A and B within households where the index patient was not treated.”

Randomized, Placebo-Controlled Studies of Inhaled Zanamivir in the Treatment of Influenza A and B: Pooled Efficacy Analysis.

Monto, AS, Webster, A, Keene, O. Randomized, Placebo-Controlled Studies of Inhaled Zanamivir in the Treatment of Influenza A and B: Pooled Efficacy Analysis. Journal of Antimicrobial Chemotherapy. 1999;44:23-29.

“Zanamivir, a potent, highly selective inhibitor of influenza virus A and B neuraminidase, has been evaluated in seven, similarly designed, placebo-controlled studies of the treatment of influenza. Patients with typical influenza symptoms were recruited when influenza was known to be circulating in the community. Six of these studies included a zanamivir 10 mg inhaled bd (for 5 days) treatment arm, the dose regimen submitted to regulatory agencies. Pooled analyses were conducted to evaluate efficacy more precisely in terms of the alleviation of symptoms in population subgroups and for secondary endpoints. Median time to alleviation of symptoms, the primary endpoint, was reduced from 6.0 days in the placebo group (n 1102) to 5.0 days in the zanamivir group (n 1133), $P < 0.001$. In febrile, laboratory-confirmed, influenza-positive (IP) patients, time to alleviation was reduced from 6.5 days to 5.0 days, a treatment benefit of 1.5 days ($P < 0.001$). A larger treatment benefit (3 days) was seen in IP patients who had severe symptoms at entry (n=474, $P < 0.001$), compared with 1 day in patients whose symptoms were not severe (n 1098, $P < 0.001$). Similarly, a 3 day treatment benefit ($P = 0.003$) was observed in IP patients aged 50 years (n=263), compared with 1 day ($P < 0.001$) in patients aged <50 years. In ‘high-risk’ IP patients (recruited into all treatment studies), there was a treatment benefit of 2.5 days (n=305, $P = 0.006$). Pooled analyses of secondary end-points showed statistically significant reductions in antibiotic use, time to return to normal activities and use of relief medication. In addition, reductions in symptom scores were apparent shortly after commencing zanamivir treatment. By the evening of the second day of treatment, the median total symptom score had fallen by 44% in zanamivir recipients compared with 33% in placebo recipients ($P < 0.001$). These results highlight the groups likely to show greatest benefit from zanamivir treatment, and confirm the clinical relevance of the treatment benefit.”

Effectiveness of Oseltamivir in Preventing Influenza in Household Contacts: A Randomized Control Trial.

Welliver, R, Monto, AS, Carewicz, O, Shatteman, E, Hassman, M, Hedrick, J, Jackson, HC, Huson, L, Ward, P, Oxford, JS. Effectiveness of Oseltamivir in Preventing Influenza in Household Contacts: A Randomized Control Trial. JAMA. 2001;285(6).

“Context Influenza virus is easily spread among the household contacts of an infected person, and prevention of influenza in household contacts can control spread of influenza in the community.

Objective: To investigate the efficacy of oseltamivir in preventing spread of influenza to household contacts of influenza-infected index cases (ICs).

Design and Setting: Randomized, double-blind, placebo-controlled study conducted at 76 centers in North America and Europe during the winter of 1998-1999.

Participants: Three hundred seventy-seven ICs, 163 (43%) of whom had laboratory-confirmed influenza infection, and 955 household contacts (aged 12 years) of all ICs (415 contacts of influenza-positive ICs).

Interventions: Household contacts were randomly assigned by household cluster to take 75 mg of oseltamivir (n = 493) or placebo (n = 462) once daily for 7 days within 48 hours of symptom onset in the IC. The ICs did not receive antiviral treatment.

Main Outcome Measure: Clinical influenza in contacts of influenza-positive ICs, confirmed in a laboratory by detection of virus shedding in nose and throat swabs or a 4-fold or greater increase in influenza-specific serum antibody titer between base-line and convalescent serum samples.

Results: In contacts of an influenza-positive IC, the overall protective efficacy of oseltamivir against clinical influenza was 89% for individuals (95% confidence interval [CI], 67%-97%; P .001) and 84% for households (95% CI, 49%-95%; P .001). In contacts of all ICs, oseltamivir also significantly reduced incidence of clinical influenza, with 89% protective efficacy (95% CI, 71%-96%; P .001). Viral shedding was inhibited in contacts taking oseltamivir, with 84% protective efficacy (95% CI, 57%-95%; P .001). All virus isolates from oseltamivir recipients retained sensitivity to the active metabolite. Oseltamivir was well tolerated; gastrointestinal tract effects were reported with similar frequency in oseltamivir (9.3%) and placebo (7.2%) recipients.

Conclusion: In our sample, postexposure prophylaxis with oseltamivir, 75 mg once daily for 7 days, protected close contacts of influenza-infected persons against influenza illness, prevented outbreaks within households, and was well tolerated.”

The Role of Antivirals in the Control of Influenza.

Monto, AS. The Role of Antivirals in the Control of Influenza. Vaccine. 2003.; 21:1796-1800.

“Antivirals are effective in the prophylaxis and therapy of influenza and are likely to be active against a new pandemic variant. They can be divided into the M2 inhibitors, amantadine and rimantadine, and the neuraminidase inhibitors (NIs), zanamivir and oseltamivir. The former are limited in activity to type A viruses, while the latter are also active against type B viruses. Both classes of drugs are approximately 70–90% efficacious when used as prophylaxis. However, the use of M2 inhibitors in therapy is frequently limited by side effects, more common with amantadine, by the emergence of antiviral resistance and by the lack of demonstrated prevention of complications. In contrast, the NIs are better tolerated, antiviral resistance has not emerged as a significant problem and limited evidence suggests they may reduce the frequency of influenza complications. Antiviral agents have not been widely used for either prophylaxis or treatment of annual influenza epidemics. During the early months of the next pandemic they will be the only specific agents that could be used for prevention and treatment. Their availability will depend entirely on the creation of stockpiles of these agents well in advance of the arrival of the pandemic.”

Guillain-Barré Syndrome Following Influenza Vaccination

Haber, P DeStefano, F, Angulo, FJ, Iskander, J, Shadomy, SV, Weintraub, E, Chen, RT. Guillain-Barré Syndrome Following Influenza Vaccination. JAMA. 2004;292:2478-2481.

“Context: An unexplained increase in the risk of Guillain-Barré syndrome (GBS) occurred among recipients of the swine influenza vaccine in 1976-1977. Guillain-Barré syndrome remains the most frequent neurological condition reported after influenza vaccination to the Vaccine Adverse Events Reporting System (VAERS) since its inception in 1990.

Objective: To evaluate trends of reports to VAERS of GBS following influenza vaccination in adults.

Design, Setting, and Participants: VAERS is the US national spontaneous reporting system for adverse events following vaccination. Reports of GBS in persons 18 years or older following influenza vaccination were evaluated for each influenza season from July 1, 1990, through June 30, 2003. The number of people vaccinated was estimated from the National Health Interview Survey and US census data. Beginning in 1994, active follow-up was conducted to verify GBS diagnosis and obtain other clinical details.

Main Outcome Measure: Reporting rates of GBS following influenza vaccination over time.

Results: From July 1990 through June 2003, VAERS received 501 reports of GBS following influenza vaccination in adults. The median onset interval (13 days) was longer than that of non-GBS reports of adverse events after influenza vaccine (1 day) (P .001). The annual reporting rate decreased 4-fold from a high of 0.17 per 100 000 vaccinees in 1993-1994 to 0.04 in 2002-2003 (P .001). A GBS diagnosis was confirmed in 82% of reports. Preceding illness within 4 weeks of vaccination was identified in 24% of reported cases.

Conclusions: From 1990 to 2003, VAERS reporting rates of GBS after influenza vaccination decreased. The long onset interval and low prevalence of other preexisting illnesses are consistent with a possible causal association between GBS and influenza vaccine. These findings require additional research, which can lead to a fuller understanding of the causes of GBS and its possible relationship with influenza vaccine.

Influenza Vaccination and Guillain Barre Syndrome.

Geier,MR, Geier, DA, Zahalskyc, AC. Influenza Vaccination and Guillain Barre Syndrome. Clinical Immunology. 2003.

“Acute and severe Guillain Barre Syndrome (GBS) cases reported following influenza vaccine to the Vaccine Adverse Events Reporting System (VAERS) database from 1991 through 1999 were examined. Endotoxin lysate assay in influenza vaccines. There were a total of 382 cases of GBS reported to the VAERS database following influenza vaccination (male/female ratio, 1.2). The median onset of GBS following influenza vaccine was 12 days (interquartile range, 7 days to 21 days). There was an increased risk of acute GBS (relative risk, 4.3; 95% confidence interval, 3.0 to 6.4) and severe GBS (relative risk, 8.5; 95% confidence interval, 3.7 to 18.9) in comparison to an adult tetanus– diphtheria (Td) vaccine control group. There were maximums in the incidence of GBS following influenza vaccine that occurred approximately every third year (1993, 1996, and 1998) and statistically significant variation in the incidence of GBS among different influenza manufacturers. Influenza vaccines contained from a 125- to a 1250-fold increase in endotoxin concentrations in comparison to an adult Td vaccine control and endotoxin concentrations varied up to 10-fold among different lots and manufacturers of influenza vaccine. The biologic mechanism for GBS following influenza vaccine may involve the synergistic effects of endotoxin and vaccine-induced autoimmunity. There were minimal potential reporting biases in the data reported to the VAERS database in this study. Patients should make an informed consent decision on whether to take this optional vaccine based upon its safety and efficacy and physicians should vigilantly report GBS following influenza vaccination to the VAERS in the United States so that continued evaluation of the safety of influenza vaccine may be undertaken.”

Mitochondrial dysfunction, impaired oxidative-reduction activity, degeneration, and death in human neuronal and fetal cells induced by low-level exposure to thimerosal and other metal compounds.

D.A. Geier, P.G. King, and M.R. Geier. Toxicological & Environmental Chemistry

Vol. 91, No. 4, June 2009, 735–749.

“Thimerosal (ethylmercurithiosalicylic acid), an ethylmercury (EtHg)-releasing compound (49.55% mercury (Hg)), was used in a range of medical products for more than 70 years. Of particular recent concern, routine administering of Thimerosal-containing biologics/childhood vaccines have become significant sources of Hg exposure for some fetuses/infants. This study was undertaken to investigate cellular damage among in vitro human neuronal (SH-SY-5Y neuroblastoma and 1321N1 astrocytoma) and fetal (nontransformed) model systems using cell vitality assays and microscope-based digital image capture techniques to assess potential damage induced by Thimerosal and other metal compounds (aluminum (Al) sulfate, lead (Pb)(II) acetate, methylmercury (MeHg) hydroxide, and mercury (Hg)(II) chloride) where the cation was

reported to exert adverse effects on developing cells. Thimerosal-associated cellular damage was also evaluated for similarity to pathophysiological findings observed in patients diagnosed with autistic disorders (ADs). Thimerosal-induced cellular damage as evidenced by concentration- and time-dependent mitochondrial damage, reduced oxidative–reduction activity, cellular degeneration, and cell death in the in vitro human neuronal and fetal model systems studied. Thimerosal at low nanomolar (nM) concentrations induced significant cellular toxicity in human neuronal and fetal cells. Thimerosal-induced cytotoxicity is similar to that observed in AD pathophysiologic studies. Thimerosal was found to be significantly more toxic than the other metal compounds examined. Future studies need to be conducted to evaluate additional mechanisms underlying Thimerosal-induced cellular damage and assess potential co-exposures to other compounds that may increase or decrease Thimerosal-mediated toxicity.”

Prenatal and Postnatal Mercury Exposure, Breastfeeding and Neurodevelopment During the First 5 Years.

Marques RC, Dorea JG, Bernardi VE, Bastos WR, Malm O. Prenatal and postnatal mercury exposure, breastfeeding and neurodevelopment during the first 5 years. *Cog Behav Neurol* 2009;22:134-41.

"OBJECTIVE: We evaluated the association between infant hair-Hg and Gesell schedules (GS).
BACKGROUND: Longitudinal assessment of prenatal and postnatal Hg exposure during the first 60 months.
METHODS: We used hair-Hg as a marker of postnatal Hg exposure (inorganic and methyl-Hg from breast milk, and ethyl-Hg from thimerosal) and GS measured at 6, 36, and 60 months. RESULTS: Hair-Hg at 6 months responded to events related to Hg exposure and breastfeeding. However, most neurodevelopment delays observed at 6 months were overcome with infant growth; at 60 months 87% of children showed adequate GS (>85). Length of lactation and hair-Hg were each significantly correlated with 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. Multiple regression models showed that the GS outcome at 60 months depended on GS at 36 months that in turn was influenced by infants' developmental and Hg exposure variables. GS at 6 months was significantly influenced by prenatal (maternal and infant hair-Hg at birth) and postnatal Hg exposure at 6 months..."

Principal Component Analysis and Discrimination of Variables Associated with Pre- and Post-Natal Exposure to Mercury.

Marques RC, Barnardi 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;211:606-14.

"The variance of variables associated with neurodevelopment at 180 days, pre-natal variables (Hg in placenta, blood and hair) and post-natal Hg exposure (including Thimerosal-containing vaccines, TCV) were examined in 82 exclusively breastfed infants using principal component analysis (PCA). This multivariate method was applied to identify hierarchy and sets of interrelated variables. The PCA yielded a two-factor solution, explaining 92% of variance and summarizing most of the relevant information in the dataset matrix: 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. The third component explained only 3% of the remaining 8% variance. Beside CNS priming by breastfeeding, infant development (birth weight) and time of immunization with TCV should be considered in epidemiological studies. PCA can classify sets of variables related to vaccination and neuromotor development schedules, clearly discriminating between earlier and later TCV exposures of exclusively breastfed infants. In conclusion, the incommensurable concept of the chance of toxic risk caused by TCV-EtHg exposure against the proven benefit of immunization is in no way disputed here. However, 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."

Thimerosal and Children's Neurodevelopmental Disorders.

Maya L, Luna F. Thimerosal and children's neurodevelopmental disorders. An Fac Med Lima 2006;67:243-62.

"The causal relationship between thimerosal (ethylmercury), preservative in pediatric vaccines, and the increase in children's neurodevelopmental disorders as a result of the increase in immunization schemes is evaluated. The scientific information on thimerosal was reviewed, and evidence found in epidemiological, ecological, biomolecular, toxicology, biosafety, fetal toxicology and reproductive health studies signal the possible causal association of thimerosal exposure and neurodevelopmental disorders of the child. Such neurotoxicity occurs in infants and fetuses of vaccinated pregnant women, due to cumulative doses of mercury. The various types of evidence imply thimerosal as the causal agent, aggravating or triggering neurodevelopmental disorders of the child. The toxicity of mercury forced the progressive withdrawal of thimerosal. Unfortunately, in the vaccines, there was a substantial delay in demonstrating the negative impact of thimerosal. Currently, there exist vaccines without thimerosal, whose use is causing a lower incidence of children's neurodevelopmental disorders."

Thimerosal Exposure in Infants and Neurodevelopmental Disorders: An Assessment of Computerized Medical Records in the Vaccine Safety Datalink.

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;271:110-8.

"The study evaluated possible associations between neurodevelopmental disorders (NDs) and exposure to mercury (Hg) from Thimerosal-containing vaccines (TCVs) by examining the automated Vaccine Safety Datalink (VSD). A total of 278,624 subjects were identified in birth cohorts from 1990–1996 that had received their first oral polio vaccination by 3 months of age in the VSD. The birth cohort prevalence rate of medically diagnosed International Classification of Disease, 9th revision (ICD-9) specific NDs and control outcomes were

calculated. Exposures to Hg from TCVs were calculated by birth cohort for specific exposure windows from birth-7 months and birth-13 months of age. Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from TCVs. Consistent significantly increased rate ratios were observed for autism, autism spectrum disorders, tics, attention deficit disorder, and emotional disturbances with Hg exposure from TCVs. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs. Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be conducted to further evaluate the relationship between Hg exposure and NDs."

A Meta-Analysis Epidemiological Assessment of Neurodevelopmental Disorders Following Vaccines Administered from 1994 through 2000 in the United States.

Geier DA, Geier MR. A meta-analysis epidemiological assessment of neurodevelopmental disorders following vaccines administered from 1994 through 2000 in the United States. Neuro Endocrinol Lett 2006;27:401-13.

"BACKGROUND: Thimerosal is an ethylmercury-containing compound (49.6% mercury by weight) used as the preservative level in vaccines (0.005% to 0.01%). METHODS: Statistical modeling in a meta-analysis epidemiological assessment of the Vaccine Adverse Event Reporting System (VAERS) for neurodevelopment disorders (NDs) reported following Diphtheria-Tetanus-whole-cell-Pertussis (DTP) vaccines in comparison to Diphtheria-Tetanus-whole-cell-Pertussis-Haemophilus Influenzae Type b (DTPH) vaccines (administered: 1994-1997) and following Thimerosal-containing Diphtheria-Tetanus-acellular-Pertussis (DTaP), vaccines in comparison to Thimerosal-free DTaP vaccines (administered: 1997-2000), was undertaken. RESULTS: Significantly increased adjusted (sex, age, vaccine type, vaccine manufacturer) risks of autism, speech disorders, mental retardation, personality disorders, thinking abnormalities, ataxia, and NDs in general, with minimal systematic error or confounding, were associated with TCV exposure. CONCLUSION: It is clear from the results of the present epidemiological study and other recently published data associating mercury exposure with childhood NDs, additional ND research should be undertaken in the context of evaluating mercury-associated exposures, especially from Thimerosal-containing vaccines."

An Assessment of Downward Trends in Neurodevelopmental Disorders in the United States Following Removal of Thimerosal from Childhood Vaccines.

Geier DA, Geier MR. An assessment of downward trends in neurodevelopmental disorders in the United States following removal of Thimerosal from childhood vaccines. Med Sci Monit 2006;12(6):CR231-9.

The US is in the midst of an epidemic of neurodevelopmental disorders (NDs). Thimerosal is an ethylmercury-containing compound added to some childhood vaccines. Several previous epidemiological studies conducted in the US have associated Thimerosal-containing vaccine (TCV) administration with NDs.

An ecological study was undertaken to evaluate NDs reported to the Vaccine Adverse Event Reporting System (VAERS) from 1991 through 2004 by date of receipt and by date of vaccine administration. The NDs examined included autism, mental retardation, and speech disorders. Statistical trend analysis was employed to evaluate the effects of removal of Thimerosal on the proportion of NDs reported to VAERS.

There was a peak in the proportion of ND reports received by VAERS in 2001–2002 and in the proportion of ND reports by date of vaccine administration in 1998. There were significant reductions in the proportion of NDs reported to VAERS as Thimerosal was begun to be removed from childhood vaccines in the US from mid-1999 onwards.

The present study provides the first epidemiological evidence showing that as Thimerosal was removed from childhood vaccines, the number of NDs has decreased in the US. The analysis techniques utilized attempted to

minimize chance or bias/confounding. Additional research should be conducted to further evaluate the relationship between TCVs and NDs. This is especially true because the handling of vaccine safety data from the National Immunization Program of the CDC has been called into question by the Institute of Medicine of the National Academy of Sciences in 2005.

Neurodevelopmental Disorders, Maternal Rh-Negativity, and Rho(D) Immune Globulins: A Multi-Center Assessment.

Geier DA, Mumper E, Gladfelter B, Coleman L, Geier MR. Neuro Endocrinol Lett 2008;29:272-80.

"BACKGROUND: Many formulations of Thimerosal (49.55% mercury by weight)-containing Rho(D) immune globulins (TCRs) were routinely administered to Rh-negative mothers in the US prior to 2002. OBJECTIVES: It was hypothesized: (1) if prenatal Rho(D)-immune globulin preparation exposure was a risk factor for neurodevelopmental disorders (NDs) then more children with NDs would have Rh-negative mothers compared to controls; and (2) if Thimerosal in the Rho(D)-immune globulin preparations was the ingredient associated with NDs, following the removal of Thimerosal from all manufactured Rho(D)-immune globulin preparations from 2002 in the US the frequency of maternal Rh-negativity among children with NDs should be similar to control populations. METHODS: Maternal Rh-negativity was assessed at two sites (Clinic A-Lynchburg, VA; Clinic B-Rockville and Baltimore, MD) among 298 Caucasian children with NDs and known Rh-status. As controls, maternal Rh-negativity frequency was determined from 124 Caucasian children (born 1987-2001) without NDs at Clinic A, and the Rh-negativity frequency was determined from 1,021 Caucasian pregnant mothers that presented for prenatal genetic care at Clinic B (1980-1989). Additionally, 22 Caucasian patients with NDs born from 2002 onwards (Clinics A and B) were assessed for maternal Rh-negativity. RESULTS: There were significant and comparable increases in maternal Rh-negativity among children with NDs (Clinic: A=24.2%), autism spectrum disorders (Clinic: A=28.3%, B=25.3%), and attention-deficit-disorder/attention-deficit-hyperactivity-disorder (Clinic: A=26.3%) observed at both clinics in comparison to both control groups (Clinic: A=12.1%, B=13.9%) employed. Children with NDs born post-2001 had a maternal Rh-negativity frequency (13.6%) similar to controls. CONCLUSION: This study associates TCR exposure with some NDs in children."

Early Downward Trends in Neurodevelopmental Disorders Following Removal of Thimerosal-Containing Vaccines.

David A. Geier, B.A., Mark R. Geier, M.D., Ph.D. Journal of American Physicians and Surgeons; Spring 2006; 11(1).

"Contemporaneously with the epidemic rise in neurodevelopmental disorders (NDs), first observed in the United States during the 1990s, the childhood immunization schedule was

expanded by the U.S. Centers for Disease Control and Prevention (CDC) to include several additional thimerosal-containing vaccines (TCVs). On July 7, 1999, a joint recommendation was made by the American Academy of Pediatrics

(AAP) and the U.S. Public Health Service (PHS) to remove thimerosal from vaccines. A two-phase study was undertaken to evaluate trends in diagnosis of new NDs entered into the Vaccine Adverse Event Reporting System (VAERS) and the California Department of Developmental Services (CDDS) databases on a reporting quarter basis, from 1994 through 2005. Significant increasing trends in newly diagnosed NDs were observed in both databases 1994 through mid-2002. Significant decreasing trends in newly diagnosed NDs were observed in both databases from mid-2002 through 2005. The results indicate that the trends in newly diagnosed NDs correspond directly to the expansion and subsequent contraction of the cumulative mercury dose to which children were exposed from TCVs through the U.S. immunization schedule.”

Neurodevelopmental Disorders after Thimerosal-Containing Vaccines:

A Brief Communication.

Mark R. Geier, David A. Geier, Society for Experimental Medicine. 2003.

“We were initially highly skeptical that differences in the concentrations of thimerosal in vaccines would have any effect on the incidence rate of neurodevelopmental disorders after childhood immunization. This study presents the first epidemiologic evidence, based upon tens of millions of doses of vaccine administered in the United States, that associates increasing thimerosal from vaccines with neurodevelopmental disorders. Specifically, an analysis of the Vaccine Adverse Events Reporting System (VAERS) database showed statistical increases in the incidence rate of autism (relative risk [RR] = 6.0), mental retardation (RR = 6.1), and speech disorders (RR = 2.2) after thimerosal-containing diphtheria, tetanus, and acellular pertussis (DTaP) vaccines in comparison with thimerosal-free DTaP vaccines. The male/female ratio indicated that autism (1.7) and speech disorders (2.3) were reported more in males than females after thimerosal-containing DTaP vaccines, whereas mental retardation (1.2) was more evenly reported among male and female vaccine recipients. Controls were employed to determine if biases were present in the data, but none were found. It was determined that overall adverse reactions were reported in similar-aged populations after thimerosal-containing DTaP (2.4 ± 3.2 years old) and thimerosal-free DTaP (2.1 ± 2.8 years old) vaccinations. Acute control adverse reactions such as deaths (RR = 1.0), vasculitis (RR = 1.2), seizures (RR = 1.6), ED visits (RR = 1.4), total adverse reactions (RR = 1.4), and gastroenteritis (RR = 1.1) were reported similarly after thimerosal-containing and thimerosal-free DTaP vaccines. An association between neurodevelopmental disorders and thimerosal-containing

DTaP vaccines was found, but additional studies should be conducted to confirm and extend this study.”

NeuroDevelopmental Disorders Following Thimerosal-Containing Childhood Immunizations: A Follow-up Analysis

Mark R. Geier, David A. Geier, International Journal of Toxicology, 23:369-376, 2004.

“The authors previously published the first epidemiological study from the United States associating thimerosal from childhood vaccines with neurodevelopmental disorders (NDs) based upon assessment of the Vaccine Adverse Event

Reporting System (VAERS). A number of years have gone by since their previous analysis of the VAERS. The present study was undertaken to determine whether the previously observed effect between thimerosal-containing childhood vaccines and NDs are still apparent in the VAERS as children have had a chance to further mature and potentially be diagnosed with additional NDs. In the present study, a cohort of children receiving thimerosal-containing diphtheria-tetanus-acellular pertussis (DTaP) vaccines in comparison to a cohort of children receiving thimerosal-free DTaP vaccines administered from 1997 through 2000 based upon an assessment of adverse events reported to the VAERS were evaluated. It was determined that there were significantly increased odds ratios (ORs) for autism (OR=1/8, $p<.05$), mental retardation (OR=2.6, $p<.002$), speech disorder (OR=2.1, $p<.02$), personality disorders (OR=2.6, $p<.01$), and thinking abnormality (OR=8.2, $p<.01$) adverse events reported to the VAERS following thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines. Potential confounders and reporting biases were found to be minimal in this assessment of the VAERS. It was observed, even though the media has reported a potential association between autism and thimerosal exposure, that the other NDs analyzed in this assessment of the VAERS had significantly higher ORs than autism following thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines. The present study provides additional epidemiological evidence supporting previous epidemiological, clinical and experimental evidence that administration of thimerosal-containing vaccines in the United States resulted in a significant number of children developing NDs.

A Two-Phased Population Epidemiological Study of the Safety of Thimerosal-Containing Vaccines: A Follow-Up Analysis.

Mark R. Geier, David A. Geier. Med Sci Monit, 2005; 11(4): CR160-170

“Background: Thimerosal is an ethylmercury-containing preservative in vaccines. Toxicokinetic studies have shown children received doses of mercury from thimerosal-containing vaccines (TCVs) that were in excess of safety guidelines. Previously, an ecological study showing a significant association between TCVs and neurodevelopmental disorders (NDs) in the US was published in this journal.

Material/Methods: A two phased population-based epidemiological study was undertaken. Phase one evaluated reported NDs to the Vaccine Adverse Event Reporting System (VAERS) following thimerosal-containing Diphtheria-Tetanus-acellular-Pertussis (DTaP) vaccines in comparison to thimerosal-free DTaP vaccines administered from 1997 through 2001. Phase two evaluated the automated Vaccine Safety Datalink (VSD) for cumulative exposures to mercury from TCVs at 1-, 2-, 3-, and 6-months-of-age for infants born from 1992 through 1997 and the eventual risk of developing NDs.

Results: Phase one showed significantly increased risks for autism, speech disorders, mental retardation, personality disorders, and thinking abnormalities reported to VAERS following thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines. Phase two showed significant associations between cumulative exposures to thimerosal and the following types of NDs: unspecified developmental delay, tics, attention deficit disorder (ADD), language delay, speech delay, and neurodevelopmental delays in general.

Conclusions: This study showed that exposure to mercury from TCVs administered in the US was a consistent significant risk factor for the development of NDs. It is clear from these data and other recent publications linking TCVs with NDs that additional ND research should be undertaken in the context of evaluating mercury-associated exposures and thimerosal-free vaccines should be made available.”

An Assessment of Downward Trends in Neurodevelopmental Disorders in the United States Following Removal of Thimerosal from Childhood Vaccines.

Mark R. Geier, David A. Geier. Med Sci Monit, 2006; 12(6): CR231-239.

“Background: The US is in the midst of an epidemic of neurodevelopmental disorders (NDs). Thimerosal is an ethylmercury-containing compound added to some childhood vaccines. Several previous epidemiological studies conducted in the US have associated Thimerosal-containing vaccine (TCV) administration with NDs.

Material/Methods: An ecological study was undertaken to evaluate NDs reported to the Vaccine Adverse Event Reporting System (VAERS) from 1991 through 2004 by date of receipt and by date of vaccine administration. The NDs examined included autism, mental retardation, and speech disorders. Statistical trend analysis was employed to evaluate the effects of removal of Thimerosal on the proportion of NDs reported to VAERS.

Results: There was a peak in the proportion of ND reports received by VAERS in 2001–2002 and in the proportion of ND reports by date of vaccine administration in 1998. There were significant reductions in the proportion of NDs reported to VAERS as Thimerosal was begun to be removed from childhood vaccines in the US from mid-1999 onwards.

Conclusions: The present study provides the first epidemiological evidence showing that as Thimerosal was removed from childhood vaccines, the number of NDs has decreased in the US. The analysis techniques utilized attempted to minimize chance or bias/confounding. Additional research should be conducted to further evaluate the relationship between TCVs and NDs. This is especially true because the handling of vaccine safety data from the National Immunization Program of the CDC has been called into question by the Institute of Medicine of the National Academy of Sciences in 2005.”

An Evaluation of the Effects of Thimerosal on Neurodevelopmental Disorders Reported Following DTP and Hib Vaccines in Comparison to DTPH Vaccine in the United States.

Mark R. Geier, David A. Geier. Journal of Toxicology and Environmental Health, Part A, 69:1481–1495, 2006

“Thimerosal is an ethylmercury (49.55% mercury by weight) preservative historically added to some vaccines. Toxicokinetic studies showed children in the United States received doses of mercury from Thimerosal-containing vaccines (TCVs) in excess of safety guidelines. In the United States during the 1990s, diphtheria–tetanus–pertussis (DTP) and Haemophilus influenzae type b (Hib) vaccines (maximally, 50 mg mercury per joint administration) and diphtheria–tetanus–pertussis–Haemophilus influenzae type b (DTPH) vaccines (25 mg mercury per administration) were given to children in the same childhood vaccination schedule at 2, 4, 6, and 15–18 mo, so that children receiving DTP and Hib vaccines may have maximally received an additional 100 mg more mercury exposure from TCVs than children administered DTPH vaccines. A case-control epidemiological study of neurodevelopmental disorders (NDs) reported to the Vaccine Adverse Event Reporting System (VAERS) (online public access version; updated 31 August 2004) following administration of DTP vaccines in comparison to DTPH vaccines manufactured by Lederle Laboratories (Pearl River, NY) from 1994 through 1998 was undertaken. Significantly increased odds ratios for autism, speech disorders, mental retardation, infantile spasms, and thinking abnormalities reported to VAERS were found following DTP vaccines in comparison to DTPH vaccines with minimal bias or systematic error. Additional ND research should be undertaken in the context of evaluating mercury-associated exposures, especially since in 2005 the Institute of Medicine issued a report calling into question handling of vaccine safety data by the National Immunization Program of the Centers for Disease Control and Prevention.”

Hepatitis B Triple Series Vaccine and Developmental Disability in US Children Aged 1-9 Years.

Gallagher C, Goodman M. Hepatitis B triple series vaccine and developmental disability in US children aged 1-9 years. Toxicol Environ Chem 2008;90:997-1008.

"This study investigated the association between vaccination with the Hepatitis B triple series vaccine prior to 2000 and developmental disability in children aged 1–9 years (n=1824), proxied by parental report that their child receives early intervention or special education services (EIS). National Health and Nutrition Examination Survey 1999–2000 data were analyzed and adjusted for survey design by Taylor Linearization using SAS version 9.1 software, with SAS callable SUDAAN version 9.0.1. The odds of receiving EIS were approximately nine times as great for vaccinated boys (n=46) as for unvaccinated boys (n=7), 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."

Hepatitis B Vaccination of Male Neonates and Autism.

Gallagher CM, Goodman MS. Hepatitis B vaccination of male neonates and autism. AEP 2009;19:659.

"PURPOSE: Universal newborn immunization with hepatitis B vaccine was recommended in 1991; however, safety findings are mixed. The Vaccine Safety Datalink Workgroup reported no association between hepatitis B vaccination at birth and febrile episodes or neurological adverse events. Other studies found positive associations between hepatitis B vaccination and ear infection, pharyngitis, and chronic arthritis; as well as receipt of early intervention/special education services (EIS); in probability samples of U.S. children. Children with autistic spectrum disorder (ASD) comprise a growing caseload for EIS. We evaluated the association between hepatitis B vaccination of male neonates and parental report of ASD. METHODS: This cross-sectional study used U.S. probability samples obtained from National Health Interview Survey 1997–2002 datasets. Logistic regression modeling was used to estimate the effect of neonatal hepatitis B vaccination on ASD risk

among boys age 3–17 years with shot records, adjusted for race, maternal education, and two-parent household. RESULTS: Boys who received the hepatitis B vaccine during the first month of life had 2.94 greater odds for ASD (n=31 of 7,486; OR Z 2.94; $p < 0.03$; 95% CI = 1.10, 7.90) compared to later- or unvaccinated boys. Non-Hispanic white boys were 61% less likely to have ASD (OR=0.39; $p < 0.04$; 95% CI=0.16, 0.94) relative to non-white boys. CONCLUSION: Findings suggest that U.S. male neonates vaccinated with hepatitis B vaccine had a 3-fold greater risk of ASD; risk was greatest for non-white boys."

Delayed Acquisition of Neonatal Reflexes in Newborn Primates Receiving a Thimerosal-Containing Hepatitis B Vaccine: Influence of Gestational Age and Birth Weight.

Hewitson L, Houser LA, Stott C, Sackett G, Tomko JL, Atwood D, Blue L, Railey White E, Wakefield AJ. Delayed acquisition of neonatal reflexes in newborn primates receiving a thimerosal-containing Hepatitis B vaccine: Influence of gestational age and birth weight. *Neurotoxicology*. 2009 Oct 2. [Epub ahead of print]

"This study examined whether acquisition of neonatal reflexes and sensorimotor skills in newborn rhesus macaques (*Macaca mulatta*) is influenced by receipt of the single neonatal dose of Hepatitis B (HB) vaccine containing the preservative thimerosal (Th). HB vaccine containing a standardized weight-adjusted Th dose was administered to male macaques within 24h of birth (n=13). Unexposed animals received saline placebo (n=4) or no injection (n=3). Infants were raised identically and tested daily for acquisition of 9 survival, motor, and sensorimotor reflexes by a blinded observer. In exposed animals there was a significant delay in the acquisition of three survival reflexes: root, snout and suck, compared with unexposed animals. No neonatal responses were significantly delayed in unexposed animals compared with exposed. Gestational age (GA) and birth weight were not significantly correlated. Cox regression models were used to evaluate the main effects and interactions of exposure with birth weight and GA as independent predictors and time-invariant covariates. Significant main effects remained for exposure on root and suck when controlling for GA and birth weight such that exposed animals were relatively delayed in time-to-criterion. There was a significant effect of GA on visual follow far when controlling for exposure such that increasing GA was associated with shorter time-to-criterion. Interaction models indicated that while there were no main effects of GA or birth weight on root, suck or snout reflexes there were various interactions between exposure, GA, and birth weight such that inclusion of the relevant interaction terms significantly improved model fit. This, in turn, indicated important influences of birth weight and/or GA on the effect of exposure which, in general, operated in a way that lower birth weight and/or lower GA exacerbated the detrimental effect of vaccine exposure. This primate model provides a possible means of assessing adverse neurodevelopmental outcomes from neonatal Th-containing HB vaccine exposure, particularly in infants of lower GA or low birth weight. The mechanism of these effects and the requirements for Th is not known and requires further study."