

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

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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 µg mercury per joint administration) and diphtheria–tetanus–pertussis–Haemophilus influenzae type b (DTPH) vaccines (25 µg 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 µg 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.

Thimerosal, an ethylmercurial preservative (49.55% mercury by weight) historically added to many vaccines, may have represented a significant source of mercury exposure in susceptible children (Ball et al., 2001). Thimerosal is still routinely added to required vaccines administered to U.S. infants (e.g., for influenza), and the Institute of Medicine (2004) of the U.S. National Academy

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of Sciences has backed away from the stated goal issued by the American Academy of Pediatrics and U.S. Public Health Service in 1999 that Thimerosal be removed from U.S. vaccines as soon as possible (Ball et al., 2001). Furthermore, many nations still add Thimerosal to many of their pediatric vaccines. The World Health Organization (WHO) and several vaccine manufacturers still advocate the continued use of Thimerosal in pediatric vaccines.

Standard vaccine practices in the United States during the past several decades exposed many children to levels of mercury that exceeded federal safety guidelines for the oral ingestion of methylmercury, and also exposed children to levels of mercury that exceeded the U.S. Environmental Protection Agency (EPA) permissible hair mercury limit (Ball et al., 2001; Redwood et al., 2001). According to the Centers for Disease Control and Prevention (CDC), expanded required immunization schedule in the United States during the 1990s, infants may have been exposed to 12.5 μg mercury at birth, 62.5 μg mercury at 2 mo, 50 μg mercury at 4 mo, 62.5 μg mercury at 6 mo, and 50 μg mercury at approximately 18 mo, for a total of 237.5 μg of mercury during the first 18 mo of life, if all Thimerosal-containing vaccines were administered. Additionally, if 3 Thimerosal-containing influenza vaccines were administered during the first 18 mo of life, as was suggested for certain populations, then the total mercury exposure could have been as high as 275 μg mercury (Ball et al., 2001; Redwood et al., 2001).

At the same time that the CDC expanded the childhood immunization schedule in the 1990s, epidemic trends in neurodevelopmental disorders were observed in the United States (Bertrand et al., 2001; Blaxill, 2004; California Department of Developmental Services, 2003; Gerlai & Gerlai, 2003, 2004; Newschaffer et al., 2005; Yeargin-Allsopp et al., 2003). In 2004, the Department of Health and Human Services and the American Academy of Pediatrics issued an autism A.L.A.R.M. stating that presently 1 in 166 children have an autistic disorder, and 1 in 6 children have a developmental and/or behavior disorder. Autism, once a rare disorder, has now been found to be more prevalent than childhood cancer, diabetes, and Down syndrome (California Department of Developmental Services, 2003). It was suggested that immigration or shifts in diagnostic criteria cannot explain the observed increase, and phenomena are driven by factors beyond improved identification and diagnosis (Blaxill, 2004; Blaxill et al., 2003; California Department of Developmental Services, 2003; Newschaffer et al., 2005).

Autism is a neurodevelopmental syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movements (California Department of Developmental Services, 2003). While genetic factors are recognized as being important in the pathogenesis of autistic disorders, a role for environmental factors has received considerable attention. Recent studies reported that exposure to mercury produces immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autistic disorders (Bernard et al., 2001, 2002; Blaxill et al., 2004; Redwood et al., 2001).

In previous studies the effects of Thimerosal on the risks of neurodevelopmental disorders in the United States was evaluated. It was determined that an approximately two- to eight-fold significantly increased risk for neurodevelopmental disorders existed, dependent on the condition or symptoms examined, following administration of Thimerosal-containing diphtheria–tetanus–acellular pertussis (DTaP) vaccines in comparison to Thimerosal-free DTaP vaccines, based on assessment of the Vaccine Adverse Event Reporting System (VAERS) database. In addition, significant associations between administration of Thimerosal-containing vaccines and neurodevelopmental disorders have been observed based on examinations of the U.S. Department of Education database and the CDC Vaccine Safety Datalink (VSD) database (Geier & Geier, 2003a, 2003b, 2003c, 2004a, 2004b, 2004c, 2005a).

The purpose of this study was to extend our previous epidemiological studies, and further evaluate the relationship between Thimerosal-containing childhood vaccines and neurodevelopmental disorders in the United States. In order to examine the relationship between Thimerosal and neurodevelopmental disorders in this study as compared to previous studies, neurodevelopmental disorders reported to the VAERS database were evaluated following whole-cell diphtheria–tetanus–pertussis (DTP) vaccines in comparison to whole-cell diphtheria–tetanus–pertussis–*Haemophilus influenzae* type b (DTPH) vaccines. Based on this method of analysis, there should have been an approximately 100 µg additional exposure to mercury among those children receiving DTP vaccines in comparison to those receiving DTPH vaccines, because children receiving DTP vaccines were concurrently administered *Haemophilus influenzae* type b (Hib) vaccines. When these two vaccines were combined in the DTPH vaccine, children receiving it were exposed to 25 µg mercury per vaccine administration. In contrast, children receiving DTP vaccines were exposed to 25 µg mercury from the DTP vaccine and were maximally exposed to 25 µg mercury from some Hib vaccines. Thus, among the vaccines under study, children receiving separate DTP and Hib vaccines potentially received 200 µg mercury from these vaccines, whereas children receiving DTPH in the same schedule potentially received 100 µg mercury from these vaccines during the first 18 mo of life. These vaccines were administered for similar years, in the same childhood vaccination schedule at 2, 4, 6, and 15–18 mo, in the United States.

METHODS

The VAERS database is an epidemiological database that has been maintained by the CDC since 1990 as a surveillance tool, in order to evaluate vaccine safety. Specific adverse events following vaccination are required to be reported to this database as mandated by law. The VAERS Working Group of the CDC has previously stated that less than 5% of the total adverse events reported to VAERS are reported by parents (Singleton et al., 1999). The VAERS Working Group of the CDC and the FDA analyze and publish epidemiologic

studies based on analyses of VAERS. They note that VAERS is simple to use, flexible by design, and the data are available in a timely fashion, but they also warn that the potential limitations may include systematic error due to underreporting, erroneous reporting, frequent multiple exposures, multiple outcomes, and lack of precise denominators (Singleton et al., 1999).

In order to examine VAERS correctly in this study, a technique developed by Rosenthal et al. (1996) from the National Immunization Program (NIP) of the CDC was employed. This technique involves comparing two different types of vaccines that were administered to age-matched populations, and using the net number of doses distributed to estimate the number of doses administered. This process corrects for doses not distributed or returned during the period examined in the Biological Surveillance Summaries of the CDC and net number of doses distributed are used as the denominator to determine incidence rates of reported adverse events to the VAERS database. It should be noted that even though the net numbers of doses of vaccine distributed were analyzed, there is the possibility that some doses of vaccine were not administered to children, but such a limitation should be minimal and should equally affect both vaccines under study. Comparison of reported adverse event incidence data between different vaccines establishes the relative safety and risk of the various agents.

The strength of the VAERS database stems from its large reporting base (i.e., patients from the entire United States). Its potential weakness is that not all vaccine-associated adverse events experienced are reported. Therefore, the VAERS database contains a sample of adverse events that occurred following immunization, and hence, reporting of vaccine-associated adverse events must also be evaluated to determine whether systematic error or bias is present in the data examined.

Analysis Methods

In this study, a case-control epidemiological examination of VAERS database (online public access version; reports entered through 31 August 2004) was undertaken using Microsoft Access while employing Hill (1965) criteria framework to assess observed associations.

The neurodevelopmental adverse events analyzed in the present assessment of the VAERS database included: autism (Costart term = Autism), mental retardation (Costart term = Mental Retard), speech disorders (Costart term = Speech Dis), thinking abnormalities (Costart term = Thinking Abnorm), infantile spasms (Costart term = Spasm General), and ataxia (Costart term = Ataxia). Descriptions of these adverse events were based on those reporting them and were coded by VAERS technical staff into defined symptom fields contained in each report.

The Biological Surveillance Summaries of the CDC, as segregated by vaccine manufacturer, determined the number of doses of DTP and DTPH vaccines (1994 through 1998) distributed/administered. The Biological Surveillance Summaries indicated that Lederle Laboratories (Pearl River, NY) distributed

3,571,475 DTP vaccines doses and 33,084,460 DTPH vaccine doses from 1994 through 1998.

A number of controls were employed to determine if systematic error or bias was present among the reported adverse events in the childhood cohorts examined. To evaluate potential bias present in the reporting of adverse events, a series of control adverse events were employed. The control adverse events examined in the present study were selected on an a priori basis as not biologically plausibly linked to an increased risk following additional doses of mercury from Thimerosal-containing vaccines, and included the following outcomes: conjunctivitis (Costart term = Conjunctivitis), encephalitis/encephalopathy (Costart term = Enceph*), urinary-tract infection (Costart term = Infect Urin Tract), and febrile seizures (Costart term = Febrile Seizure).

The distribution, health status, and geographical dispersion of the cohorts analyzed in VAERS were examined because these factors might also affect reporting of adverse events. In determining the distribution of the populations reviewed, the total numbers of male and female reports of adverse events to VAERS were examined. In evaluating the health status of the populations reviewed, the total numbers of reports specifying a past medical history or other medications in VAERS were examined. Similarly, in reviewing the geographical dispersion of the populations analyzed, the total numbers of adverse event reports submitted to VAERS from large representative states from the western (California), central (Illinois), and eastern (Florida) regions of the United States were evaluated.

Statistical Analyses

The premise of equality between the groups examined forms the basis of the null hypothesis employed in this study. Odds ratios (OR), 95% OR confidence intervals (CIs), and *p*- values were determined from the 2 × 2 contingency tables utilized in the present study. The statistical package in StatsDirect (Version 2.4.1) and the nominal Fisher's exact test statistic were used to determine statistical significance. In order for statistical significance testing to be performed for an adverse event, 10 adverse events were required to be identified following administration of the vaccines under study, and a two-sided *p*- value <.05 was accepted as statistically significant.

RESULTS

Table 1 summarizes the population distribution, geographical dispersion, health status, and control events for reported adverse events examined in the VAERS database following DTP vaccines in comparison to DTPH vaccines. It was determined that both cohorts were populations with a similar distribution, health status, and geographical dispersion. The control adverse events of encephalopathy/encephalitis, febrile seizures, urinary-tract infection, and conjunctivitis were reported similarly to VAERS following DTP and DTPH vaccines.

TABLE 1. Summary of the Population Distribution, Geographical Dispersion, Health Status, and Control Events for Reported Adverse Events Examined in the VAERS Database Following DTP Vaccines in Comparison DTPH Vaccines

Outcomes analyzed	Reported incidence per million DTP vaccines [number of reports]	Reported incidence per million DTPH vaccines [number of reports]	Odds ratio for reported events	95% Odds ratio confidence interval for reported events
Total reports	167 [596]	160 [5293]	1.04	0.96–1.1
Male reports	91 [326]	82 [2730]	1.1	0.98–1.2
Female reports	72 [257]	74 [2454]	0.97	0.85–1.4
Past medical histories	137 [490]	133 [4413]	1.03	0.94–1.1
Other medications	111 [396]	112 [3713]	0.99	0.89–1.5
California reports	16 [56]	14 [454]	1.1	0.85–1.5
Illinois reports	3.9 [14]	4.5 [149]	0.87	0.46–1.5
Florida reports	6.7 [24]	5.8 [191]	1.2	0.73–1.8
Febrile seizures	5.0 [18]	5.8 [191]	0.87	0.51–1.4
Encephalitis/ encephalopathy	0.84 [3]	0.85 [28]	0.99	0.19–3.2
Conjunctivitis	0.84 [3]	0.82 [27]	1.03	0.20–3.3
Urinary tract infection	0.28 [1]	0.36 [12]	0.77	0.02–5.2

Note. All *p* values determined using Fisher's exact test statistic. The Biological Surveillance Summaries indicated that 3,571,475 DTP vaccines and 33,084,460 DTPH vaccines were distributed/administered by Lederle Laboratories (Pearl River, NY) from 1994 through 1998, and were used as the denominators to determine the calculated reported incidence rates of adverse events to the VAERS database. Number of reports = number of reports identified in the VAERS database.

In Table 2, a summary of neurodevelopmental disorders reported to VAERS following DTP vaccines in comparison to DTPH vaccines is presented. Specifically, a significant relationship was observed for the incidence rate of neurodevelopmental disorder adverse events reported to VAERS following DTP vaccines in comparison to DTPH vaccines, including autism, speech disorders, mental retardation, infantile spasms, and thinking abnormalities.

DISCUSSION

The results of our examination of the VAERS database showed an association between Thimerosal-containing childhood vaccines and childhood neurodevelopmental disorders. Data demonstrate that a significant risk factor for the development of neurodevelopmental disorders was the amount of mercury children received from Thimerosal-containing childhood immunizations. Importantly, all other neurodevelopmental disorders, except for mental retardation, reflected a higher odds ratio than autism. This argues strongly against the presumed media bias effect of reporting about the alleged association between Thimerosal-containing childhood vaccines and autism. Taken collectively, the increased risk associated with five of the six neurodevelopmental

TABLE 2. Summary of Neurodevelopmental Disorders Reported to VAERS Following DTP Vaccines in Comparison to DTPH Vaccines Administered From 1994 Through 1998

Type of vaccine	Autism	Speech disorders	Mental retardation	Ataxia	Thinking abnormalities	Infantile spasms
Reported incidence per million DTP vaccines [number of reports]	2.2 [8]	2.5 [9]	3.4 [12]	0.84 [3]	1.7 [6]	1.4 [5]
Reported incidence per million DTPH vaccines [number of Reports]	0.82 [27]	0.82 [27]	1.6 [53]	0.24 [8]	0.33 [11]	0.36 [12]
Odds ratio for reported adverse events	2.6 ^a	3.1 ^b	2.1 ^a	3.5	5.1 ^b	3.8 ^a
95% Odds ratio confidence interval for reported adverse events	1.1–6.2	1.3–6.8	1.02–4.0	0.59–14	1.5–15	1.1–12

Note. All *p* values determined using the Fisher's exact test statistic. The Biological Surveillance Summaries indicated that 3,571,475 DTP vaccines and 33,084,460 DTPH vaccines were distributed/administered by Lederle Laboratories (Pearl River, NY) from 1994 through 1998, and were used as the denominators to determine the calculated reported incidence rates of adverse events to the VAERS database. Number of reports = number of reports identified in the VAERS database.

^aSignificant from DTP vaccines (*p* < .05).

^bSignificant from DTP vaccines (*p* < .01).

disorders examined in the present study, any of which could independently be an indicator of possible mercury toxicity, favors an association based on this controlled assessment of the VAERS database.

In considering the CDC-developed epidemiological technique employed in this study, one notes that it continues to be used by the NIP of the CDC to evaluate the safety of vaccines in the VAERS database (Lloyd et al., 2003). Chen and Rosenthal (1996) from NIP proposed that the potential limitations in VAERS database, such as underreporting, erroneous reporting, frequent multiple exposures, multiple outcomes, and lack of precise denominators, should apply equally to both vaccines when administered to similarly aged populations, and allow for determination of accurate, relative, quantitative relationships between vaccines and adverse outcomes. Additionally, a recent review examined the utility of this method to analyze the VAERS database, and concluded that studies examining the VAERS database using the methods of analysis developed by Rosenthal et al. (1996) had good positive predictive value for determining vaccine-associated adverse events that were consistent with observations made in vaccine clinical trials and other databases, including the CDCs VSD database (Geier & Geier, 2004a).

In further considering the results of the present study, it must be noted that none of the children examined in this study from the VAERS database truly constitute a Thimerosal-free population. Within the reports, it was observed that other vaccines containing Thimerosal, such as hepatitis B vaccine or

influenza vaccine, were concurrently administered. The pivotal difference in the total amounts of mercury received from Thimerosal-containing vaccines in the reports of children with adverse events examined in the VAERS database stems from the fact that some children received an approximately 100 µg additional dose of mercury from DTP and Hib vaccines in comparison to those children receiving the combined DTPH vaccine. As a result, the increased risks observed for neurodevelopmental disorders probably represent a considerable underestimate of the true risk of additional doses of Thimerosal from vaccines.

Other sources of mercury, such as anti-Rh₀ immune globulin, seafood, manufacturing plant emissions, dental amalgams, and other pharmaceuticals, while potentially significant, probably had a limited effect on the VAERS results of this study because the populations analyzed were large, and there should have been equal exposures to other sources of mercury among the populations examined. The probability that exposures to other sources of mercury were similar in the DTP and DTPH vaccine cohorts examined in the VAERS database is further supported by the fact that there were similar geographical dispersions and health statuses.

Furthermore, the fact that neurodevelopmental disorders evaluated were underreported following DTP and DTPH vaccines in this examination of the VAERS database in comparison to the published population frequency of these conditions is significant. For example, autism has been reported to affect 1 in approximately 300 children in the United States (Yeargin-Allsopp et al., 2003), whereas based on analysis of the VAERS database, it was found that autism was reported following 1 in 454,545 DTP immunizations. This means that since, on average, children receive about 5 DTP immunizations, about 1 in 90,909 children had autism based on reports submitted to the VAERS database, and thus, the occurrence of autism in children reporting to the VAERS database was about 0.33% of the actual population autism frequency. In considering that neurodevelopmental disorders examined may have been underreported to the VAERS database, it should be noted that the present study was not designed to determine the absolute frequency of neurodevelopmental disorders following immunization, but instead was designed to employ a case-control epidemiological methodology for evaluating the risk of neurodevelopmental disorders following additional doses of mercury from Thimerosal-containing vaccines. The methodology employed in this study, despite the fact that only a fraction of the actual neurodevelopmental disorders that occurred in the populations examined was reported to the VAERS database, should still provide accurate, relative, quantitative relationships between vaccines and adverse outcomes, as long as there was no systematic error or bias present in the data examined in the VAERS database. A series of control adverse events, as well as the distribution, health status, and geographical dispersion of the cohorts analyzed in VAERS, was examined to determine whether there was systematic error or bias present in the data examined in the VAERS database. The results of the present study showed that systematic error or bias were minimally present in the data examined in the VAERS database, and, as such,

suggest that the increased risks observed for neurodevelopmental disorders following additional doses of mercury from Thimerosal-containing vaccines represent genuine phenomena.

In this study, chance significant associations between Thimerosal and neurodevelopmental disorders were also minimized. First, since only a limited number of specific neurodevelopmental disorders were evaluated in the present study (i.e., only 6 neurodevelopmental disorders were examined, and only 18 outcomes all told were examined), and since a p value $< .05$ was considered significant (1 in 20 outcomes would be expected to found significant by chance), one would expect that less than one of the types of outcomes examined in the present assessment of VAERS would by chance be found to be significantly associated with Thimerosal. Second, a series of different types of neurodevelopmental disorders was examined, in VAERS, involving different types of symptoms/syndromes. It was observed that five out of six types of neurodevelopmental disorders examined in VAERS were significantly associated with Thimerosal. This consistency of observation across multiple types of neurodevelopmental disorders argues against the present observations resulting from a mere chance statistical association, or even a simple reporting bias stemming from a presumed association between Thimerosal and a given outcome that resulted in an overreporting of a single type of adverse event.

The present case-control epidemiological assessment of VAERS shows that very specific adverse affects were attributable to Thimerosal. Thimerosal was associated with an increased risk of neurodevelopmental disorders, and potential systematic error or confounding was found to be minimal in VAERS.

Other large population-based epidemiological studies conducted outside the United States that have not shown an apparent relationship between Thimerosal-containing childhood vaccines and neurodevelopmental disorders were conducted in countries (e.g., England, Denmark, and Sweden) utilizing very different exposures to mercury from Thimerosal-containing childhood vaccines (Andrews et al., 2004; Heron et al., 2004; Hviid et al., 2003; Madsen et al., 2003; Stehr-Green et al. 2003). In these countries, children were exposed to doses of mercury from Thimerosal-containing childhood vaccines that were approximately one-third as much as those administered in U.S. childhood vaccines. Additionally, the children in these countries received Thimerosal-containing childhood vaccines on a much less rigorous schedule (i.e., in the United States, mercury dosing from Thimerosal-containing childhood vaccines began on the day of birth, and continued, at periodic intervals, throughout the first 6 mo of life). Additionally, the studies in Denmark have been shown to suffer from the fact that (1) only inpatient diagnosed autistics were initially identified, and then subsequently in these studies both inpatient and outpatient diagnosed autistics were identified, (2) different diagnosis codes of neurodevelopmental maladies, that is, psychosis infantilis posterior (ICD-8 299.01) versus atypical (i.e., regressive) autism (ICD-10 F84.1), before and during the presumed increase in autism incidence, respectively, were used, and (3) data from additional clinics with a significant portion

of the autistics in the entire country were added as the studies progressed (Geier & Geier, 2004c, 2005a).

Outside of the authors' epidemiological examinations of the relationship between Thimerosal-containing vaccines and neurodevelopmental disorders, the only other study conducted in the United States is by Verstraeten et al. (2003) from the CDC, who initially found a significant relationship between Thimerosal-containing childhood vaccines and some types of neurodevelopmental disorders, but on further examination of a different data set did not find a consistent effect. The lead author concluded that their study was neutral (i.e., could neither accept nor reject a causal relationship) regarding the relationship between Thimerosal and neurodevelopmental disorders (Verstraeten, 2004).

In addition to large population-based epidemiological studies on Thimerosal, additional extensive clinical and molecular studies were conducted that support the plausibility of Thimerosal-containing childhood vaccines inducing neurodevelopmental disorders in children. Bradstreet et al. (2003) showed following chelation that there were approximately six times significantly greater urinary mercury concentrations among autistics matched to neurotypical children, whereas autistics and matched neurotypical children had similar urinary cadmium and lead concentrations. Similar urinary mercury concentrations were observed among matched vaccinated and unvaccinated neurotypical children following chelation. Similarly, Holmes et al. (2003) demonstrated the ability to find excreted mercury in first baby haircuts was inversely proportional to the severity of autistics, which on the whole was very low compared to nonautistic matched controls. Together, these clinical observations suggest that autistics have significantly higher body burdens of mercury than neurotypical children following prenatal/infant exposures to mercury.

The biochemical and genomic bases for the increased body burden of mercury in autistic children were identified. James et al. (2004) evaluated the methionine cycle and transsulfuration metabolites in autistic children in comparison to age- and gender-matched control children. It was determined that there were significant decreases in the plasma concentration of cysteine (19% reduction) and glutathione (46% reduction), and autistic children had significantly increased oxidative stress (threefold decrease in glutathione/oxidized glutathione redox ratio) in comparison to control children. Researchers also identified specific genomic polymorphisms for enzymes in the methionine cycle and transsulfuration pathways in autistic children that help to account for the distinct transsulfuration metabolite profiles observed by James et al. (2004) in autistic children (Boris et al., 2004).

The inability to properly eliminate mercury is particularly troubling since it was shown by Gasset et al. (1975) and confirmed by Slikker (2000) from the Food and Drug Administration (FDA) that Thimerosal crosses the blood-brain and placental barriers, and results in appreciable mercury content in tissues, including the brain. Burbacher et al. (2005) reported on the half-life of mercury from Thimerosal in the brain of infant monkeys following injection of

doses of mercury comparable to the dosing schedule (weight- and age-adjusted) U.S. children received during the 1990s. They determined that the overall half-life of total mercury (inorganic and organic) in the brain was approximately 24 d. In addition, it was determined that the inorganic mercury levels in the brains averaged 16 ppb following the dosing schedule, and the half-life of the inorganic mercury was found to be very long in the monkey brains (>120 d).

Furthermore, in molecular studies, Baskin et al. (2003) and Humphrey et al. (2005) demonstrated that micromolar concentrations of Thimerosal induced membrane and DNA damage, and initiated caspase-3-dependent apoptosis in human neurons and fibroblasts within hrs of exposure. Leong et al. (2001) also demonstrated that nanomolar concentrations of inorganic mercury markedly disrupted membrane structure and linear growth rates of imaged neurites. Similar results were observed in tissue culture systems with Thimerosal (Brunner et al., 1991; Parry, 1993; Wallin & Hartely-Asp, 1993).

In addition, it has been reported that the neurotoxicity of Thimerosal is associated with depletion of glutathione. The ethylmercury in Thimerosal binds to cysteine thiol (-SH) groups on intracellular proteins and inactivates their function. The cysteine -SH group of glutathione binds mercury and protects essential proteins from functional inactivation. Glutathione is the major mechanism of mercury excretion, and individuals with genetic deficiencies in glutathione synthesis are less able to excrete mercury and are more sensitive to its adverse effects (James et al., 2005).

Waly et al. (2004) reported that methylation events play a critical role in the ability of growth factors to promote normal development. The authors found that insulin-like growth factor-1 (IGF-1)-stimulated and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells occurred via a PI3-kinase- and MAP-kinase-dependent mechanism. Thimerosal at 1 nM significantly inhibited both IGF-1- and dopamine-stimulated methylation, and reduced MS activity. The authors concluded that the discovery of the PI3-kinase/MAP-kinase/MS pathway, and of its potent inhibition by Thimerosal, a vaccine component, provides a molecular explanation for how increased use of vaccines might promote an increase in the incidence of autism and attention deficit hyperactivity disorder (ADHD). In addition, Deth and Waly (2004) reported that folate-dependent phospholipid methylation in the lymphoblasts of autistics was, in a dose-response manner, significantly more sensitive to Thimerosal exposure than in unaffected siblings.

Parran et al. (2005) reported that signaling through neurotrophic receptors is necessary for differentiation and survival of the developing nervous system. Parran et al. (2005) examined the effects of Thimerosal on nerve growth factor signal transduction and cell death in a human neuroblastoma cell line (SH-SY5Y cells). It was evident that at that concurrent exposure to increasing low nanomolar concentrations of Thimerosal and nerve growth factor resulted in a concentration-dependent decrease in nerve growth factor signal transduction. Additionally, it was determined that significant neuronal

death was observed following 48 h of exposure to Thimerosal at concentrations as low as 4.35 nM.

In addition to molecular studies, Hornig et al. (2004) administered Thimerosal to mice, mimicking the U.S. routine childhood immunization schedule of the 1990s (weight and age adjusted), and observed autistic symptoms in a susceptible mouse strain that included growth delay, reduced locomotion, exaggerated response to novelty, increased brain size, decreased numbers of Purkinje cells, significant abnormalities in brain architecture, affecting areas subserving emotion and cognition, and densely packed hyperchromic hippocampal neurons with altered glutamate receptors and transporters. In addition, Digar et al. (1987) showed that exposure to Thimerosal from injection of a single 50- μ g mercury dose at specific prenatal developmental stages in an animal model resulted in significant fetal lethality and teratogenicity compared to controls.

Sager et al. (1984) demonstrated that males are considerably more sensitive than females to the neurotoxic effects of mercury. It has been observed in some human fetal/infant populations exposed to low-dose alkylmercury that males were more sensitive than females to psychomotor retardation, suggesting an interaction between testosterone and mercury toxicity (Clarkson et al., 1985; Geier & Geier, 2005b; Grandjean et al., 1998). It should also be noted that neurodevelopmental disorders in the United States are significantly more prevalent in males than females (Blaxill, 2004; Blaxill et al., 2003; California Department of Developmental Services, 2003; Newschaffer et al., 2005).

Recently, the Environmental Working Group (EWG) issued a report following an extensive investigation into the relationship between mercury exposure, especially mercury exposure from Thimerosal-containing childhood vaccines, and autistic disorders (Environmental Working Group, 2004). They reported that a signature metabolic impairment or biomarker in autistic children suggests that these children would be susceptible to the harmful effects of mercury exposure. The EWG concluded that these new findings significantly strengthen the possibility that mercury might induce or contribute to autism and other neurodevelopmental disorders, by identifying a metabolic imbalance common to nearly all autistic children that would make these children poorly equipped to mount a defense against a number of neurotoxic compounds, including mercury. In addition, they concluded that these findings raise serious concerns about the studies that have allegedly proven the safety of mercury in vaccines.

CONCLUSION

Despite a conclusion by the Institute of Medicine in 2004 that there is no relationship between Thimerosal and autism, and that no further studies should be conducted to evaluate the relationship between Thimerosal and autism, it is clear from these data, and other recently emerging data that have been published, that additional neurodevelopmental disorder research should be

undertaken in the context of evaluating mercury-associated exposures. This is especially true in light of another Institute of Medicine report in 2005 that has called into question handling of vaccine safety data by the National Immunization Program of the CDC.

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