An assessment of downward trends in neurodevelopmental disorders in the United States following removal of thimerosal from childhood vaccines

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Summary

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.

key words: Autism Spectrum Disorder • Merthiolate • Thimerasol • Thiomersal

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**BACKGROUND**

Thimerosal is an ethylmercury-containing compound (49.6% mercury by weight) that was historically added to many vaccines at the preservative level (0.005 to 0.01%). The Centers for Disease Control and Prevention (CDC) from the late 1980s through the 1990s expanded the number of doses of Thimerosal-containing vaccines to be administered to US infants. The routine childhood immunization was gradually expanded from administration of five doses of Thimerosal-containing Diphtheria-Tetanus-whole-cell-Pertussis (DTP) vaccine (the first dose being administered at two months of age) to eventually include three doses of Thimerosal-containing hepatitis B vaccine (the first dose administered on the day of birth), and four doses of Thimerosal-containing Haemophilus Influenzae type b (Hib) vaccine (the first dose administered at two months of age). Additionally, the CDC also began to recommend that three doses of Thimerosal-containing influenza vaccine be administered to certain infant populations (the first dose administered at sixth months of age). As a result under the expanded childhood immunization schedule of the CDC, if infants received all Thimerosal-containing vaccines, their total mercury exposure could have been as high as 200 micrograms (μg) of mercury during the first six months of life [1]. In response to theoretical concerns about the cumulative doses of mercury children received from Thimerosal-containing vaccines, on July 7, 1999 the American Academy of Pediatrics and the United States Public Health Service issued a joint statement calling for the removal of Thimerosal from all childhood vaccines. It has been estimated that the last Thimerosal-containing hepatitis B, Diphtheria-Tetanus-acellular-pertussis (DTaP) and Hib vaccines were manufactured in 2000-2001, and expired at the end of 2002 (or early 2003) [1]. Table 1 summarizes the recent significant historical dates in the use of Thimerosal in pediatric vaccines administered in the US.

In evaluating the dose of mercury some children received from Thimerosal-containing vaccines in the US, when factoring in significant environmental exposure (i.e. mercury in breast milk), it has been estimated the mercury in Thimerosal-containing vaccines represented almost 50% of the total mercury dose infants received [2]. As a result, it has been determined that some infants receiving 187.5 μg of mercury from Thimerosal-containing vaccines during the first sixth months from the routine childhood vaccination schedule, in combination with environmental exposure from mercury in breast milk (164 μg of mercury), were exposed to cumulative doses of mercury during the first sixth months of life in excess of the methylmercury safety guidelines established by the US Environmental Protection Agency (EPA), Health Canada, the World Health Organization (WHO), the Agency for Toxic Substances Disease Registry (ATSDR), and the US Food and Drug Administration (FDA) [2]. It was also determined that these same infants (with no additional exposure to mercury from any source) were in excess of the methylmercury guidelines established by the EPA, Health Canada, WHO, and the ATSDR for the entire first year of life [2].

Despite this fact, Thimerosal is still routinely added to required vaccines administered to US infants (e.g. influenza), as well as to several other vaccines (e.g. Tetanus-diphtheria, meningitis, and monovalent tetanus). In 2004, the Institute of Medicine (IOM) of the United States National Academy of Sciences has backed away from the stated goal issued by the American Academy of Pediatrics and US Public Health Service in 1999 that Thimerosal be removed from US vaccines as soon as possible [3]. Furthermore, many nations still add Thimerosal to many of their pediatric vaccines. The WHO and several vaccine manufacturers still advocate the continued use of Thimerosal in pediatric vaccines.

In a series of previous epidemiological studies various databases, including the Vaccine Adverse Event Reporting System (VAERS), US Department of Education, and the Vaccine Safety Datalink (VSD) database, have been examined, and 2- to 8-fold significantly increased risks, depending upon the symptoms or outcomes examined, for neurodevelopmental disorders have been observed following administration of Thimerosal-containing childhood vaccines [4-10]. In these studies it was hypothesized that the removal of Thimerosal from childhood vaccines in the United States would reduce the number of neurodevelopmental disorders in the United States.

Outside of our own studies, there has only been one epidemiological study conducted in the United States that examined the relationship between Thimerosal-containing vaccines and neurodevelopmental disorders [11]. This study, by Verstaeten et al. from the CDC, initially found a significant relationship between Thimerosal-containing childhood vaccines and some types of neurodevelopmental disorders, but upon further examination of a different dataset, it did not find a consistent effect. The lead author concluded that this study was neutral (i.e. could neither accept nor reject a causal relationship) regarding the relationship between Thimerosal and neurodevelopmental disorders [12].

In light of the fact that now for the first time, there are a number of children that have received reduced doses of mercury from Thimerosal-containing childhood vaccines for a number of years, the present retrospective study was undertaken to see if there was a statistical correlation between decreasing doses of mercury from Thimerosal-containing vaccines with decreasing trends in new neurodevelopmental disorders in the United States.

**MATERIAL AND METHODS**

The Vaccine Adverse Events Reporting System (VAERS) database

In order to conduct the present epidemiological study, an evaluation of the VAERS database was undertaken. The VAERS database is an epidemiological database that has been maintained by the CDC since 1990 as a surveillance tool 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 reported that less than 5% of the total adverse events reported to VAERS are reported by parents. The VAERS Working Group of the CDC and the Food and Drug Administration (FDA) analyze and publish epidemiologic studies based upon analyses of VAERS. They note that VAERS is simple to use, flexible by design, and the data are available in a timely fashion, but warn that the potential lim-
In this study, the online public access VAERS database (updated through December 31, 2004) was examined using Microsoft Access™. The entire database was surveyed for duplicate reports (i.e., having the same VAERS ID number), and these were eliminated.

In analyzing the VAERS database, an ecological epidemiological methodology was employed to evaluate neurodevelopmental disorder adverse events reported following immunizations whilst employing a Bradford Hill criteria framework to assess observed statistical correlations [14].

The neurodevelopmental disorders examined in the present study include autism (Costart Term = Autism), mental retardation (Costart Term = Mental Retard), and speech disorders (Costart Term = Speech Dis among children ≤5 years-old). Descriptions of these adverse events were based upon those reporting them, and coded by VAERS technical staff into defined symptom fields contained in each report. In evaluating the neurodevelopmental disorder adverse events analyzed in the present study, the total number of neurodevelopmental disorder adverse event reports received by the VAERS database on a yearly basis (1991 through 2004) and the total number of neurodevelopmental disorder adverse event reports by year of vaccine administration in the VAERS database (1991 through 2004) were determined.

In order to adjust for potential yearly differences in reporting rates to the VAERS database, the total number of specific...
ic types of neurodevelopmental disorders adverse event reports received by VAERS on a yearly basis was divided by the yearly total number of adverse event reports received by the VAERS database on a yearly basis from 1991 through 2004 among children ≤5 years-old. Similarly, the total number of specific types of neurodevelopmental disorders by year of vaccine administration analyzed in the VAERS were divided by the yearly total number of adverse event reports reported to the VAERS database by year of vaccine administration reported to the VAERS database from 1991 through 2004 among children ≤5 years-old. The resulting proportion was then compared against other years in order to evaluate potential trends for neurodevelopmental disorders in the VAERS database.

Controls

In order to examine if there were biases/confounding present in the data examined in VAERS, a control adverse event of accidental injury (Costart Term = Injury Accid; among children ≤5 years-old) was selected on an a priori basis as not being biologically plausibly linked to mercury exposure, and was analyzed using the same analytical methods employed to evaluate neurodevelopmental disorder adverse events.

Statistical analysis

Table 2 summarizes the raw data analyzed from the VAERS database in the present study. The yearly proportions of neurodevelopmental disorders were statistically evaluated to determine if the removal of Thimerosal from childhood vaccines produced a discernable trend in the VAERS database. The null hypothesis employed in the present study was that the yearly proportion of neurodevelopmental disorders should not be affected by the removal of Thimerosal from childhood vaccines. The statistical package contained in StatsDirect (Version 2.4.2) was employed, and 2×2 contingency tables and the nominal Yates χ² statistical test was utilized to determine odds ratios (OR), 95% CIs, and p-values when comparing the yearly proportion of neurodevelopmental disorders reported to the VAERS database. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Figure 1 summarizes the yearly proportion of neurodevelopmental disorders reported to the VAERS database by date received. It was found that the maximum proportion of neurodevelopmental disorder adverse events occurred among reports entered into the VAERS database around 2001–2002. It was observed that following 2002, there was a sharp decrease in proportion of neurodevelopmental disorders entered into the VAERS database. It was observed that 2003 and 2004 had significantly reduced proportions of autism adverse events in comparison to 2002. Specifically, it was determined that there were significantly reduced proportions of autism adverse events received by the VAERS database for 2003 (OR=0.63, p<0.0001 95% CI=0.50–0.78, χ²=17.6) and 2004 (OR=0.46, p<0.0001, 95% CI=0.36–0.58, χ²=42) in comparison to the maximum year of 2002, and that there was a significantly reduced proportion of autism adverse events received by the VAERS database for 2004 in comparison to 2003 (OR=0.73, p<0.02, 95% CI=0.56–0.94, χ²=5.5). Similar results were observed for speech disorders and mental retardation.
Figure 2 summarizes the yearly proportion of neurodevelopmental disorders reported to the VAERS database by date of vaccine administration. It was observed that the maximum proportion of neurodevelopmental disorder adverse events occurred among reports indicating vaccines that were administered in 1998. It was observed that vaccines administered post-1998 had progressively decreasing proportions of neurodevelopmental disorders reported to the VAERS database. It was observed that, for vaccines administered from 1999 through 2001, there were significant decreases in the proportion of autism adverse events reported to the VAERS database in comparison to vaccines administered in 1998. It was also observed that vaccines administered in 1997 (OR=0.50, p<0.003, 95% CI=0.35–0.72, \( \chi^2=13.5 \)) had significant decreases in the proportion of autism adverse events reported to the VAERS database in comparison to vaccines administered in 1999, and that vaccines administered in 2000 (OR=0.64 p<0.02, 95% CI=0.46–0.90, \( \chi^2=6.31 \) ) and 2001 (OR=0.46, p<0.0001, 95% CI=0.32–0.65, \( \chi^2=18.8 \) ) had significant decreases in the proportion of autism adverse events reported to the VAERS database in comparison to vaccines administered in 1999, and that vaccines administered in

**DISCUSSION**

The results of the present study indicate that the removal of Thimerosal from childhood vaccines has been corre-
lated with a substantial reduction in neurodevelopmental disorders reported to the VAERS database following childhood immunizations. Specifically, it is observed that there was an overall reduction in total reports of neurodevelopmental disorders reported to the VAERS database following vaccination, and a significant reduction in the proportion of neurodevelopmental disorders reported to the VAERS following vaccination, in both cases, in the time period when Thimerosal was being removed from childhood vaccines in the United States.

The ecological methodology employed in the present study represents a novel way in which to monitor adverse event reports to the VAERS database. In this study, both the date the report was received for an adverse event and the date when a vaccine was administered in adverse event reports were examined.

Analyzing the date on which the report was received for an adverse event represents a method which accounts for the potential time-lag necessary for a particular individual to be diagnosed with a condition and to submit an adverse event report to the VAERS database. It would be expected that whatever the time-lag necessary, it should apply equally to all years under study. In the present study, it appears that there is a 3- to 4-year lag between a when a vaccine is administered and when a neurodevelopmental disorder adverse event report is submitted to the VAERS. It was observed reports entered into the VAERS database during the early- to mid-1990s had comparatively few reports of neurodevelopmental disorders. It was only during the late 1990s and early 2000s that a substantial increase in the proportion of neurodevelopmental disorders reported to the VAERS database was noted. The maximum years for neurodevelopmental disorders reported to the VAERS database were in 2001–2002. Then, following this maximum, in 2003 and 2004, the total number of neurodevelopmental disorders decreased. Assuming that the aforementioned pattern of neurodevelopmental disorders yearly entered into the VAERS database is related to the amount of Thimerosal in childhood vaccines, and based upon the assumption that there is a 3- to 4-year lag in the reporting of neurodevelopmental disorders, this would mean that reports of neurodevelopmental disorders entered into the VAERS database in the early- to mid-1990s reflect doses of mercury from Thimerosal-containing vaccines administered to children during the first 6 months of life (i.e. with 3 DTP immunizations, and dosing beginning at 2 months of age). Then, during the mid- to late-1990s, as was stated previously, this amount rose because of the implementation of a new policy to administer Hib and hepatitis B as part of the routine childhood immunization schedule. This resulted in children being exposed to approximately 187.5 micrograms of mercury during the first six months of life, if all Thimerosal-containing vaccines were administered (i.e. with the addition of Hib and hepatitis B vaccines, and dosing beginning at birth), and some children even receiving 200 micrograms of mercury during the first 6 months of life (i.e. if influenza vaccines were administered, as was recommended for certain populations). In mid-1999, it was recommended that Thimerosal begin to be removed from childhood vaccines, and certain Thimerosal-containing childhood vaccines (i.e. hepatitis B vaccines) be delayed for at least several months [1]. Based upon the observations made in this study, this was correlated with a substantial significant decrease in neurodevelopmental disorders reported to VAERS.

The aforementioned assumptions seem to have been borne-out regarding the necessary lag-time between immunization and when a neurodevelopmental disorder is entered into the VAERS database, based upon the analysis in this study that was conducted for neurodevelopmental disorders reported to the VAERS database by the date of vaccine administration. It was observed that the number of neurodevelopmental disorders increased following vaccines administered in the early 1990s through 1998, when a maximum was reached for neurodevelopmental disorders in VAERS. In the years for vaccine administration since 1998 in the VAERS, it was observed that significant decreases occurred in the number of neurodevelopmental disorders. It is apparent that the decreases observed in neurodevelopmental disorders following vaccines administered post-1998 in VAERS, appear to be reflected in the data for neurodevelopmental disorders reports received by VAERS in 2003 and 2004.

As a result of the 3- to 4-year lag-time necessary for a neurodevelopmental disorder to be entered into VAERS, one should be cautious in interpreting the post-2001 date of vaccine administration data. The documented further decreases in neurodevelopmental disorders observed in the post-2001 vaccine administration data may in part reflect that not enough time has elapsed between vaccine administration and the date a neurodevelopmental disorder report will be received by VAERS, assuming there is a causal relationship.

The 3- to 4-year lag-time between the date of vaccine administration and the date the neurodevelopmental disorder report is received by the VAERS database is consistent with previous epidemiological studies that have examined the age at which neurodevelopmental disorders are diagnosed (i.e. because most primary childhood vaccines are administered during the first 1.5 years of life and neurodevelopmental disorders are usually diagnosed by age 3 to 4 years-old). For example, the CDC has previously published that the approximate median age of diagnosis for autism (3.9 years), speech or language delay (2.9 years), and coordination disorder (3.8 years) are all consistent with the 3- to 4-year lag-time between the date of vaccine administration and the date a neurodevelopmental disorder report is received by the VAERS database [11].

In further considering the methodology employed in the present study, the proportion of neurodevelopmental disorders was evaluated for each year examined in the VAERS database, in addition to simply analyzing the overall crude number of reports in VAERS. As a result of analyzing the yearly proportion of neurodevelopmental disorders, it was possible to take into account potential yearly variations in the number of reports submitted to the VAERS database. It was observed that similar trends were seen regardless of whether the yearly total number of neurodevelopmental disorders or the proportion of neurodevelopmental disorders was examined in VAERS.
Also, in considering the results from the present study, it appears that they do not reflect mere chance or biased/confounded results. It could be hypothesized that knowledge, on the part of either parents or physicians, of an association between Thimerosal and autism, could have resulted in an increased/decreased willingness to report such conditions following immunization as Thimerosal was introduced/removed from childhood vaccines. The results of the present study argue against biases or confounding being potentially responsible for the initial increase and subsequent decrease in neurodevelopmental disorders reported to the VAERS database. First, by examining neurodevelopmental disorders such as speech disorders and mental retardation, in addition to just autism in the VAERS database, it was possible to detect whether similar increases/decreases have occurred for other types of neurodevelopmental disorder conditions that have never been associated, in the public’s mind, with Thimerosal exposure. The results of our study indicate that there were not only substantial initial increases and subsequent decreases in autism adverse events reported to the VAERS database, but also substantial initial increases and subsequent decreases for the other types of neurodevelopmental disorders examined in VAERS, and in some cases, the initial increases and subsequent decreases in these disorders was even more significant than for autism. Second, the specific temporal time periods during which the increases and decreases in neurodevelopmental disorders were observed in the VAERS database, appear to correlate the amounts of mercury children presumably received from Thimerosal-containing vaccines. Third, additional observations in the VAERS database for data that has began to be reported for 2005 indicate that similar decreasing trends, in neurodevelopmental disorders reports received, have occurred. For example, in extrapolating data from the online public VAERS database (updated through March 31, 2005, and extrapolated through the end of the year 2005 by multiplying the total number of reports observed for each type of neurodevelopmental disorder by four) results indicate that only 80 autism, 32 speech disorder, and 32 mental retardation adverse events will be received by the VAERS database for the year 2005. Fourth, the observed odds ratios, when comparing the proportions of neurodevelopmental disorders reported following the years in which Thimerosal was reduced in comparison to the years prior to its reduction, are similar to those that have been reported in previous epidemiological studies examining the correlation between Thimerosal-containing vaccines and neurodevelopmental disorders [4-10]. Fifth, it was observed when examining the control adverse event of accidental injury that was selected on an a priori basis for not biologically plausibly being linked to mercury exposure from Thimerosal-containing vaccines, that it was reported to the VAERS database in a different pattern than for the neurodevelopmental disorders evaluated in the VAERS database. The combination of these factors argue strongly against fluctuations in neurodevelopmental disorders evaluated in the VAERS database simply reflecting mere chance or biased/confounded observations.

In considering the utility of the VAERS database to evaluate vaccine safety by examining trends in reported adverse events, studies by FDA/CDC researchers have previously employed such methods to review and draw epidemiological conclusions about the safety of vaccines based upon analysis of the VAERS [15–20]. In addition, we have authored a recent review examining the utility the VAERS database to detect vaccine safety concerns and have concluded that studies examining the VAERS database offer good positive predictive value for determining vaccine-associated adverse events. These predictive values were consistent with observations made in vaccine clinical trials and other databases, including the CDC’s VSD database [21].

Some large-population based epidemiological studies conducted outside the United States have not shown an apparent relationship between Thimerosal-containing childhood vaccines and neurodevelopmental disorders [22–26], but these have been criticized for their applicability to the US experience with Thimerosal-containing vaccines and for their methodology [5,9,10,27–30].

The observed trends in neurodevelopmental disorders determined from the results of the present study appear to be compatible with population observations in trends in neurodevelopmental disorders. 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 [31–38]. 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 [35]. 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 [35–38].

In considering neurodevelopmental disorders, autism is a neurodevelopmental syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, and stereotypic abnormal movements [39]. While genetic factors are recognized as being important in the pathogenesis of autistic disorders, a role for environmental factors has received considerable attention. Researchers have previously reported that exposure to mercury can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autistic disorders, and the similarities extend to neuroanatomy, neurotransmitters, and biochemistry [39–42]. Furthermore, in recent research observing children’s communicative, social, affective, repetitive behaviors, and toy play coded from videotapes of the toddlers’ first and second birthday parties that there are significant numbers of children with regressive autistic disorders that manifest between the ages of 12 and 24 months of age [43], a temporal period directly coinciding with administration of mercury from Thimerosal-containing childhood vaccines.

The biological plausibility of the present results are further supported by recently emerging extensive toxicokinetic, molecular, and animal model studies showing that administration of Thimerosal-containing childhood vaccines correlated with a significant number of neurodevelopmental disorders in children [44–55].

Burbacher et al. have evaluated infant monkeys following injection of doses of mercury comparable to the dosing schedule (weight- and age-adjusted) [44]. These researchers con-
confirmed that Thimerosal crosses the blood-brain barrier and results in appreciable mercury content in tissues including the brain. They 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) US children received during the 1990s. They determined that the overall half-life of mercury in the brain of the infant monkeys examined was approximately 24 days. In addition, it was determined that the percentage of inorganic mercury in the brains of the Thimerosal-treated infant monkeys averaged 16 parts-per-billion following the dosing schedule, and the half-life of this inorganic mercury was found to be very long in the monkey brains (>120 days).

In a series of molecular studies with neurons it has now been shown that nanomolar (nM) to micromolar (μM) concentrations of Thimerosal are capable of inducing neuronal death, neurodegeneration, membrane damage, and DNA damage within hours of exposure [45–50]. Additionally, it has also been shown that nM to μM concentrations of Thimerosal are capable of disrupting critical signaling pathways/biochemical events necessary for neurons to undergo normal neuronal development [51–53].

Hornig et al. administered Thimerosal to mice, mimicking the United States’ 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 [54]. In addition, Diggel et al. showed exposure to Thimerosal from injection of a single 50 μg of mercury dose at specific prenatal developmental stages in an animal model resulted in significant fetal lethality and teratogenicity compared to controls [55].

CONCLUSIONS

Despite conclusions by the IOM in 2004, largely based upon examination of vaccine safety data from the National Immunization Program (NIP) of the CDC, 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 [3], it is clear from these data and other emerging data that have been recently published, that additional neurodevelopmental disorder research should be undertaken in the context of evaluating mercury-associated exposures, especially from Thimerosal-containing vaccines. Furthermore, studies should also be undertaken to evaluate additional databases registries to evaluate the compatibility of the present results with trends in neurodevelopmental disorders in other US populations. This is especially true in light of the fact that the handling of vaccine safety data by the NIP of the CDC has recently been called into question by the IOM in 2005 [56].

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