Research Article

The risk of neurodevelopmental disorders following a Thimerosal-preserved DTaP formulation in comparison to its Thimerosal-reduced formulation in the vaccine adverse event reporting system (VAERS)

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Abstract: Mercury (Hg) exposure in human infants and fetuses has long been known to be significantly associated with neurodevelopmental disorders (NDs). Thimerosal (49.55% Hg by weight) is an ethyl-Hg containing compound added to many childhood vaccines as a preservative. A hypothesis testing case-control study was undertaken in the Vaccine Adverse Event Reporting System (VAERS) database (updated through September 2013) by examining 5,591 adverse event reports entered following Thimerosal-preserved Diphtheria-Tetanus-acellular-Pertussis (DTaP) (Tripedia™, Sanofi) administered from 1997-1999 (exposed) and following Thimerosal-reduced DTaP (Tripedia™, Sanofi) administered from 2004-2006 (unexposed). Cases were defined as individuals with adverse event reports with the outcomes of autism, speech disorder, mental retardation, or ND (at least of one these aforementioned specific outcomes being mentioned in the adverse event report). Controls were defined as individuals with adverse event reports without any mention of the specific case outcomes examined. Cases reported with the outcomes of autism (odds ratio = 7.67, $p < 0.0001$), speech disorders (odds ratio = 3.49, $p < 0.02$), mental retardation (odds ratio = 8.73, $p < 0.0005$), or ND (odds ratio = 4.82, $p < 0.0001$) were significantly more likely than controls to have received Thimerosal-preserved DTaP vaccine (exposed) in comparison to Thimerosal-reduced DTaP vaccine (unexposed). Though routine childhood vaccination is considered an important public health tool to reduce the morbidity and mortality associated with certain infectious diseases, this study supports a significant relationship between increased organic-Hg exposure from Thimerosal-preserved childhood vaccines and the child's subsequent risk of a ND diagnosis.

Keywords: autistic, developmental delay, ethylmercury, merthiolate, language, thiomersal

Introduction

Mercury (Hg) is a heavy metal that is widespread and persistent in the environment, and infants in the US are exposed to significant levels of environmental Hg through air, water, and breast milk [1]. In addition to environmental Hg exposure and maternal exposures from the mother's Hg body burden, dietary intakes, and Hg-containing pharmaceuticals administered to the mother while the child is developing in utero, and injected organic-Hg from

Thimerosal-preserved childhood vaccines have been and, in many countries, remain a significant source of Hg exposure for many infants during the first year of life [1, 2].

Thimerosal is an organic-Hg compound (49.55% Hg weight) added to vaccines as a preservative, typically at concentrations from 0.005% to 0.01% (12.5 µg Hg to 25 µg Hg per 0.5 mL vaccine dose) [3]. Thimerosal is known to rapidly dissociate into ethyl-Hg chloride, ethyl-Hg hydroxide, and sodium thiosalicylate in saline solutions. As a result of adherence to the recommended routine childhood vaccination schedule in the US during the 1990s infants may have been exposed to bolus doses of Hg nominally ranging from 12.5 µg Hg to 62.5 µg Hg that collectively amounted to nominally 200 µg Hg from Thimerosal-containing childhood vaccines during the first six months of life (> 50% of all Hg exposure when considering environmental sources of Hg) [2, 3]. This dosing pattern continues unabated in many developing nations to the present day. Moreover, despite a call for the removal of Thimerosal from all vaccines in the United States on July 7, 1999 by the American Academy of Pediatrics (AAP) and US Public Health Service (USPHS) [4], many children in the US continue to receive significant doses of Hg from the routinely recommended administration of Thimerosal-preserved inactivated-influenza vaccines (where more than 50% of all doses of inactivated-influenza vaccine continue to contain 0.01% Thimerosal) to pregnant women, infants, and young children [3].

The purpose of the present study was to evaluate concerns about the toxic effects of organic-Hg exposure from Thimerosal-preserved childhood vaccines by conducting a hypothesis-testing case-control study in the Vaccine Adverse Event Reporting System (VAERS) database.

Methods

The VAERS is an epidemiological database that has been maintained jointly by the US Centers for Disease Control and Prevention (CDC) and US Food and Drug Administration (FDA) 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, but other adverse events that occur following vaccine administration are passively reported to VAERS. The VAERS Working Group of the CDC has previously acknowledged that less than 5% of the total adverse events reported to VAERS are reported by parents. Specific serious adverse events and deaths reported to VAERS are followed-up by the CDC/FDA. The VAERS Working Group of the CDC and the FDA have repeatedly analyzed and published epidemiologic studies based upon VAERS [5, 6].

The VAERS Working Group notes that VAERS is simple to use, flexible by design, and the data are available in a timely fashion, but it also warns that the potential limitations may include systematic error due to underreporting, erroneous reporting, frequent multiple exposures, multiple outcomes and lack of precise denominators. In addition, when evaluating data from VAERS, it is important to note that, for any reported event, no cause and effect relationship has been established. VAERS is interested in all potential associations between vaccines and adverse events. Therefore, VAERS collects information on any adverse event following vaccination reported by an individual associating the adverse event with vaccination, be it coincidental or truly caused by a vaccine [5, 6].

Determining the Population at Risk

An analysis of the VAERS updated through September 17, 2013 was undertaken using the CDC Wonder online computer interface (http://wonder.cdc.gov/vaers.html). This portal provides a direct method for independent investigators to rapidly analyze up-to-date data in VAERS. Adverse event reports associated with Diphtheria-Tetanus-acellular-Pertussis (DTaP) (Tripedia™, Sanofi, VAERS Code = 242) vaccine administered from January 1997 through 1999 and from January 2004 through September 2006 with a listed residence in the United States and a specified gender were used to identify cases and controls in the present study. Overall, a total of 5,591 adverse event reports (2,995 adverse event reports in males and a total of 2,596 adverse event reports in females) were examined in the present study.

Determining Cases

The cases were selected from the 5,591 total adverse event reports examined in the present study, and were defined as biologically plausibly Hg-associated adverse event reports in VAERS with outcomes specified as autism (VAERS code: 10003805), speech disorder (VAERS code: 10041466), mental retardation (VAERS code: 10027378), and neurodevelopmental disorder (ND) (defined as one or more of the following VAERS codes in an adverse event report: 10003805, 10041466, or 10027378). In addition, cases were also selected from the 5,591 total adverse event reports examined in the present study, and were defined as biologically non-plausibly Hg-associated adverse event reports in VAERS with outcomes specified as injury (VAERS code: 10022116), pneumonia (VAERS code: 10035664), or injection site pain (VAERS code: 10022086). Table 1 summarizes the gender breakdown for each type of each case type examined in the present study.

Determining Controls

The controls were selected from the 5,591 total adverse event reports examined in the present study. The controls were selected for each type of case outcome examined by including only those adverse event reports that did not include the specific type of case outcome under study as controls. Table 1 summarizes the gender breakdown of controls for each case type of examined.

Determining exposure

Exposure was determined in the present study based upon the date of DTaP vaccine administration. It was

Table 1 - A summary of various types of cases and controls examined in the present study

Group Examined (VAERS Code)	Number Males	Number Females	Male/Female Ratio
Neurodevelopmental Disorders:			
Autism Cases (10003805)	24	4	6
Controls	2,971	2,592	1.15
Speech Disorder Cases (10041466)	20	4	5
Controls	2,975	2,592	1.15
Mental Retardation Cases (10027378)	10	11	0.91
Controls	2,985	2,585	1.15
Neurodevelopmental Disorder Cases[1] (10003805, 10041466, 10027378)	44	18	2.4
Controls	2,951	2,578	1.15
Non-Mercury Related Disorders:			
Injury (10022116) - Cases	15	7	2.14
Controls	2,980	2,589	1.15
Pneumonia (10035664) - Cases	16	14	1.14
Controls	2,979	2,582	1.15
Injection Site Pain (10022086) - Cases	146	156	0.94
Controls	2,849	2,440	1.17

[1] Among the neurodevelopmental disorder cases, overall there were a total of 11 cases (10 males and 1 female) with more than one of the neurodevelopmental disorder diagnoses examined in the present study.

presumed for the adverse event reports included in the present study that all DTaP vaccines administered from January 1997 through December 1999 were Thimerosal-preserved (exposed) because these were the only US FDA-licensed Tripedia™ vaccines available at that time, and all Tripedia™ DTaP vaccines administered from January 2004 through September 2006 were Thimerosal-reduced (unexposed) because the in-date Tripedia™ doses available in this time period should have been doses of the Thimerosal-reduced Tripedia™ DTaP vaccine licensed by the US FDA on March 7, 2001 to replace the Thimerosal-preserved Tripedia™ DTaP vaccine previously licensed.

Table 2 - A summary of exposure to Thimerosal-containing DTaP vaccines and Thimerosal-reduced DTaP vaccines among neurodevelopmental disorders cases and controls

Group Examined	Number of Cases (%)	Number of Controls (%)	Odds Ratio (95% CI)	p-value
Experiment I - Autism				
Thimerosal-containing DTaP Vaccine (Exposed)	25 (89.3)	2,897 (52.1)		
			7.67 (2.33-39.7)	< 0.0001
Thimerosal-reduced DTaP Vaccine (Unexposed)	3 (10.7)	2,666 (47.9)		
Experiment II - Speech Disorder				
Thimerosal-containing DTaP Vaccine (Exposed)	19 (79.2)	2,903 (52.1)		
			3.49 (1.26-12)	< 0.02
Thimerosal-reduced DTaP Vaccine (Unexposed)	5 (20.8)	2,664 (47.9)		
Experiment III - Mental Retardation				
Thimerosal-containing DTaP Vaccine (Exposed)	19 (90.5)	2,903 (52.1)		
			8.73 (2.10-77)	< 0.0005
Thimerosal-reduced DTaP Vaccine (Unexposed)	2 (9.5)	2,667 (47.9)		
Experiment IV - Neurodevelopmental Disorder				
Thimerosal-containing DTaP Vaccine (Exposed)	52 (83.9)	2,870 (51.9)		
			4.82 (2.42-10.6)	< 0.0001
Thimerosal-reduced DTaP Vaccine (Unexposed)	10 (16.1)	2,659 (48.1)		

Statistical analyses

The Fisher's exact test contained in the StatsDirect (version 2.7.8) statistical software package was utilized for statistical analyses, and a two-sided p-value < 0.05 was considered to be statistically significant. The null hypothesis was that there would be no difference in exposure to Thimerosal among cases and controls examined in the present study.

Results

Table 2 displays the relationship between exposure to Thimerosal-preserved and Thimerosal-reduced Tripedia™ DTaP vaccines among neurodevelopmental disorder cases and controls. It was observed that autism cases (odds ratio = 7.67, $p < 0.0001$), speech disorder cases (odds ratio = 3.49, $p < 0.02$), mental retardation cases (odds ratio = 8.73, $p < 0.0005$), and ND cases (odds ratio = 4.82, $p < 0.0001$) were significantly more likely than controls to be exposed to Thimerosal-preserved Tripedia™ in comparison to Thimerosal-reduced Tripedia™.

Table 3 reveals the relationship between exposure to Thimerosal-preserved DTaP vaccines and Thimerosal-reduced DTaP vaccines among cases with non-biologically plausibly Hg-related outcomes and controls. It was observed that injury cases (odds ratio = 1.32, $p > 0.65$), pneumonia cases (odds ratio = 1.37, $p > 0.45$), and injection site pain cases (odds ratio = 0.90, $p > 0.35$) were statistically no more likely than controls to be exposed to Thimerosal-preserved

Table 3 - A summary of exposure to Thimerosal-containing DTaP vaccines and Thimerosal-reduced DTaP vaccines among cases with non-biologically plausibly mercury-related outcomes and controls

Group Examined	Number of Cases (%)	Number of Controls (%)	Odds Ratio (95% CI)	p-value
Experiment I - Injury				
Thimerosal-containing DTaP Vaccine (Exposed)	13 (59.1)	2,909 (52.2)		
			1.32 (0.52-3.51)	> 0.65
Thimerosal-reduced DTaP Vaccine (Unexposed)	9 (40.9)	2,660 (47.8)		
Experiment II - Pneumonia				
Thimerosal-containing DTaP Vaccine (Exposed)	18 (60)	2,904 (52.2)		
			1.37 (0.62-3.13)	> 0.45
Thimerosal-reduced DTaP Vaccine (Unexposed)	12 (40)	2,657 (47.8)		
Experiment III - Injection Site Pain				
Thimerosal-containing DTaP Vaccine (Exposed)	150 (49.7)	2,772 (52.4)		
			0.9 (0.71-1.14)	> 0.35
Thimerosal-reduced DTaP Vaccine (Unexposed)	152 (50.3)	2,517 (47.6)		

Tripedia™ in comparison to Thimerosal-reduced Tripedia™.

Discussion

The present study evaluated the potential relationship between organic-Hg exposure from a Thimerosal-preserved childhood vaccine and a subsequent risk of a ND diagnosis using a hypothesis-testing case-control study methodology to examine adverse event reports in the VAERS database. The present study revealed the children diagnosed with each of the types of ND examined were significantly more likely than controls to be exposed to Thimerosal-preserved Tripedia™ DTaP vaccines in comparison to Thimerosal-reduced Tripedia™ DTaP vaccines. By contrast, for children diagnosed with each type of cases with non-biologically plausibly Hg-related outcomes, they were no more likely than controls to be exposed to Thimerosal-preserved Tripedia™ DTaP vaccines in comparison to Thimerosal-reduced Tripedia™ DTaP vaccines.

It is interesting to consider the specific methods employed to evaluate the potential relationship between organic-Hg exposure from Thimerosal-preserved childhood Tripedia™ DTaP vaccines and the risk of ND in the present study. In 1997, the Advisory Committee on Immunization Practices (ACIP) recommended that Thimerosal-preserved Tripedia™ vaccine be routinely administered to US infants in a schedule at 2, 4, 6 months [7]. As mentioned previously, on July 7, 1999 the AAP and the USPHS called for the removal of Thimerosal from all vaccines in the United Statas soon as possible. On March 7, 2001, the US FDA licensed Thimerosal-reduced Tripedia™, and by early 2003, the last remaining doses of Thimerosal-preserved Tripedia™ on the shelf had either been used or expired [8]. As a consequence, in the period post-2003, it was possible for infants to receive the Tripedia™ vaccine in the same routinely recommended vaccine schedule with a similar formulation (i.e., the vaccines contained the same number and amount of antigens, aluminum-based adjuvant, trace constituents, etc), except that the concentration of Thimerosal had been reduced from a preservative level (25 µg Hg per dose) to a trace level (< 0.3 µg Hg per dose), an 83-plus-fold reduction. In addition, since NDs are diagnosed in a non-uniform distribution following birth and may take many years to be fully diagnosed following birth of a child (in some instances > 5 years), any study that fails to consider the lag-time between birth and age of initial ND diagnosis will likely not be able to observe the true relationship between exposure and the subsequent risk of an ND diagnosis [9].

As a result of the aforementioned considerations, children examined in the present study that received Tripedia™ vaccine administered from January 1997 through December 1999 were considered to be exposed (i.e., they received Thimerosal-preserved DTaP vaccine), and children in the present study that received Tripedia™ vaccine administered from January 2004 through September 2006 were considered to be unexposed (i.e., they received Thimerosal-reduced DTaP vaccine). In addition, based upon the ending of last period of vaccine administration (September 2006) studied, and the fact that the VAERS database examined was last updated in September of 2013, the youngest children examined in the present study had more than 7 years of follow-up.

Despite the difference in the methods of examining the potential relationship between Thimerosal exposure and NDs employed in the present study in comparison to previous studies, the results observed in the present study are supported by a number of previous epidemiological studies finding a significant relationship between organic-Hg exposure from Thimerosal-containing vaccines and NDs, using several epidemiological methods in various databases.

For example, investigators conducted a two-phase (hypothesis generating/hypothesis testing) study with documented exposure to varying levels of Thimerosal from vaccinations [9]. The cohort study was undertaken to evaluate the relationship between exposure to organic-Hg from a Thimerosal-preserved DTaP vaccine in comparison to a Thimerosal-free DTaP vaccine administered, from 1998 through 2000, for the risk of autism as reported in the VAERS database. A hypothesis testing case-control study was undertaken to evaluate the relationship between organic-Hg exposure from Thimerosal-containing hepatitis B vaccines administered at specific intervals in the first six months of life among cases diagnosed with autism and controls born between 1991 through 1999 in the Vaccine Safety Datalink (VSD) database. That study found a significantly increased risk ratio for the incidence of autism reported following the Thimerosal-preserved DTaP vaccine in comparison to the Thinerosal-free DTaP vaccine. That study also observed that cases diagnosed with an ASD were significantly more likely than the controls to have received increased organic-Hg from Thimerosal-containing hepatitis B vaccine administered within the first, second, and sixth month of life.

Other investigators also reported on the results of a meta-analysis using statistical modeling to evaluate the relationship between exposure to organic-Hg exposure from Thimerosal-preserved childhood vaccination and the reported rates of ND adverse events in the VAERS database [10]. Consistent with the results observed in the present study, that study found significantly increased risks of autism, speech disorders, mental retardation, personality disorders, thinking abnormalities, ataxia, and NDs in general, which were associated with the administration Thimerosal-preserved vaccine.

Investigators, using an ecological study design, evaluated the relationship between the birth cohort prevalence of specific ND and birth cohort exposures to organic-Hg from Thimerosal-preserved childhood vaccines in the VSD database [11]. Consistent with the results observed in the present study, infants receiving an additional 100 µg organic-Hg from Thimerosal-preserved childhood

vaccines from birth to 7 months of age and birth to 13 months of age, had significantly increased risk for being diagnosed with autism, autism spectrum disorder, tics, attention deficit disorder, developmental disorders, and emotional disturbances.

As another example, Gallagher and Goodman [12] evaluated the relationship between administration of Thimerosal-containing hepatitis B vaccine administration and the subsequent risk of individual being diagnosed with autism or NDs, based upon assessment of the National Health Interview Survey (NHIS) data sets. They reported that boys diagnosed with an autistic disorder in comparison to controls had a 3-fold significantly greater odds ratio for receiving a Thimerosal-containing hepatitis B vaccine during the first month of life, and, in comparison to controls, boys diagnosed with a ND had a 9-fold significantly greater odds ratio for receiving three Thimerosal-containing hepatitis B vaccines in comparison to those who were not given any hepatitis B vaccine doses [13].

The results of the present study differ from several other studies that failed to find a statistically significant relationship between NDs and organic-Hg exposure from Thimerosal-containing childhood vaccines [9]. This may have occurred, in part, because other studies examined cohorts with significantly different childhood vaccine schedules and with different diagnostic criteria for outcomes. This difference may have also occurred because these other studies employed different epidemiological methods, especially with respect to the issue of follow-up period for individuals in the cohorts examined. The method used to measure follow-up period for individuals is a critical issue in all studies examining the relationship between exposures and the subsequent risk of an ND diagnosis. This is the case because the risk of an individual being diagnosed with an ND is not uniform throughout his/her lifetime. Therefore, any follow-up method that fails to explicitly address lag-time between birth and age of initial ND diagnosis will likely not be able to observe the true relationship between exposure and the subsequent risk of an ND diagnosis [9].

Importantly, many recent studies support the biologically plausible role of organic-Hg exposure from Thimerosal-containing vaccines in the pathogenesis of ND [3]. Investigators have examined the distribution of organic-Hg following administration of Thimerosal to animals and infants. For example, administering Thimerosal mimicking the US vaccine schedule of the 1990s to infant monkeys, researchers found that significant levels of Hg were present in the brain (about 40 -50 parts-per-billion), and a significant fraction of the Hg was present as inorganic-Hg (about 16 parts-per-billion) that was projected to not significantly decline 120 days following the last dose of Thimerosal [14]. Other investigators undertook further evaluations of the speciation of Hg present in rat tissues following administration of Thimerosal [15]. Interestingly, these researchers observed that administration of Thimerosal resulted in significant brain levels of Hg with 63% present in the form or inorganic-Hg, 13.5% as ethyl-Hg, and, unexpectedly, 23.7% as methyl-Hg.

In addition, other investigators have found that Thimerosal-containing vaccine administration to human infants significantly increased the vaccinated infants' blood Hg levels (with some infants having total blood mercury levels in excess of the safety limits established by the US Environmental Protection Agency) [16-18] and also significantly increased the vaccinated infants' hair ethyl-Hg levels (with some infants having total hair Hg levels in excess of the safety limits established by the US Environmental Protection Agency) [19]. Finally, in further research on the distribution of Hg species within the body, researchers recently demonstrated that ethyl-Hg is transported across neuronal cellular membranes to the same degree as methyl-Hg [20, 21], and ethyl-Hg and methyl-Hg species were shown to be actively transported into neuronal cells at the same rate by the L-type neutral amino acid carrier transport (LAT) system [21].

Studies have also evaluated the potential for organic-Hg exposure from Thimerosal-containing vaccine administration to induce ND pathology or clinical symptoms in animal model systems. These studies have yielded significant pathology or clinical symptoms in mice, rats, hamsters, and monkeys which are consistent with those observed in ND following exposure to Thimerosal-containing vaccines mimicking the US routine childhood vaccination schedule [3].

Strengths/Limitations

A strength of the present study was the consistency of the results observed with respect to the association between ND adverse events and Thimerosal-preserved DTaP vaccine exposure in the VAERS database. For each type of ND adverse event outcome examined, the risks from exposures to the Thimerosal-preserved Tripedia™ DTaP vaccine were significantly elevated in comparison to exposures to the Thimerosal-reduced Tripedia™ DTaP vaccine. By contrast, none of the *a priori* selected non-Hg related adverse events examined generated a statistically significant risk associated with exposures to the Thimerosal-preserved Tripedia™ DTaP vaccine.

The study design used to evaluate the relationship between exposure and outcome was another significant strength of the present study. The method employed to examine VAERS ensured that the exposures to the various types of vaccines studied occurred prior to the outcomes described in the adverse event reports, since those reporting the subsequent adverse outcomes associated those outcomes with the vaccines listed in the adverse event reports.

The length of follow-up time from the last vaccine administration was another strength of the present study. The youngest children vaccinated in the present study were administered their doses of these vaccines > 7 years before

the last update of the VAERS database. As a result, the present study should have had a very high likelihood of capturing the diagnostic outcomes of the studied for cases and controls examined.

Another strength of the study was that the VAERS data were collected independently of the study design used in the present study. Among those reporting the adverse event reports examined in the present study, it was highly unlikely that any of them could have envisioned methods of analysis used to evaluate the potential relationship between Thimerosal and NDs in the present study. Furthermore, any potential differences in reporting between vaccine manufacturers or vaccine types was minimized, since the exact same vaccine (Tripedia™) was examined, and the difference in exposure was based upon the presence/absence of the Thimerosal at a preservative or 83-plus-fold-lower level as a result of the specific periods of vaccine administration.

However, the results of the present study may have a number of potential limitations. It is possible the results observed may have occurred from unknown biases or cofounders present in the datasets examined. This seems unlikely because none of the *a priori* selected non-Hg related adverse events examined revealed an association with Thimerosal-preserved Tripedia™ DTaP vaccine exposure, whereas each of the ND adverse events did show an association with Thimerosal-preserved Tripedia™ DTaP vaccine exposure.

An additional potential limitation of the present study of the VAERS database is that VAERS may have shortcomings, such as underreporting, difficulty in determining causal relationship, and a lack of precise denominators. Nevertheless, as previously described by investigators from the CDC, almost all of these types of shortcomings would apply equally to VAERS reports after vaccines administered to similar populations [22], such as the Thimerosal-preserved Tripedia™ DTaP and Thimerosal-reduced Tripedia™ DTaP vaccines examined in the present study. As a result, the comparison of vaccines administered to similar populations should provide accurate relative qualitative and quantitative relationships between vaccine exposures and adverse outcomes [22].

Another potential limitation of the present study is that the results observed may be the result of statistical chance. However, such a possibility would be unlikely given the limited number of statistical tests performed, the highly significant results observed (most p-values observed were < 0.01), and the consistency in the direction and magnitude of the results observed.

Still, other potential limitations of the present study include the possibilities that some of the individuals in VAERS may have had more subtle neurological dysfunction that was not brought to the attention of their healthcare providers, healthcare providers may have misdiagnosed some individuals, or some vaccine exposures may not have been appropriately classified. These limitations, while possibly present in the data examined in the current study, should not have significantly impacted the results observed because it is unclear how differential application would have occurred to the study cohorts examined based upon the Thimerosal doses that the individuals received. Moreover, misclassification occurring in the data examined would tend to bias any results observed toward the null hypothesis, since such effects would result in individuals being placed in the wrong exposure and/or outcome categories examined, and result in decreased statistical power to determine true potential exposure-outcome relationships.

In addition, another potential limitation of the present study is that exposures to other sources of Hg were not evaluated. It is very likely that among the individuals examined in the present study, they incurred other organic-Hg exposures from other Thimerosal-preserved childhood vaccines, dental amalgams, fish, or other environmental sources. While these other sources of Hg may play a significant involvement in the pathogenesis ND, these unaccounted for Hg exposures would actually tend to bias the results observed towards the null hypothesis because they potentially would confound the specific exposure classifications of Hg examined. For example, individuals classified as having lower organic-Hg exposure from Thimerosal-preserved vaccines may have actually received high doses of Hg from other sources, and individuals having higher organic-Hg exposure from Thimerosal-preserved vaccines may have actually received low doses of Hg from other sources, with the net result tending to minimize the magnitude of the associations observed.

It is also possible that the findings may be the result of other components of the vaccines studied which, in isolation or synergistically, interacted with the organic-Hg exposures examined in the present study. The former seems remote, since in our study the composition of the DTaP vaccines in VAERS were, except for the level of Thimerosal, very similar with respect to the levels of all of the antigens, the aluminum-based adjuvant, and the other constituents.

Finally, the current study suffers from the potential limitation that analyses were not conducted to further explore the precise timing and cumulative doses of organic-Hg from all Thimerosal-containing childhood vaccines associated with maximum adverse consequences. In future studies it would be worthwhile to further explore these precise-timing and cumulative-doses phenomena. In addition, it would be valuable to evaluate other NDs, as well as other covariates such as gender, race, birth weight, etc., that may further affect the magnitude of the adverse effects found.

Conclusion

The present study provides additional compelling epidemiological evidence supporting a significant relationship between increased organic-Hg exposure from Thimerosal-preserved childhood vaccines and the

subsequent risk of a ND diagnosis. Using a hypothesis-testing, epidemiological analytical methodology, organic-Hg exposure from Thimerosal-preserved Tripedia™ DTaP vaccine was determined to be a significant risk factor for the subsequent diagnosis of a ND. In addition, the present VAERS database studied placed special emphasis on requiring an adequate follow-up period in the analysis (> 7 years from the last vaccine administration). The cases and controls were followed for a sufficient period to ensure that they were appropriately classified with respect to their exposures and outcomes, thus helping to ensure the potential for a cause-and-effect relationship between exposure and outcome was not biased or confounded. Future studies should be completed to further evaluate the relationship between other sources of organic-Hg exposure from Thimerosal-containing childhood vaccines and other chronic disorders, and to further explore potential subpopulations and the timing of exposure to organic-Hg from Thimerosal-containing vaccine administration associated with adverse outcomes.

As previously described, routine childhood vaccination is recognized public health tool used to reduce the morbidity and mortality associated with certain infectious diseases. However, it is also a public health imperative to "do no harm" by ending the unnecessary addition of organic-Hg to vaccines in the form of Thimerosal used as a preservative, based on an ever-increasing body of toxicological and epidemiological data showing an association between its administration and adverse outcomes.

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Competing interests

All of the investigators on the present study have been involved in vaccine/biologic litigation.

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