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Thimerosal exposure in infants and neurodevelopmental disorders: An assessment of computerized medical records in the Vaccine Safety Datalink

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Abstract

The study evaluated possible associations between neurodevelopmental disorders (NDs) and exposure to mercury (Hg) from Thimerosal-containing vaccines (TCVs) by examining the automated Vaccine Safety Datalink (VSD). A total of 278,624 subjects were identified in birth cohorts from 1990–1996 that had received their first oral polio vaccination by 3 months of age in the VSD. The birth cohort prevalence rate of medically diagnosed International Classification of Disease, 9th revision (ICD-9) specific NDs and control outcomes were calculated. Exposures to Hg from TCVs were calculated by birth cohort for specific exposure windows from birth-7 months and birth-13 months of age. Poisson regression analysis was used to model the association between the prevalence of outcomes and Hg doses from TCVs. Consistent significantly increased rate ratios were observed for autism, autism spectrum disorders, tics, attention deficit disorder, and emotional disturbances with Hg exposure from TCVs. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs. Routine childhood vaccination should be continued to help reduce the morbidity and mortality associated with infectious diseases, but efforts should be undertaken to remove Hg from vaccines. Additional studies should be conducted to further evaluate the relationship between Hg exposure and NDs.

Keywords: ADD; ADHD autistic disorder; ASD; Ethylmercury; Merthiolate; Thiomersal

1. Introduction

In the last few decades, vaccines—one of the greatest breakthroughs in health sciences—have helped to accomplish striking reductions of infection and disease worldwide [1]. From the 1930s through the early 2000s, many routinely administered childhood vaccines in the United States contained Thimerosal [2]. Thimerosal is an organic mercury-containing compound that is 49.55% mercury (Hg) by weight, and initially metabolized to ethylmercury compounds and thiosalicylate [3].

The American Academy of Pediatrics and the US Public Health Service in 1999 [4] published a joint statement that urged "all government agencies to work rapidly toward reducing children's exposure to mercury from all sources." The statement recommended that Thimerosal be removed from vaccines as soon as possible as part of this overall process. Between 1999 and 2001, many vaccines recommended for children ≤6 years of age were made available in

Abbreviations: ADD, Attention Deficit Disorder; ADHD, Attention Deficit Hyperactivity Disorder; ASD, Autism Spectrum Disorder; IRB, Institutional Review Board; ICD-9, International Classification of Disease, 9th revision; Hg, Mercury; μg, micrograms; NDs, neurodevelopmental disorders; TCVs, Thimerosal-containing vaccines; US Agency for Toxic Substances and Disease Registry; CDC, US Centers for Disease Control and Prevention; EPA, US Environmental Protection Agency; FDA, US Food and Drug Administration; VAERS, Vaccine Adverse Event Reporting System; VSD, Vaccine Safety Datalink.

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Thimerosal-free or Thimerosal-reduced formulations in the US [5]. Exposures to Thimerosal through pediatric vaccines, however, still occur in the US and worldwide. Thimerosal continues to remain in most formulations of influenza vaccine recommended for administration to pregnant women and infants in the US, and in many of the childhood vaccines used in other countries where multiple-dose vaccine vials are utilized [6].

Hg exposure from Thimerosal-containing vaccines administered to American infants in the 1990s, in conjunction with environmental Hg exposure, resulted in some infants receiving cumulative Hg doses for the first 6 to 12 months of life that were in excess of the US Environmental Protection Agency (EPA), the US Food and Drug Administration (FDA), and the US Agency for Toxic Substances and Disease Registry (ATSDR) safety guidelines established for methylmercury, a closely related chemical compound to the ethylmercury found in Thimerosal [7]. It is important to note that the National Research Council of the US National Academy of Science in 2000 determined that there is a causal relationship between childhood methylmercury exposure and neurodevelopmental disorders [8].

The purpose of the present study was to epidemiologically evaluate concerns regarding the potential neurotoxic effects of Thimerosal in vaccines. In this study, a large group of children with documented exposure to varying levels of Thimerosal from vaccines in several health maintenance organizations (HMOs) were examined.

2. Materials and methods

The study protocol employed was approved by the US Centers for Disease Control and Prevention (CDC), the Institutional Review Board (IRB) of Kaiser North–West, and the IRB of Kaiser Northern California. The data were analyzed at the secure Research Data Center of the National Center for Health Statistics in Hyattsville, MD. The views expressed in this study do not necessarily reflect those of the US CDC or those of Kaiser Permanente.

The study was conducted based upon a retrospective ecological assessment of neurodevelopmental disorders that were identified a priori as possibly related to Hg exposure. Pre-existing HMO administered databases collected for the Vaccine Safety Datalink (VSD) project were evaluated for associations between neurodevelopmental disorders and cumulative Thimerosal exposure.

2.1. Determining the population at risk

A cohort of infants enrolled in the VSD project (updated through 2000) from Kaiser North–West, Kaiser Northern California, and Kaiser Colorado were examined. The VSD project was created in 1991 by the National Immunization Program of the CDC and VSD methods were previously described [9–11]. The project links medical event information, specific vaccine history, and selected demographic information from the computerized databases of several HMOs.

Only those individuals who had a non-missing date of birth and were born before January 1, 1997 were examined. This date was chosen to allow for at least 4 years of follow-up for each member of the cohort which was believed to be an adequate amount of time to observe the outcomes of interest.

Only those individuals who had a recorded oral polio vaccine within the first 3 months after birth were examined. A 3 month window was chosen in order to maximize chances of following children with complete records for both vaccine exposure and outcome, and because the first oral polio vaccine is administered at a 2 month visit. One month additional was allowed to account for late vaccinations. The oral polio vaccine file was chosen because this vaccine was the only vaccine that was consistently administered to all children during the time period of 1990–1996, which is the time period of this study. All children who received an oral polio vaccine within 3 months of their birth date and who were born before January 1, 1997 were used as the denominator or population at risk for this study. Table 1 summarizes the demographic information for the population examined.

2.2. Determining outcomes

The outcome files (inpatient and outpatient diagnoses) from this population were then reviewed to find the first instance of diagnosis of the disorders of interest. If there were multiple instances of the same diagnosis in a child, only the first instance was counted. Then the total numbers of each diagnosis for each disorder of interest were determined by birth cohort. The counts of each diagnosis of interest represented the numerator or outcomes for this study.

Table 1
Demographic information for cohort (n=278,624)

Gender	%
Male	51.1
Female	48.9
Birth cohort	%
1990	0.6
1991	14.8
1992	15.9
1993	16.3
1994	16.7
1995	18.4
1996	17.4
Race[1]	**%**
White	60.0
Black	8.6
Hispanic	16.5
Asian	13.5
Other	1.4
Birth characteristics[1]	Mean (standard error)
Gestational age (weeks)	39.3 (0.01)
Birth weight (grams)	3,422 (1.5)
Maternal age	29.0 (0.02)

[1]Not for total cohort-only those with birth file (n=163,793).

Table 2 summarizes the specific medically diagnosed neurodevelopmental disorder International Classification of Disease, 9th revision (ICD-9) codes selected a priori as having a biologically plausible link to Hg exposure. Table 2 also summarizes specific medically diagnosed control disorder ICD-9 codes selected a priori as not having biologically plausible links to Hg exposure.

The prevalence of each diagnosis was then calculated by birth cohort by dividing the count of a diagnosis in that birth year by the total number of children from the study population that were born in that same year. Because of concern that the cohorts from 1995–1996 had only 4–6 years of follow-up, frequency distributions of age at diagnosis were examined for all years. This revealed that for some of the disorders a sizable proportion of children were diagnosed after 4.5 years. Adjustments were made for counts of cases as needed for birth cohorts depending upon the disorder examined to correct for under ascertainment that occurred due to shorter follow-up times. These adjustments were made for all disorders including the control disorders as appropriate based on the age distribution.

For example, 37% of autism cases in the study were diagnosed after 5 years old with about 50% diagnosed after 4.5 years old. This is a conservative estimate since it includes the 2 years (1995–1996) that had shorter follow-up times. Examination of the distribution of age of diagnosis by birth year for autism revealed that only about 15% of cases were diagnosed after 5 years of age in the 1995 birth cohort while the 1996 cohort had no cases diagnosed after 5 years of age and only 3.5% of cases diagnosed between 4.5 and 5 years of age. Based on the average age at diagnosis for all cohorts, the 1995 count of autism cases was increased by 45 cases with the assumption that all of these would have been added in the 5 year+ age group (bringing this percentage close to the overall average of 37% diagnosed after 5 years of age). The same was done for 1996, but the number of cases was augmented by 80 because it was assumed that these would be diagnosed in the 4.5 to 5 and 5+ groups essentially bringing the percentage diagnosed after age 4.5 close to the overall average of 50% diagnosed after 4.5 years of age. The new augmented frequency counts of cases in 1995 and 1996 birth cohorts were then used as the new case counts in the analysis.

In analyzing the adjustments made for follow-up corrections, varying levels of imputing additional cases were modeled to assess the sensitivity of the results to the assumptions made when imputing additional cases in specific birth cohorts. Sensitivity analyses revealed that point estimates were similar even when imputing 50% fewer cases than would be expected using the average age distributions as noted above. In addition, confidence intervals showed little variation and maintained statistical significance when imputing as low as 25% fewer cases than would be expected using the average age distributions.

2.3. Determining exposure

Because the study protocol did not permit us to match data across vaccine files, exposure was determined in aggregate by birth cohort for each vaccine and then summed across the birth cohorts. The routine childhood vaccines of interest were Haemophilus Influenza Type b (Hib), hepatitis B vaccine, acellular Diphtheria–Tetanus–acellular-Pertussis (DTaP), and whole-cell Diphtheria–Tetanus–Pertussis (DTP) vaccines. The following Hg content from Thimerosal, as detailed by the US FDA [12], were assumed for the following routine childhood vaccines under study: Hib = 25 micrograms (μg) Hg/dose, DTaP/DTaPH = 25 μg Hg/dose, whole-cell DTP/DTPH = 25 μg Hg/dose, and hepatitis B = 12.5 μg Hg/dose.

Table 2
Medical diagnoses examined in the VSD database

ICD-9 codes:	Diagnosis	N	% Male	Median Age at Initial Diagnosis (yrs)	Adjusted[1] Overall Prevalence Rate
Neurodevelopmental disorders[2]					
299.00, 299.01	Autism	583	80.1	4.5	25.4/10,000
299.00, 299.01, 299.80, 299.81	Autism spectrum disorders	817	80.4	4.7	36.7/10,000
314.00, 314.01, 314.1, 314.2, 314.8, 314.9	Hyperkinetic syndrome of childhood (ADD/ADHD)	5712	77.7	6.4	25.1/1000
315.9	Developmental disorder/Learning disorder—not otherwise specified	2248	64.3	3.9	94.8/10,000
313.0, 313.1, 313.21, 313.22, 313.23, 313.3, 313.81, 313.82, 313.83, 313.89, 313.9	Disturbance of emotions specific to childhood and adolescence	1694	74.7	6.0	76.2/10,000
307.20, 307.21, 307.22, 307.23	Tics	804	76.2	6.0	38.9/10,000
Control disorders[3]					
486	Pneumonia	33,648	53.3	3.1	13.2/100
759.9	Congenital anomalies	1643	52.0	2.5	63.2/1,000
783.40, 783.41, 783.42, 783.43	Failure to thrive	4754	56.0	1.7	18.5/1,000

ADD/ADHD = Attention Deficit Disorder/Attention Deficit Hyperactivity Disorder.
[1]Based upon the age of diagnosis and length of follow-up time in the VSD for each birth cohort.
[2]Outcomes selected a priori as having biologically plausible association with Hg exposure.
[3]Outcomes selected a priori as not having biologically plausible association with Hg exposure.

The vaccine datasets were subset in a similar way to the population datasets in that an individual had to have the specific vaccine within 3 months of birth and be born before January 1, 1997. This ensured that the exposure population was as similar as possible to the outcome population. Each vaccine file was then searched to determine the number and specific type of vaccine that was administered within the first 13 months from date of birth which had been chosen as the exposure period of interest.

Within each vaccine file, the cumulative Hg dose for each individual was calculated based on the number of each type of vaccine received. The cumulative dose of Hg was then aggregated over a birth cohort resulting in a total Hg dose for a particular vaccine by year of birth. The total Hg doses for each of the vaccines were then added together to obtain a total Hg dose for all vaccines by year of birth. The total Hg dose by year of birth was then divided by the population at risk for each birth cohort which was previously defined above. This calculation resulted in an average Hg dose per person for each birth cohort which served as the exposure variable. Because of interest in particular windows of exposure, Hg doses from vaccine exposure were calculated for the following periods: 1) birth to 7 months; and 2) birth to 13 months.

2.4. Statistical analysis

Graphs plotting the Hg dose by birth cohort as well as prevalence of a particular disorder by birth cohort were constructed. Poisson regression analysis was used to model the association between prevalence of event of interest and Hg dose. Poisson regression analysis is commonly used as a technique for modeling the occurrence of rare events, such as the count of new cases of disease developing in some population over a period of time. Parameter estimates from Poisson regression models were used to obtain rate ratios. Hg dose was modeled as a continuous variable and rate ratio estimates and 95% confidence intervals were calculated to determine the change in prevalence rate of each diagnosis per unit increase in Hg dose from Thimerosal-containing vaccines. Chi-square statistics and corresponding p-values were also generated to assess statistical significance. A two-tailed p-value <0.05 was considered statistically significant.

3. Results

Table 3 presents the rate ratios and 95% confidence intervals for each diagnosis assuming a μg increase in mercury exposure from Thimerosal-containing vaccines administered from birth to 7 months and birth to 13 months. It was observed that there were significantly increased rate ratios for the neurodevelopmental disorders of autism, autism spectrum disorder (ASD), hyperkinetic syndrome of childhood (attention deficit disorder/attention deficit hyperactivity disorder), developmental disorder/learning disorder—not otherwise specified, disturbance of emotions specific to childhood and adolescence, and tics following additional Hg exposure from Thimerosal-containing childhood vaccines. For example, in the birth to 7 month period, the rate of tics was approximately 3.4 times higher given a 100 microgram increase in Hg exposure in TCVs. The increased rate ratios ranged from a low of 1.73 (developmental disorder/learning disorder-not otherwise specified) for a 100 μg increase in Hg exposure in the birth to 7 month period to a high of 4.51 (hyperkinetic syndrome of childhood) for a 100 μg increase in Hg exposure in the birth to 13 month period. By contrast, no significantly increased rate ratios for the control disorders of pneumonia, congenital anomalies, and failure to thrive were

Table 3
Rate ratios (95% confidence intervals) for a 100 μg **difference in mercury exposure** for each diagnosis from Thimerosal-containing vaccines administered from birth to 7 months and from birth to 13 months

Diagnosis	100 μg Hg difference Birth to 7 months Rate ratio (95% CI)	100 μg Hg difference Birth to 13 months Rate ratio (95% CI)
Neurodevelopmental disorders		
Autism[1]	2.87 (1.19–6.94)	2.62 (1.15–6.01)
Autism spectrum disorders[1]	2.44 (1.16–5.10)	2.20 (1.10–4.40)
Hyperkinetic syndrome of childhood (ADD/ADHD)[3]	3.15 (2.38–4.17)	4.51 (3.48–5.84)
Developmental disorder/Learning disorder—not otherwise specified[1]	1.73 (1.08–2.75)	1.81 (1.17–2.80)
Disturbance of emotions specific to childhood and adolescence[2]	2.27 (1.36–3.80)	2.91 (1.81–4.68)
Tics[3]	3.39 (1.64–6.79)	4.11 (2.12–7.94)
Control disorders		
Pneumonia	0.98 (0.86–1.11)	0.92 (0.82–1.04)
Congenital anomalies	0.62 (0.34–1.14)	0.57 (0.33–1.00)
Failure to thrive	1.05 (0.74–1.47)	0.92 (0.67–1.27)

ADD/ADHD = Attention Deficit Disorder/Attention Deficit Hyperactivity Disorder.
[1] $p<0.05$.
[2] $p<0.01$.
[3] $p<0.001$.

observed with increasing Hg exposure from Thimerosal-containing vaccines.

4. Discussion

The results of the present study showed a significant association between Hg exposure from Thimerosal-containing vaccines and neurodevelopmental disorders. The strength of the present study stems from the database that was examined.

First, the VSD contains medical records for patients that were collected on a prospective basis, as part of the routine treatment course of physician care. The VSD requires no reporting of adverse events or having a physician associate an outcome with an exposure.

Second, the outcomes examined were entered into the VSD using ICD-9 coding. The use of ICD-9 coding allows for a consistent and specific physician diagnosed disease status to be examined among the patients evaluated in the present study. This is in contrast to other databases that use more descriptive coding or are non-physician based, and the coding employed in the VSD is consistent with the nearly universal medical standard across the US.

Third, the study design employed in the present study helps to strengthen the observed results. The medical conditions examined were selected a priori as biologically or not biologically plausibly linked to Hg exposure from Thimerosal-containing vaccines administered during specific exposure windows. There were significantly increased rate ratios for the two exposure windows examined between Hg doses and each type of neurodevelopmental disorder. By contrast, no significantly increased rate ratios for either of the exposure windows examined were observed between Hg doses and the control conditions evaluated. Additionally, the study design also allowed us to be certain that virtually all exposures to Hg preceded the diagnoses of the diseases examined (i.e., allowing for a potential cause–effect relationship between exposure and disease). This was ensured because only children receiving a vaccine by age 3 months were examined, and as Table 2 shows the median age of initial diagnosis for the conditions examined in the present study ranged from 1.7 to 6.4 years-old.

Fourth, the methods of ensuring capture of Hg exposure from Thimerosal-containing vaccines and outcomes appear to have yielded results consistent with previous studies. For example, the US CDC previously published that the overall prevalence of ASDs in children, born for similar birth years as examined in the present study, was 3.4 per 1000 children in the metropolitan Atlanta, Georgia area [13]. The adjusted overall prevalence of ASDs (3.67 per 1000 children) in the present assessment of the VSD was consistent with the previous observation made by the US CDC. Additionally, Ball et al. [12] reported average Hg doses from Thimerosal-containing vaccines in a cohort of 85,000 children consistent with those observed in the present study [12].

Fifth, the birth cohort years examined in the VSD help to strengthen the results observed. The birth cohort years examined from 1990 through 1996 occurred many years prior to the raising of concern about potential problems with Thimerosal in childhood vaccines by the American Academy of Pediatrics and the US Public Health Service, so that their announcement to remove Thimerosal from childhood vaccines in July of 1999 should have had virtually no impact on physicians' thoughts about Thimerosal in childhood vaccines. Additionally, the years examined in the VSD help to ensure that changes in diagnostic criteria for outcomes such as autism that came into effect in 1994 would have minimally impacted the present study, since most children examined were diagnosed post-1994 with autistic disorders.

Finally, another significant strength of the present study stems from the trends in birth cohort Hg exposure and outcomes. As shown in Fig. 1 for autism, it was observed there were increasing/decreasing trends in exposures and outcomes across the birth cohort years examined, and that for the neurodevelopmental disorders there were significant associations between birth cohort mean Hg exposure and disease prevalence rates. It is important to note that the increasing/decreasing trends in Hg exposure were not simply the result of random yearly fluctuations in vaccine uptake rates or even simply the result of increasing exposure to vaccine antigens, but instead reflect known changes in the Hg content of the US childhood vaccine schedule. Namely, in the late 1980s/early 1990s the Hg dose from vaccines increased with the addition of hepatitis B (12.5 μg Hg/dose) and Hib (25 μg Hg/dose) vaccines to the routine childhood schedule during the first year of life. Subsequently, starting from 1992, the Hg dose from vaccines decreased with the addition of combination whole-cell DTP-Hib (25 μg Hg/dose) vaccine, instead of the 50 μg Hg per joint administration of whole-cell DTP and Hib vaccines (each contained 25 μg Hg/dose) in separate immunizations. This was finally followed by in the mid-1990s the replacement of whole-cell

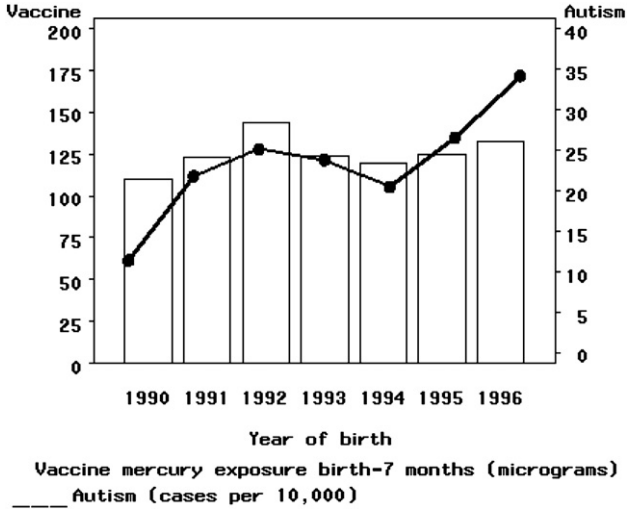

Fig. 1. An analysis of the time-trend in the birth cohort prevalence of autism in comparison to the birth cohort average Hg dose from Thimerosal-containing vaccines administered from birth to 7 months of age.

DTP vaccines with acellular DTaP vaccines (25 μg Hg/dose). For the most part these vaccines were not made in combination with Hib vaccine.

In considering potential limitations for the present study, because of the ecological nature of the study design, we were not able to link vaccine exposures across individual patient records. Individual vaccine doses could not be directly attributed to individual patients. Hence, the results of the present study represent the aggregate doses of Hg and aggregate prevalence of disorders for a given birth cohort year, and not analyses of individual children. While this information would have been useful for additional analyses, given the magnitude and robustness of the observed effects, this limitation appears to have had a limited impact on the strength of the results.

Another possible limitation of the present study was the potential for under ascertainment of a child's total Hg exposure. The present study was only able to detect differences in Hg exposure from vaccines that were recorded in the VSD. Other potential sources of Hg such as fish consumption or environmental exposure, while potentially significant to the risk of a child being diagnosed with a neurodevelopmental disorder, could not be examined in the present study. We believe that these other exposures to Hg should not have biased the effects observed. In actuality, such sources of Hg exposure would potentially minimize the significance of the effects observed.

This study was also not able to analyze medical conditions that were not entered into the VSD. It not clear how this would have biased the results of the present study, but precludes us from being able to evaluate the potential association between Hg exposure from Thimerosal-containing vaccines and more subtle neurodevelopmental effects that were not observed/diagnosed by physicians.

In addition, the reliability of the ICD-9 diagnosis codes for the outcomes of interest are not known since paper-review of patient medical records is not available to outside researchers examining the VSD database. This may make it difficult to assess the accuracy of the rates of various neurodevelopmental outcomes, but the CDC has previously published that there was good agreement between the automated records in the VSD database and the paper-review of patient medical records [9–11]. Furthermore, it is not clear how differences between patient medical records and automated medical records would have added biases towards observing a relationship between Thimerosal exposure and NDs, but not be present for the control conditions examined.

The study was also limited to a maximum of 4 years of follow-up time for the latest birth cohorts. This likely caused the rates of various outcomes to be lower than if all cohorts had longer follow-up periods, particularly for outcomes such as emotional disturbance and attention deficit disorder which appear to have later median ages of diagnosis. The study attempted to account for the truncated follow-up periods by imputing additional outcomes based on patterns for the longest followed cohorts. Sensitivity analyses of the imputations were performed, and it was noted that point estimates and confidence intervals were reliable even when using very conservative imputation methods.

Finally, the present study was not able to adjust for potential factors that might have resulted in vaccine avoidance but may have predisposed one towards the neurodevelopmental disorders under study. Specifically, Fine and Chen reported that there are several social and medical attributes associated with avoidance or delay of vaccination and an increased risk of neurological adverse events, and that confounding of this sort is a general problem for studies of adverse reactions to prophylactic interventions, as they may be withheld from some individuals precisely because they are already at high risk of the adverse event [14]. They described that studies that fail to control adequately for such confounding factors are likely to underestimate the risks of adverse events attributable to vaccination. This effect may have been detected in the present study, because the control conditions examined, while not significantly, did indeed trend towards decreasing risks with increasing exposure to Hg from Thimerosal-containing vaccines. As a result, the effects observed in the present study may represent an underestimate of the true effects of Hg exposure from Thimerosal-containing vaccines on the risk of neurodevelopmental disorders.

In considering the results of the present study in the context of previous epidemiological findings, two previous cohort studies have examined the relationship between Hg exposures from Thimerosal-containing vaccines and diagnosed neurodevelopmental disorders in the VSD. One study found results consistent with those observed in the present study. That study observed significant increasing dose–response effects between vaccine Hg exposures during the first 6 months of life and the subsequent risk of neurodevelopmental disorders [15]. The second study by Verstraeten et al. found significant increasing dose–response effects between Hg exposure and some types of neurodevelopmental disorders in the first phase of the study, but the effects observed were not consistent in the second phase of the study [16]. It was concluded that the study was neutral with respect to causality [17].

In addition, the results of a meta-analysis of adverse events reported to the US Vaccine Adverse Event Reporting System (VAERS) was recently published. The study examined about 400 neurodevelopmental disorder adverse events reported to VAERS following about 100 million doses of vaccines distributed from 1994 through 2000. It was observed, consistent with the results from the present study, that neurodevelopmental disorder adverse events were significantly more likely to be reported to VAERS following vaccines containing higher Hg doses than those containing lower Hg doses [18]. Finally, two ecological studies found a correlation between increasing cumulative doses of vaccine Hg and neurodevelopmental disorders. One examined children born from the mid-1980s through the mid-1990s,

and found a significant correlation between the birth cohort prevalence of autistic disorders from the US Department of Education and the estimated Hg dose children received from Thimerosal-containing vaccines [19]. The second study correlated increased cumulative doses of Hg exposure from Thimerosal-containing childhood vaccines with the increasing population prevalence of children diagnosed with autism-like disorders seeking special education services for autism in California from 1987 to 1998 by birth-year cohort [20].

Several epidemiological studies conducted outside the US have examined the relationship between vaccine Hg exposure and neurodevelopmental disorders. Two studies in the United Kingdom showed significant increasing dose–response effects for some neurodevelopmental problems such as tics or behavioral problems and increasing vaccine Hg exposure, but the results for other conditions showed no significant association [21,22]. The researchers concluded that they could not finding convincing evidence that early exposure to Hg doses from Thimerosal-containing vaccines had deleterious effects on neurodevelopmental outcomes. Other studies in Canada [23], Sweden [20], and Denmark [24] evaluated the relationship between Hg exposure from Thimerosal-containing vaccines and autism. These studies found no significant relationship. In considering these studies, concerns have been raised regarding their applicability to the US experience with Thimerosal-containing vaccines. In many of these countries, alternate vaccines in different vaccine schedules and different diagnostic measures were used, and many countries apparently had very different neurodevelopmental disorder prevalence rates than in the US.

Mercury exposure was also observed to be significantly associated with neurodevelopmental disorders and autism in a series of epidemiological studies evaluating prenatal/early postnatal environmental Hg exposure [25–32]. In the most recent epidemiological study published from California (supported by the US CDC), 283 children with autistic disorders and 657 controls, born in 1994 in the San Francisco Bay area, were examined [32]. These researchers assigned exposure level by census tract of birth residence for 19 chemicals. Among the 19 chemicals examined to which children were exposed, mercury was found to be the single largest risk factor associated with autistic disorders. When comparing high mercury relative to low mercury exposure, there was a statistically significant increase in risk, which was about double, for having an autistic disorder.

The biological plausibility of the results observed in the present study are supported by Faustman et al. [33] who reported on the effects of mercury on neuronal development: "…mercury exposure altered cell number and cell division; these impacts have been postulated as modes of action for the observed adverse effects in neuronal development. The potential implications of such observations are evident when evaluated in context with research showing that altered cell proliferation and focal neuropathologic effects have been linked with specific neurobehavioral deficits (e.g., autism)." In addition, the US National Institutes of Environmental Health Sciences have described a role for mercury exposure in the pathogenesis of learning disabilities, intellectual retardation, dyslexia, attention deficit/hyperactivity disorder, autism, and propensity to violence [34].

Burbacher et al. evaluated infant monkeys following injection of doses of Thimerosal comparable to the dosing schedule (weight- and age-adjusted) US children received during the 1990s [35]. They determined that the maximum ethylmercury content in the brains of the Thimerosal-treated infant monkeys ranged from about 40 to 50 parts-per-billion (ppb). In addition, post-dosing-schedule testing found the concentration of inorganic mercury (formed from the ethylmercury entering the brain) averaged 16 ppb in the brains of the Thimerosal-treated infant monkeys. Moreover, the half-life of this inorganic mercury in the monkeys' brains was too long to estimate a value from the available data (no significant measurable decline was detectable by 120 days).

Additionally, Hornig et al. [36] observed features reminiscent of autism in a susceptible mouse strain [37] following Thimerosal exposure through vaccines mimicking the US childhood vaccine schedule of the 1990s (weight- and age-adjusted). The symptoms observed included growth delay, reduced locomotion, exaggerated response to novelty, increased brain size, decreased numbers of Purkinje cells, significant abnormalities in brain architecture affecting areas sub-serving emotion and cognition, and densely packed hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Similar symptoms have been observed in other animal models following perinatal, low-dose methylmercury administration [38].

A series of clinical studies on patients diagnosed with neurodevelopmental disorders have revealed significant elevations in Hg concentrations [39–48] and potential biochemical and genomic susceptibility factors to Hg poisoning [49–53]. For example, Cheuk and Wong in patients diagnosed with attention-deficit hyperactivity disorder [40] and Desoto and Hitlan in patients diagnosed with autistic disorders [41], both found significant elevations in blood mercury levels in comparison with controls. Adams et al. observed significant increases in the mercury levels of baby teeth in patients with autistic disorders in comparison with controls [39]. Several recent brain pathology studies have even revealed elevations in mercury concentrations and mercury-associated oxidative stress markers in patients diagnosed with autistic disorders in comparison with controls [46–48].

5. Conclusions

The results of the present ecological study show an association between increased Hg exposure from Thimerosal-containing vaccines and neurodevelopmental disorders. The observed effects were consistent with several previous epidemiological studies on the potential adverse effects of prenatal/early postnatal Hg exposure and are also supported by the known adverse effects of Hg exposure on human

neurodevelopment. Despite the findings from the present study indicating that the Hg additive, Thimerosal, was associated in some children with significant adverse outcomes, children should still continue to receive routine childhood vaccines. However, efforts should be undertaken to remove Thimerosal from all vaccines as rapidly as possible, and further efforts should be undertaken to evaluate adverse effects of Thimerosal and other mercurial compounds on human neurodevelopment.

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