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A comparative evaluation of the effects of MMR immunization and mercury doses from thimerosal-containing childhood vaccines on the population prevalence of autism

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Potential conflict of interest: Dr. Mark Geier has been an expert witness and a consultant in cases involving adverse reactions to vaccines before the U.S. Vaccine Compensation Act and in civil litigation. David Geier has been a consultant in cases involving adverse reactions to vaccines before the U.S. Vaccine Compensation Act and in civil litigation.

Background:	Summary The purpose of the study was to evaluate the effects of MMR immunization and mercury from thimerosal-containing childhood vaccines on the prevalence of autism.
Material/Methods:	Evaluations of the Biological Surveillance Summaries of the Centers for Disease Control and Prevention (CDC), the U.S. Department of Education datasets, and the CDC's yearly live birth estimates were undertaken.
Results:	It was determined that there was a close correlation between mercury doses from thimerosal-containing childhood vaccines and the prevalence of autism from the late 1980s through the mid-1990s. In contrast, there was a potential correlation between the number of primary pediatric measles-containing vaccines administered and the prevalence of autism during the 1980s. In addition, it was found that there were statistically significant odds ratios for the development of autism following increasing doses of mercury from thimerosal-containing vaccines (birth cohorts: 1985 and 1990–1995) in comparison to a baseline measurement (birth cohort: 1984). The contribution of thimerosal from childhood vaccines (>50% effect) was greater than MMR vaccine on the prevalence of autism observed in this study.
Conclusions:	The results of this study agree with a number of previously published studies. These studies have shown that there is biological plausibility and epidemiological evidence showing a direct relationship between increasing doses of mercury from thimerosal-containing vaccines and neurodevelopmental disorders, and measles-containing vaccines and serious neurological disorders. It is recommended that thimerosal be removed from all vaccines, and additional research be undertaken to produce a MMR vaccine with an improved safety profile.
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BACKGROUND

We have previously published that both pediatric Measles-Mumps-Rubella (MMR) vaccine and thimerosal-containing childhood vaccines were statistically significantly associated with childhood neurodevelopmental disorders [1–4]. Specifically, we determined that pediatric MMR vaccination was associated with an increased risk of serious neurological disorders [1]. In contrast, our assessments of thimerosal-containing vaccines and neurodevelopmental disorders have shown both, when we analyzed thimerosal-containing childhood vaccines against thimerosal-free childhood vaccines administered as part of the same childhood immunization schedule, and based upon a population assessment of the average amount of mercury administered to children as part of their routine childhood immunization schedules, that there were statistically significant associations between increasing doses of mercury from thimerosal-containing childhood vaccines and childhood neurodevelopmental disorders [2–4]. The purpose of this analysis was to further determine the effects of thimerosal-containing childhood vaccines and pediatric MMR vaccine on the prevalence of neurodevelopmental disorders based upon a population assessment of autism in the United States' Department of Education dataset.

MATERIAL AND METHODS

In order to undertake our analysis, we analyzed the U.S. Department of Education datasets [5–7]. In these datasets, we determined the number of children at various ages that had developed the neurodevelopmental disorder of autism. Autism was defined by those within the U.S. Department of Education. Once we determined the various ages of children that had developed autism, we used this information to determine the number of children in each birth cohort that had developed autism. The birth cohorts we examined were: 1981 through 1985 and 1990 through 1996. We determined the prevalence of autism in each birth cohort by determining the number of live births in each birth cohort as per the Centers for Disease Control and Prevention (CDC)'s yearly live birth surveillance data [8]. The amount of mercury that each child was on average administered as part of their respective birth cohort was based upon the number of doses of thimerosal-containing vaccines distributed/administered (this number subtracts out the number of doses returned or not distributed) in the Biologic Surveillance Summaries of the CDC, and the number of births in each birth cohort as per the CDC's live birth surveillance data (total mercury from all thimerosal-containing vaccines in the birth cohort/number of live births in the birth cohort). The thimerosal-containing vaccines analyzed in our study included: Diphtheria-Tetanus-whole-cell-Pertussis (DTwCP), Diphtheria-Tetanus-acellular-Pertussis (DTaP), Diphtheria-Tetanus-whole-cell-Pertussis-*Haemophilus influenzae* Type b (DTwCPH), Diphtheria-Tetanus-acellular-Pertussis-*Haemophilus influenzae* Type b (DTaPH), *Haemophilus influenzae* Type b (Hib), and pediatric hepatitis B. In approximating the number of pediatric hepatitis B vaccines administered, we used the number

of pediatric hepatitis B vaccines administered/distributed by SmithKline, and the total number of hepatitis B vaccine administered/distributed by Merck (there were a relatively small number of doses administered/distributed by Merck, and Merck did not distinguish between pediatric and adult hepatitis B vaccine doses). We determined the amount of mercury in each respective vaccine based upon the 2001 report of the Institute of Medicine (IOM) of the U.S. National Academy of Sciences [9]. The mercury doses per vaccine that we calculated were as follows: DTwcP, DTaP, DTwcPH, DTaPH, and Hib all had 25 micrograms per dose, whereas pediatric hepatitis B had 12.5 micrograms per dose. We determined using the statistical package contained in Microsoft Excel the slope and linear regression coefficient of the plot we generated, comparing the prevalence of autism against the average mercury dose that children received in their birth cohort from thimerosal-containing childhood vaccines.

An evaluation of the effect of MMR vaccine was undertaken in the U.S. Department of Education datasets for birth cohorts from 1982, 1985, and from 1991 through 1996. We undertook our analysis by determining the prevalence of autism in the U.S. Department of Education data for each birth cohort. The number of measles-containing vaccines administered in each respective birth cohort was based upon the CDC estimates for the coverage of primary pediatric measles-containing vaccine for the birth cohorts of 1982 (67% coverage), 1985 (61% coverage), 1991 (82.0% coverage), 1992 (82.5% coverage), 1993 (84.1% coverage), 1994 (90% coverage), 1995 (87.8% coverage), and 1996 (90.5% coverage) [10,11]. We determined, using the statistical package contained in Microsoft Excel, the slope and linear regression coefficient of the plot we generated.

Lastly, we established the 1984 birth cohort as a baseline measurement from the U.S. Department of Education datasets and the Biological Surveillance Summaries of the CDC, and compared the prevalence of autism and additional mercury doses that children received from childhood vaccines in comparison to the birth cohorts of 1985, and 1990 through 1996. We determined the slope and regression coefficient of the line using the statistical package contained in Microsoft Excel, and we used the statistical package contained in SISA [12,13] to construct 2x2 contingency tables to determine the odds ratios and 95% confidence intervals for each successive birth cohort examined in comparison to the 1984 baseline measurement. In establishing 1984 as a baseline measurement, it was our aim that all subsequent measurements of the number of cases of autism measured from the U.S. Department of Education dataset would come from a single report of the U.S. Department of Education, and potential report year variation within different U.S. Department of Education datasets would be minimized. Our null hypothesis was that a similar prevalence of autism should be found in each birth cohort examined, and a p-value of 0.05 was considered significant in our statistical analyses.

In selecting the birth cohorts for analysis, we selected birth cohorts that had complete information from all

Table 1. A description of the data used in our epidemiological analyses.

Birth Cohort	Number of Children	Number of Autism Cases [reference] (prevalence per 100,000 children)	Total Number of Thimerosal-containing Vaccines Administered (micrograms of mercury)	Average Mercury Dose per Child (micrograms)	Total Primary Pediatric Measles-Containing Vaccine Doses
1981	3,629,238	1,392 [5] (38)	(19,616,797 × 25 micrograms)	135	–
1982	3,680,537	1,755 [6] (48)	(19,241,059 × 25 micrograms)	131	2,465,960
1983	3,638,933	2,099 [6] (58)	(19,828,895 × 25 micrograms)	136	–
1984	3,669,141	2,460 [6] (67)	(17,012,295 × 25 micrograms)	116	–
1985	3,760,561	3,090 [7] (82)	(21,280,620 × 25 micrograms)	142	2,293,942
1990	4,158,212	6,631 [7] (159)	(30,277,875 × 25 micrograms) + (1,591,886 × 12.5 micrograms)	187	–
1991	4,110,907	8,748 [7] (213)	(37,588,377 × 25 micrograms) + (1,800,950 × 12.5 micrograms)	234	3,370,944
1992	4,065,014	9,974 [7] (245)	(37,664,199 × 25 micrograms) + (9,315,209 × 12.5 micrograms)	260	3,353,636
1993	4,000,240	11,641 [7] (291)	(38,434,995 × 25 micrograms) + (16,304,571 × 12.5 micrograms)	291	3,364,202
1994	3,952,767	11,379 [7] (288)	(33,698,181 × 25 micrograms) + (15,242,226 × 12.5 micrograms)	261	3,557,490
1995	3,899,589	11,121 [7] (285)	(29,447,582 × 25 micrograms) + (16,595,214 × 12.5 micrograms)	242	3,432,839
1996	3,891,494	10,813 [7] (278)	(27,265,936 × 25 micrograms) + (22,030,609 × 12.5 micrograms)	246	3,502,345

the necessary sources examined in this study. In Table 1, we present a complete summary of all the raw data that was used in our epidemiological analyses.

RESULTS

In Figure 1, we plotted the prevalence of autism per 100,000 children in comparison to the average mercury dose that children received from thimerosal-containing childhood vaccines (birth cohorts: 1981 through 1985, and 1990 through 1996). We determined that the slope of the line was 1.6, and the linear regression coefficient for the line was 0.94. The prevalence of autism in Figure 1 was shown to have increased approximately 6-fold, from approximately 50 cases per 100,000 children (i.e. 1 in 2,000 children) to approximately 300 cases per 100,000 children (i.e. 1 in 333 children).

In Figure 2 we plotted the average mercury dose per child in comparison to the prevalence of autism per 100,000 children for successive birth cohorts (birth cohorts: 1981 through 1985 and 1990 through 1996). Figure 2 shows that as the prevalence of autism increased from the birth cohorts from the late 1980s through the early 1990s a corresponding increase in the average mercury dose per child occurred. A maximum occurred in the birth cohort of 1993 in both the average mercury dose per child and the prevalence of autism. A decrease in both the prevalence of autism and the average mercury dose per child occurred from 1993 through 1996.

Figure 3 shows the number of doses of primary pediatric measles-containing vaccine in comparison to the prevalence of autism for each birth cohort examined (birth cohorts: 1982, 1985, and 1991 through 1996). This figure shows that there was a potential correlation between increasing doses of primary pediatric measles-containing vaccine and an increasing prevalence of autism during the 1980s. We determined that the slope of the line was 4831, and the linear regression coefficient for the line was 0.91.

Figure 4 presents the odds ratios ±95% confidence intervals in comparison to the average increased mer-

cury dose that children received from thimerosal-containing childhood vaccines for the birth cohorts of 1985 and 1990 through 1996 based upon a 1984 baseline-birth cohort. In Figure 4, each birth cohort examined had a statistically increased odds ratio for the prevalence of autism in comparison to the 1984 baseline-birth cohort, and each statistically significant odds ratio examined correlated with increasing mercury doses that children received from thimerosal-containing childhood vaccines. The slope of the line from Figure 4 was 0.023 and the linear regression coefficient was 0.89.

DISCUSSION

The results of this analysis showed that there was a close correlation between increasing doses of thimerosal-containing childhood vaccines and autism based upon evaluation of the U.S. Department of Education datasets. We have observed for birth cohorts from the mid-to-late 1980s through the early 1990s that as there was an increase in the average mercury dose from thimerosal-containing childhood vaccines a corresponding increase in the prevalence of autism occurred. A maximum in mercury from thimerosal-containing childhood vaccines and the prevalence of autism occurred for the birth cohort of 1993, and from 1993 through 1996, as the average mercury dose from thimerosal-containing vaccines decreased, a corresponding decrease in the prevalence of autism also ensued. We believe that we are among the first authors to observe, as has been previously hypothesized by a number of authors [2–4], that as the mercury dose from thimerosal-containing vaccines decreased, a corresponding decrease in the prevalence of autism would also ensue. It was also shown there were statistically significantly increased odds ratios for the prevalence of autism that closely correlated with increasing doses of mercury from thimerosal-containing childhood vaccines in comparison to a baseline measurement.

In 2001, the IOM published a report stating that it was biologically plausible for mercury from thimerosal-containing childhood vaccines to cause childhood neurode-



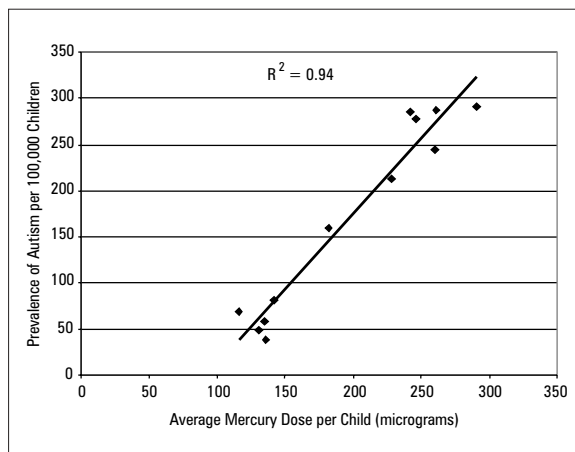


Figure 1. The prevalence of autism in comparison to the average mercury dose from thimerosal-containing childhood vaccines (birth cohorts: 1981 through 1985 and 1990 through 1996).

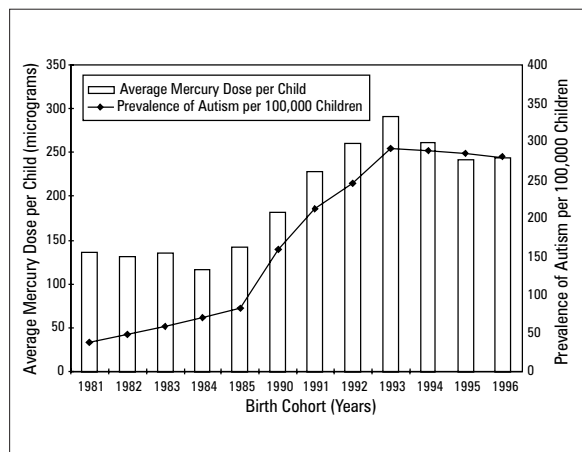


Figure 2. A birth cohort evaluation of the prevalence of autism in comparison to the average mercury dose from thimerosal-containing childhood vaccines.

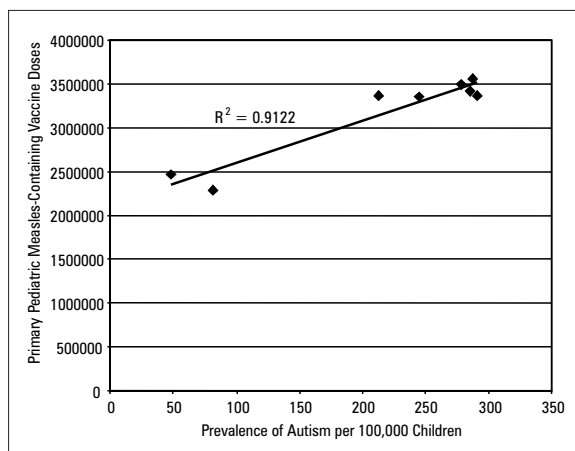


Figure 3. Number of primary pediatric measles-containing vaccine in comparison to the prevalence of autism for each birth cohort examined (birth cohorts: 1982, 1985, and 1991 through 1996).

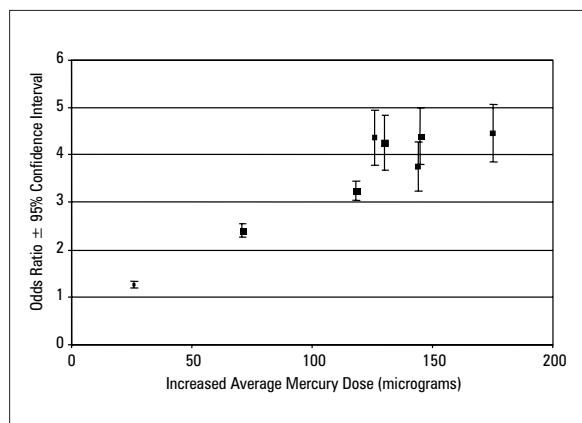


Figure 4. Odds ratios \pm 95% confidence intervals in comparison to the average increased mercury doses from thimerosal-containing childhood vaccines based upon a 1984 baseline-birth cohort (birth cohorts: 1985, 1990 through 1996).

velopmental disorders [9]. Bernard et al. have compared the similar biological abnormalities commonly found in autism and the corresponding pathologies arising from mercury exposure [14]. Distinct similarities were found between autism and mercury exposure in their effects upon biochemistry, the immune system, the central nervous system structure, neuro-chemistry, and neurophysiology. Holmes et al. [15] have observed that there was a statistically significantly approximately 7-times reduced levels of mercury in the first baby hair-cuts of children with autism in comparison to a matched normal control population. Similarly, Bradstreet et al. [16] in a case-control study examining mercury urinary excretion concentrations among children with autistic spectrum disorders in comparison to a matched normal control population based upon a three-day treatment with an oral chelating agent, meso-2, 3-dimercaptosuccinic acid (DMSA) have show statistically significantly approximately 6-times greater urinary mercury concen-

trations in cases in comparison to controls. Both of these articles hypothesize that children with autistic spectrum disorders have a decreased ability to excrete mercury in comparison to normal controls.

This is a cause for concern considering that a recent article by Baskin et al. has examined the toxic effects of micromolar concentrations of thimerosal in cultured human cerebral cortical neurons and in normal human fibroblasts [17]. The results demonstrated that thimerosal in micromolar concentrations induced membrane and DNA damage, and initiated caspase-3 dependent apoptosis in human neurons and fibroblasts, and in addition, the authors reported that thimerosal toxicity may occur at even lower doses than those utilized by Baskin et al, with longer times of exposure. Another recent article by Makani et al. has also demonstrated high cellular toxicity of thimerosal in low micromolar concentrations in cells incubated with thimerosal for 24

hours [18]. Therefore, the results of our study appear to be supported by both previous epidemiological and biological plausibility data.

Several recent studies have analyzed the prevalence of autism from the mid-1980s through 2002 in the U.S. and the United Kingdom [19–23]. The consensus of these studies is that the prevalence of autism has risen from one in about 2,500 children in the mid-1980s to as common as about one in 250 by the mid-1990s. These studies show that the rise in the prevalence in autism reflects genuine phenomena, and is not the result of population migration, differences in autism diagnoses, or other potential confounders. This suggests that the significant rise in autism observed in the U.S. Department of Education datasets analyzed by us in the present study represents a genuine phenomena and is not the result of potential confounding.

The strength of this study regarding mercury from thimerosal-containing childhood vaccines stems from the fact that it provides a large overall picture of the effects of administration of tens of millions of doses of thimerosal-containing childhood vaccines to millions of children. In addition, the children in the birth cohorts examined in this study were all at least six years of age, allowing for sufficient elapse of time so that a diagnosis of autism could be made, and all diagnoses of autism were all made by the same organization, namely the U.S. Department of Education, minimizing any potential differential diagnoses of autism. The weakness of this study stems from the fact that individual children were not examined. The result is that broad universal effects could only be detected in this study, but the strength of the signal generated by mercury from thimerosal-containing childhood vaccines on the prevalence of the neurodevelopmental disorder of autism was such that we were still able to detect its effects in this study (i.e. >50% increase in the prevalence of autism). This indicates that mercury from thimerosal-containing childhood vaccines has a very significant relationship with autism.

The results of this analysis regarding mercury from thimerosal-containing childhood vaccines and autism also provide evidence to show the validity of the Vaccine Adverse Event Reporting System (VAERS) database. In our previous analyses of the VAERS database, we compared the dose response effects of increasing doses of mercury from thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines on the incidence of autism. We determined that the odds ratio of autism increased by 0.029 per microgram of mercury [4]. This result is in very good agreement with the odds ratio slope from this study, where we found that the odds ratio of autism increased by 0.023 per microgram of mercury, as shown in Figure 3. In addition, our previous dose response effects from the VAERS database of increasing doses of mercury from thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines have shown increasing risks for autism comparable to the ones we have observed in this study [2–4]. This mutual confirmation between independent data

sources offers additional compelling evidence that the VAERS database is a useful dataset, and that there is truly an association between mercury from thimerosal-containing childhood vaccines and the childhood neurodevelopmental disorder of autism.

The results of our MMR vaccine analysis showed that the number of doses of measles-containing vaccines administered in the 1980s did affect the population prevalence of autism. In examining the number of pediatric measles-containing vaccines we observed an increase in coverage occurred from the early-to-mid 1980s (~60%) to the early-1990s (~90%), and this increase in coverage did correspond to the prevalence of autism (~30% increase) in the U.S. Department of Education dataset. The results of this study are in agreement with a number of previous studies on the relationship between MMR and measles-containing vaccines and serious neurological disorders including autism.

The British National Encephalopathy Study (NCES) identified prospectively the first 1,000 cases of encephalopathy reported to the British National Encephalopathy Registry [24]. The NCES identified, between 1 January 1977 and 31 December 1978, that there was a statistically significant increased relative risk of 2.93 (95% CI: 1.20 to 7.96) for serious neurological disorders within 14 days following measles vaccination. Weibel et al. have reported that the clustering of reactions on days eight and nine following measles-containing vaccines suggests that there is a probable causal relationship between measles vaccine and encephalopathy followed by permanent brain injury or death [25]. The U.S. National Vaccine Injury Compensation Program (NVICP) has established that encephalopathy, with neurological symptoms lasting for at least 6 months, and occurring within 5 to 15 days following MMR vaccination is among the vaccine-table injuries [26]. In addition, as was previously stated, our own epidemiological analyses showed that there was an increased risk for serious neurological disorders including autism, permanent brain damage, ataxia and mental retardation following pediatric MMR immunization [1].

Wakefield et al. [27] have clinically investigated a consecutive series of children with chronic enterocolitis and regressive developmental disorder among 12 children. Onset of behavioral symptoms was associated, by the parents, with measles, mumps, and rubella vaccination in eight of the 12 children, with measles infection in one child, and otitis media in another. The authors reported that they identified associated gastrointestinal disease and developmental regression in a group of previously normal children, which was generally associated in time with possible environmental triggers. Subsequently, Wakefield et al. [28] have described some of the endoscopic and pathological characteristics in a group of children with developmental disorders that were associated with behavioral regression and bowel symptoms, and compared them with pediatric controls. The authors observed that ileal lymphoid nodular hyperplasia was present in 54 of 58 (93%) affected children and in five of 35 (14.3%) controls ($p < 0.001$). Colonic lymphoid nodu-

lar hyperplasia was present in 18 of 60 (30%) affected children and in two of 37 (5.4%) controls ($p < 0.01$). Histologically, reactive follicular hyperplasia was present in 46 of 52 (88.5%) ileal biopsies from affected children and in four of 14 (29%) with ulcerative colitis, but not in non-inflammatory bowel disease controls ($p < 0.01$). Active ileitis was present in four of 51 (8%) affected children but not in controls. Chronic colitis was identified in 53 of 60 (88%) affected children compared with one of 22 (4.5%) controls and in 20 of 20 (100%) with ulcerative colitis. Scores of frequency and severity of inflammation were significantly greater in both affected children and those with ulcerative colitis, compared with controls ($p < 0.001$). Uhlmann et al. [29] have even investigated the presence of persistent measles virus in the intestinal tissue of these patients (new variant inflammatory bowel disease) and a series of controls by molecular analysis, and determined that 75 of 91 patients with a histologically confirmed diagnosis of ileal lymphonodular hyperplasia and enterocolitis were positive for measles virus in their intestinal tissue compared with 5 of 70 control patients. In addition, Singh et al. [30] have analyzed serum samples of 125 autistic children and 92 control children. ELISA analysis showed a significant increase in the level of MMR antibodies in autistic children. Immunoblotting analysis revealed the presence of an unusual MMR antibody (immunopositive for measles hemagglutinin protein, which is unique to the measles subunit of MMR vaccine) in 75 of 125 (60%) autistic sera but not in control sera. Furthermore, over 90% of MMR antibody-positive autistic sera were also positive for Myelin Basic Protein autoantibodies, reported by the authors as suggesting a strong association between MMR and Central Nervous System autoimmunity in autism.

It has been suggested that at present considering that the three live viral components of MMR vaccine work synergistically to produce more severe adverse reactions among vaccine recipients than would be expected based upon the individual components of the vaccine, that one might consider presently taking each of the components separately [1,31]. In addition, it has been suggested that additional research be conducted in order to improve the safety of MMR immunization by producing a new killed MMR vaccine [1].

Overall, in evaluating all the information available, we were able to show that MMR vaccine potentially contributed to a rise in the prevalence of autism in the United States during the 1980s, and that mercury doses of thimerosal-containing vaccines contributed to a rise and a subsequent fall in the prevalence of autism in the United States during the late 1980s and 1990s. From our analysis, it appears that thimerosal in childhood vaccines contributed more to the raise in the prevalence in autism observed in this study than did the MMR vaccine. The sources used for analysis in this study did not provide available information about either of the vaccines under study or the number of children with autism during the 1970s. It may be useful to in subsequent studies to analyze data from the 1970s to further examine the relationship between MMR vaccine and mercury from thimerosal on the prevalence rate of autism. This

study was also unable to analyze the potential interactions that may exist between MMR vaccine and thimerosal, which would be another useful area for subsequent research.

CONCLUSIONS

In conclusion, this study provides additional epidemiological evidence showing that there is a direct correlation between increasing mercury from thimerosal-containing childhood vaccines and the childhood neurodevelopmental disorder of autism. In addition, it showed that there were statistically significantly increased odds ratios for the prevalence of autism that correlated with increasing doses of mercury from thimerosal-containing childhood vaccines in comparison to a baseline measurement. Similarly, this study also provides additional epidemiological evidence showing that there is a direct correlation between increasing primary pediatric measles-containing immunizations and the prevalence of autism. Despite the negative results of this study, we believe that continued vaccination is justified. We believe that every effort should be made to produce vaccines that are thimerosal-free, and research should be undertaken to design a safer MMR vaccine. We believe one might consider the option, in order to alleviate some of the adverse effects of MMR vaccine, of taking each component of this vaccine separately. In the U.S. those children that are adversely affected by the current MMR vaccine should report their adverse reaction to the Vaccine Adverse Events Reporting System (VAERS) database, so that more information may be generated about the safety profile of MMR vaccine, and such unfortunate children should be entitled to rapid and generous justice before the U.S. NVICP.

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