Prenatal and Infant Exposure to Thimerosal From Vaccines and Immunoglobulins and Risk of Autism

Article *in* PEDIATRICS · October 2010

DOI: 10.1542/peds.2010-0309 · Source: PubMed

14 authors, including:

William W Thompson
Centers for Disease Control and Prevention
123 PUBLICATIONS **18,639** CITATIONS
SEE PROFILE

Barbara Goodson
Abt Associates
24 PUBLICATIONS **1,057** CITATIONS
SEE PROFILE

Eric Weintraub
Centers for Disease Control, Lesotho
200 PUBLICATIONS **16,547** CITATIONS
SEE PROFILE

Ned Lewis
Kaiser Permanente
202 PUBLICATIONS **14,455** CITATIONS
SEE PROFILE

Prenatal and Infant Exposure to Thimerosal From Vaccines and Immunoglobulins and Risk of Autism

WHAT'S KNOWN ON THIS SUBJECT: Most previous research has not revealed an increased risk of autism associated with receipt of thimerosal-containing vaccines. Evidence is limited, however, on the timing of vaccination, especially prenatal exposure, and associations with different subtypes of autism.

WHAT THIS STUDY ADDS: This study revealed no increased risk of ASD associated with receipt of thimerosal-containing vaccines. No increased risk was found for subtypes of ASD, including ASD with regression, and prenatal exposure was not associated with a risk of ASD.

AUTHORS: Cristofer S. Price, ScM,[a] William W. Thompson, PhD,[b] Barbara Goodson, PhD,[a] Eric S. Weintraub, MPH,[c] Lisa A. Croen, PhD,[d] Virginia L. Hinrichsen, MS, MPH,[e] Michael Marcy, MD,[f] Anne Robertson, PhD,[a] Eileen Eriksen, MPH,[f] Edwin Lewis, MPH,[d] Pilar Bernal, MD,[g] David Shay, MD, MPH,[h] Robert L. Davis, MD, MPH,[i] and Frank DeStefano, MD, MPH[c]

[a]Abt Associates Inc, Cambridge, Massachusetts; [b]National Center for Chronic Disease Prevention and Health Promotion, [c]Immunization Safety Office, and [h]Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia; [d]Division of Research, Kaiser Permanente Northern California, Oakland, California; [g]Department of Psychiatry and Behavioral Sciences, Kaiser Permanente ASD Center San Jose Northern California Region, Stanford University, Palo Alto, California; [e]Department of Population Medicine, Harvard Pilgrim Health Care Institute, Harvard Medical School, Boston, Massachusetts; [f]Southern California Kaiser Permanente, and Center for Vaccine Research, University of California, Los Angeles, California; and [i]Center for Health Research Southeast, Kaiser Permanente, Atlanta, Georgia

KEY WORDS
thimerosal, mercury, vaccines, immunoglobulins, autism

ABBREVIATIONS
CDC—Centers for Disease Control and Prevention
MCO—managed care organization
ASD—autism spectrum disorder
TCI—thimerosal-containing injection
AD—autistic disorder
ADI-R—Autism Diagnostic Interview-Revised
ADOS—Autism Diagnostic Observation Schedule
SCQ—Social Communication Questionnaire
OR—odds ratio
Hib—*Haemophilus influenzae* type b

The views in this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

www.pediatrics.org/cgi/doi/10.1542/peds.2010-0309

doi:10.1542/peds.2010-0309

Accepted for publication Jun 9, 2010

Address correspondence to Frank DeStefano, MD, MPH, Immunization Safety Office, MS D-26, Centers for Disease Control and Prevention, Atlanta, GA 30333. E-mail: fxd1@cdc.gov

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: *Dr Marcy received honoraria for speaking for Merck and GlaxoSmithKline within the last 5 years and grant support for studies on Gardasil and ProQuad from Merck within the last 5 years; Mr Lewis received grant support from Medimmune, Sanofi Pasteur, Chiron, Wyeth, Merck, and GlaxoSmithKline; and Dr Bernal received research funding from the CDC, the National Institute of Mental Health, Health Resources and Service Administration, and Autism Speaks. The other authors have no financial relationships relevant to this article to disclose.*

abstract

FREE

OBJECTIVE: Exposure to thimerosal, a mercury-containing preservative that is used in vaccines and immunoglobulin preparations, has been hypothesized to be associated with increased risk of autism spectrum disorder (ASD). This study was designed to examine relationships between prenatal and infant ethylmercury exposure from thimerosal-containing vaccines and/or immunoglobulin preparations and ASD and 2 ASD subcategories: autistic disorder (AD) and ASD with regression.

METHODS: A case-control study was conducted in 3 managed care organizations (MCOs) of 256 children with ASD and 752 controls matched by birth year, gender, and MCO. ASD diagnoses were validated through standardized in-person evaluations. Exposure to thimerosal in vaccines and immunoglobulin preparations was determined from electronic immunization registries, medical charts, and parent interviews. Information on potential confounding factors was obtained from the interviews and medical charts. We used conditional logistic regression to assess associations between ASD, AD, and ASD with regression and exposure to ethylmercury during prenatal, birth-to-1 month, birth-to-7-month, and birth-to-20-month periods.

RESULTS: There were no findings of increased risk for any of the 3 ASD outcomes. The adjusted odds ratios (95% confidence intervals) for ASD associated with a 2-SD increase in ethylmercury exposure were 1.12 (0.83–1.51) for prenatal exposure, 0.88 (0.62–1.26) for exposure from birth to 1 month, 0.60 (0.36–0.99) for exposure from birth to 7 months, and 0.60 (0.32–0.97) for exposure from birth to 20 months.

CONCLUSIONS: In our study of MCO members, prenatal and early-life exposure to ethylmercury from thimerosal-containing vaccines and immunoglobulin preparations was not related to increased risk of ASDs. *Pediatrics* 2010;126:656–664

Thimerosal has been used as a preservative in vaccines since the 1930s.[1] It is 49.6% mercury by weight and is metabolized into ethylmercury and thiosalicylate.[2] In 1999, the US Food and Drug Administration estimated that infants who were immunized according to the recommended schedule might have received amounts of ethylmercury that exceed Environmental Protection Agency limits for exposure to methylmercury.[1] As a precautionary measure, the US Public Health Service and the American Academy of Pediatrics urged vaccine manufacturers to remove thimerosal from all infant vaccines as soon as practical and recommended that studies be conducted to investigate the risks associated with ethylmercury exposure from thimerosal-containing vaccines.[3] In response, the Centers for Disease Control and Prevention (CDC) planned studies to examine potential links between ethylmercury exposure and developmental outcomes. The first, a screening analysis that used computerized databases from 3 large managed care organizations (MCOs), examined relationships between ethylmercury exposure from childhood vaccines and several neurodevelopmental conditions. No significant associations with autism spectrum disorder (ASD) were found.[4] Two subsequent CDC-sponsored studies examined neuropsychological outcomes, but ASD was not assessed in either of them.[5,6]

Our current study was designed to examine the relationships between ethylmercury exposure from thimerosal-containing injections (TCIs), which include thimerosal-containing vaccines and immunoglobulin preparations, and any of 3 ASD outcomes: ASD; autistic disorder (AD); and ASD with regression. We used state-of-the-art assessment tools to confirm ASD outcomes and evaluated both prenatal and postnatal exposure.

METHODS

We performed a case-control study in 3 MCOs that participate in the CDC's Vaccine Safety Datalink.[7–9] The institutional review boards of the 3 MCOs, CDC, and Abt Associates Inc approved the study. The study protocol was developed before data collection in consultation with a panel of external consultants that included autism advocates and experts in autism, child development, toxicology, epidemiology, biostatistics, and vaccine safety. All subgroup analyses and interaction tests were specified in the study protocol before data collection.

Data sources included MCO computerized data files, abstraction of maternal and child medical charts, and standardized telephone interviews with the children's biological mothers. Case-children underwent standardized in-person assessments to verify case status. Additional details regarding study design, analyses, and results can be found in technical reports available online.[10,11]

Study Population

Children from each MCO were eligible to participate if they were born between January 1, 1994, and December 31, 1999; had been continuously enrolled in the MCO from birth until their second birthday and were currently enrolled at the time of sample selection; and lived within 60 miles of a study assessment clinic. Children were 6 to 13 years old at the time of data collection. Children had to have lived with their biological mother since birth, and their family had to be fluent in English. Parents provided written consent to participate in the study. Children were excluded if they had the following medical conditions with known links to ASD traits: fragile X syndrome; tuberous sclerosis; Rett syndrome; congenital rubella syndrome; or Angelman syndrome. Recruitment was attempted for all eligible case-children within the MCO populations. Control children were randomly selected from the MCO populations to match case-children within matching strata defined by birth year, gender, and MCO.

Case Enrollment and Verification

Potential case-children were identified by searching the MCO computerized records for relevant ASD *International Classification of Diseases, Ninth Revision*, codes (299.0-ASD or 299.8-PDD NOS), supplemented by text-string searches at 1 MCO, and text strings and autism registries at another. Mothers of case-children were administered the Autism Diagnostic Interview-Revised (ADI-R),[12] and case-children were directly assessed by using the Autism Diagnostic Observation Schedule (ADOS).[13]

ASD consists of qualitative abnormalities in reciprocal social interactions and communication and restrictive, repetitive, and stereotyped patterns of behavior. Children who meet study criteria for ASD had ADOS scores that indicated abnormalities in all 3 areas and had ADI-R scores that indicated abnormalities in reciprocal social interactions and either communication or patterns of behavior. Children who met study criteria for AD were a subset of ASD children who had higher scores on all 3 areas of the ADOS, had ADI-R scores that indicated abnormalities in all 3 areas, and had onset at younger than 36 months. Using items from the ADI-R, ASD with regression was defined as the subset of case-children with ASD who reported loss of previously acquired language skills after acquisition. For additional details on case-ascertainment criteria, see the technical report.[10]

Assessors were trained and assessed for reliability using procedures developed by Dr Catherine Lord, 1 of the developers of the ADOS and ADI-R instruments. Assessors were blinded with respect to the thimerosal exposure status of the child and mother.

Controls

To reduce the likelihood that the control group included children with undiagnosed ASD, the lifetime form of the Social Communication Questionnaire (SCQ)[14] was administered as part of the maternal interview for children who had indications of neurodevelopmental difficulties.[10] Seven control-group children with SCQ scores higher than 15 were excluded from the analyses (C. Lord, PhD, personal verbal communication, 2004).

Sample

Physician consent was required before families could be recruited. Consent was requested for all case-children who met the eligibility requirements that could be ascertained from MCO records, before recruitment and eligibility calls, and for a randomly selected sample of controls that were matched to case-children within birth year, gender, and MCO matching strata. This sampling stage resulted in a pool of controls with physician consent (Fig 1). As case-children were confirmed as eligible and enrolled as study participants, random samples of matched controls were selected for recruitment from the pool of controls. The targeted control to case ratio was 3 to 1 within each matching stratum. Controls who were matched to case-children who later did not meet the study's clinical assessment criteria for ASD were excluded from the analyses.

Ethylmercury Exposure From TCIs

Children's histories of TCI receipts were obtained from computerized immunization records and abstracted medical charts. Mercury content of the TCIs was determined by linking the manufacturer, lot number, and year of receipt information to published data[15–17] and manufacturer records. Maternal receipt of immunoglobulins, tetanus toxoids, and diphtheria-tetanus during pregnancy was primarily ascertained from medical charts (81 receipts) and less often from maternal interviews (6 receipts). Maternal receipt of flu vaccine during pregnancy was infrequently recorded in medical charts (2 receipts) and primarily came from maternal report (36 receipts). We defined postnatal exposure as micrograms of ethylmercury divided by the weight of the child (in kilograms) at the time of administration of each TCI. Exposures were summed over the time periods of interest. Prenatal exposure was defined as the cumulative ethylmercury amount (in micrograms) of all TCIs received by the mother during her pregnancy with the child.

Covariates

Covariates tested for inclusion in the statistical models were child and family characteristics (maternal and paternal age at birth of child, maternal education level, family income, single-parent status, birth order, twin/triplet, breastfeeding duration); maternal exposures during pregnancy (exposure to mercury from fish, from cosmetics or medicines, or from dental fillings; use of tobacco, alcohol, or illegal drugs; use of folic acid or valproic acid; viral infections; lead exposure); child birth conditions (birth weight, Apgar score, birth asphyxia, respiratory distress syndrome, hyperbilirubinemia); early-childhood health conditions (anemia, lead exposure, pica, encephalitis); and maternal health care–seeking behavior (Kotelchuck prenatal care index, cholesterol and Papanicolaou test screenings).

Statistical Analysis

We used the SAS 9.1 (SAS Institute Inc, Cary, NC) PHReg procedure to fit conditional logistic regression models[18] that accounted for matching within strata defined by birth year, gender, and MCO to estimate the odds ratios (ORs) for ASD outcomes associated with increases in ethylmercury exposure for 4 different periods: prenatal; birth to 1 month; birth to 7 months; and birth to 20 months. Models were fit with and without covariates. Covariates were retained in the final models if they satisfied a change-in-estimate[19] criterion evaluated by dropping terms that resulted in a $<10\%$ change in exposure coefficients relative to a full model with all potential covariates.

All tests were 2-tailed, and statistical significance was set at $P < .05$. To facilitate interpretation of results, we present ORs in 2 forms. The first is the OR associated with an increase of 1 unit of exposure, in which 1 unit equals 1 μg of ethylmercury for prenatal exposure or 1 μg of ethylmercury per kilogram of body weight for postnatal exposure. The second, which is used as an indication of the difference between low and high exposure, is the OR for a difference in exposure equal to 2 SDs for each particular exposure measure of interest. A 2 SD increase in exposure can be thought of as roughly the difference between the 10th and 90th percentiles on these measures. For the measure of prenatal ethylmercury exposure, 2 SDs is equal to 16.34 μg or a little more than the amount in typical Rhogam injections in use during the years included in our study. Two SDs of the birth-to-1-month measure is 4.08 μg/kg, and 2 SDs for the birth-to-7-month and the birth-to-20-month measures are 15.56 and 17.82 μg/kg, respectively.

For the ASD outcome, for each 2 SD increase in mercury received in the prenatal, birth-to-1-month, birth-to-7-month, and birth-to-20 month periods,

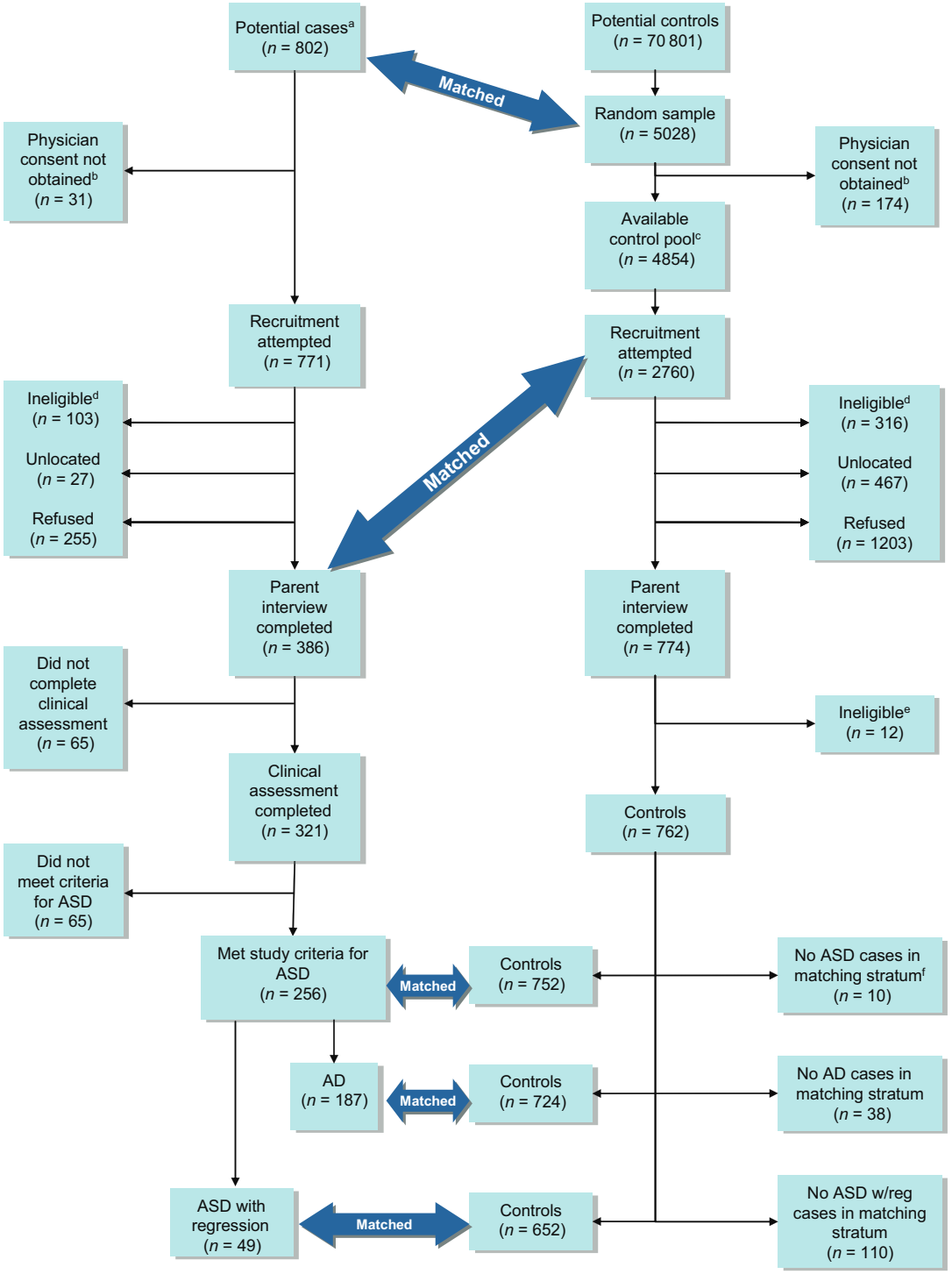

FIGURE 1

Sample flow diagram. [a] Potential case-children had a diagnosis of ASD in their medical charts (see text for eligibility criteria). [b] Before recruitment, physician consent was required. [c] Physician consent was obtained for 4854 potential controls. From this group, random samples of controls (totaling 2760) were drawn, as needed, to match participating case-children within birth year, gender, and MCO matching strata. [d] Ineligibility was determined during recruitment or eligibility calls. [e] Ineligibility was determined from information obtained from parent interview, SCQ, or medical chart abstraction. [f] Controls were matched to case-children by birth year, gender, and MCO. If there were no potential case-children who met study criteria for ASD within a birth year, gender, and MCO matching stratum, the controls in that stratum could not be used in the analysis.

posthoc calculations indicate that the study had ~80% power to detect ORs of 1.5, 1.7, 2.1, and 2.2, respectively.

In addition, by adding model terms to test for interactions, we examined whether the effect of postnatal thimerosal exposure on the risk of the 3 ASD outcomes was modified by the gender of the child, concurrent

antibiotic use, or prenatal thimerosal exposure.

RESULTS

Characteristics of the Children

Of 771 potential case-children and 2760 controls selected for recruitment, 103 case-children (13.4%) and 316 controls (11.4%) were found to be ineligible (Fig 1). Among the 668 case-children and 2444 controls remaining, 321 case-children (48.1%) and 774 controls (31.7%) participated in all phases of the study. Reasons for nonparticipation included inability to locate (cases: $n = 27$ [4.0%]; controls: $n = 467$ [19.1%]), refusal to participate (cases: $n = 255$ [38.2%]; controls: $n = 1203$ [49.2%]), and difficulty scheduling or completing the clinical assessment (cases: $n = 65$ [9.7%]). Ninety-four control mothers and 14 case-mothers participated in a refusal survey. Among control mothers, lack of time (62%) and distrust or ambivalence toward research (23%) were stated as primary reasons for nonparticipation. For case-mothers, the primary reasons were lack of time (50%), belief that child was ineligible (14%), and maternal health (14%). Among the 774 control participants, 12 (1.6%) were excluded because the analysis of their medical charts and parent interview data revealed they had exclusionary conditions. In addition, 10 controls were not included in the analysis because there were no case-children who met study criteria for ASD within the relevant birth year, gender, and MCO matching strata (Fig 1).

Of the 321 potential case-children who participated in standardized assessments, 256 (79.8%) met study criteria for ASD (Fig 1). Among those who met criteria for ASD, 187 (73%) met the stricter criteria for AD, and 49 (19%) met criteria for ASD with regression.

Children were 6 to 13 years old at the time of data collection, 85% were male, and 7% had low birth weight (Table 1). Maternal age, maternal education, maternal marital status, and paternal age were similar for case-children and controls.

TABLE 1 Characteristics of Study Participants

Characteristic	Children With ASD ($N = 256$), %	Controls ($N = 752$), %	P[a]
MCO			
MCO-A	4	4	.49
MCO-B	43	46	
MCO-C	54	49	
Child's year of birth			
1994	14	16	.90
1995	15	15	
1996	16	18	
1997	21	19	
1998	19	17	
1999	14	14	
Child's age at time of interview/assessment, y			
6	10	5	.04[b]
7	21	18	
8	17	18	
9	21	19	
10	14	17	
11	14	17	
12	2	5	
13	0.4	0.3	
Gender			
Female	13	15	.37
Male	87	85	
Birth weight, g			
<1000	2	0.3	.16
1000–1499	0.4	1	
1500–2499	7	5	
2500–3999	76	79	
>4000	16	15	
Biological mother's age at birth of child, y			
<20	2	1	.27
20–24	5	9	
25–29	23	23	
30–34	36	36	
≥35	35	30	
Biological father's age at birth of child, y			
<20	1	1	.65
20–29	20	24	
30–39	60	56	
40–49	17	17	
≥49	2	2	
Mother's education level			
No diploma	3	3	.75
High school graduate	15	15	
Some college	19	22	
College graduate	63	60	
Single parent			
No	82	85	.29
Yes	18	15	

Percentages of cases and controls were not exactly identical on matching variables (birth year, gender, MCO) because we did not always get exactly 3 matched controls per case within each matching stratum.

[a] P for χ^2 test of independence between row (characteristic of study participant) and column (ASD case versus control).

[b] Recruitment of controls lagged behind case-children so that controls could be recruited to match case-children who had agreed to participate within birth year, gender, and MCO matching strata. The lagged recruitment meant that controls were an average of 3 months older than case-children at the time of interview/assessment.

Relationships of ASD Outcomes to Ethylmercury Exposure

On average, case-children and control children had similar cumulative ethyl-

TABLE 2 Cumulative Exposure to Ethylmercury According to Exposure Period

Case/Control Comparison/Exposure Period	Cumulative Exposure Amount, μg					
	Case-Children			Controls		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Case-children with ASD ($n = 256$) vs controls ($n = 752$)						
Prenatal	2.70	0	74.00	2.35	0	100.00[a]
Birth to 1 mo (28 d)	9.01	0	45.00	8.99	0	50.00[b]
Birth to 7 mo (214 d)	101.13	0	190.83[d]	103.54	0	187.50[c]
Birth to 20 mo (609 d)	133.58	0	300.00	137.00	0	262.50
Case-children with AD ($n = 187$) vs controls ($n = 724$)						
Prenatal	2.96	0	62.75	2.28	0	100.00
Birth to 1 mo (28 d)	9.40	0	45.00	9.01	0	50.00
Birth to 7 mo (214 d)	101.42	0	190.83	104.65	0	187.50
Birth to 20 mo (609 d)	134.64	0	253.33	138.54	0	262.50
Case-children with ASD with regression ($n = 49$) vs controls ($n = 652$)						
Prenatal	3.34	0	25.00	1.86	0	37.75
Birth to 1 mo (28 d)	9.08	0	45.00	8.92	0	50.00
Birth to 7 mo (214 d)	101.09	0	190.83	103.28	0	187.50
Birth to 20 mo (609 d)	140.12	0	253.33	136.80	0	262.50

Ethylmercury from thimerosal-containing vaccines and immunoglobulins. For descriptive purposes, the postnatal exposure amounts shown here were not divided by weight at time of vaccine receipt. Most vaccines in use at the time that case-children were infants contained 0, 12.5, or 25 μg of ethylmercury per dose. Among case-children with ASD, mean prenatal ethylmercury exposure was 2.70 and ranged from 0 to 74 μg of ethylmercury from thimerosal-containing vaccines and immunoglobulins received by the mother during her pregnancy with the study child.

[a] Maximum from maternal receipt of 2 immunoglobulins during pregnancy, each containing 50 μg of ethylmercury.

[b] Maximum from child receipt of hepatitis B immunoglobulin (25 μg) and hepatitis B vaccine (12.5 μg) at birth and hepatitis B vaccine (12.5 μg) at 28 days of age.

[c] Maximum from child receipt of 3 hepatitis B (12.5 μg), 3 diphtheria-tetanus-acellular pertussis (25 μg), and 3 Hib (25 μg) vaccines in first 7 months.

[d] Maximum from child receipt of 2 hepatitis B (12.5 μg), 1 rabies (20 μg), 3 diphtheria-tetanus toxoids-pertussis (24.27, 23.28, and 23.28 μg), and 3 Hib (25 μg) vaccines in first 7 months.

mercury exposures at the end of each exposure period (Table 2). Variation among children's exposure amounts was attributable to variation in mercury content of TCIs (eg, *Haemophilus influenzae* type b [Hib] vaccines in use at the time contained 0, 12.5, or 25 μg of ethylmercury), use of combined versus separate vaccines (eg, separate receipts of diphtheria-tetanus toxoids-pertussis and Hib vaccines could result in twice the mercury exposure as receipt of a combined diphtheria-tetanus toxoids-pertussis-Hib vaccine) and variation in the number of TCIs received.

Exposure to ethylmercury from TCIs prenatally or in the first month of life was not significantly associated with any of the ASD outcomes (Table 3). The prenatal and birth-to-1-month results were similar even when adjusted for other covariates. In the adjusted analyses, however, increased cumulative exposures in the age ranges from birth to 7 months and birth to 20 months were both associated with decreased risk of all 3 ASD outcomes.

We found no significant differences in exposure effects between boys and girls for any of the ASD outcomes, no evidence that higher prenatal exposure exacerbated the effects of postnatal exposure, and no evidence that concurrent ethylmercury exposure and antimicrobial use was associated with risk of ASDs (for full model results, see the technical report).[10]

DISCUSSION

We found no evidence that increasing ethylmercury exposure from TCIs was associated with increased risk of ASD, AD, or ASD with regression. The unadjusted model results showed no significant associations between exposure and risk of ASD or AD. In the covariate adjusted models, we found that an increase in ethylmercury exposure in 2 of the 4 exposure time periods evaluated was associated with decreased risk of each of the 3 ASD outcomes. We are not aware of a biological mechanism that would lead to this result. Analyses to explore potential explanations are presented in the technical report.[10,11] For example, there were no significant differences between case-children and controls in the numbers of vaccines received up to ages 7 or 20 months. Case-children were more likely to have received thimerosal-free or combined Hib vaccines than controls and more likely to have received thimerosal-free hepatitis B vaccines, resulting in the slightly lower cumulative exposure amounts. Knowledge that a child had ASD was not likely to have influenced choice of vaccines because none of the case-children had ASD diagnoses by 7 months old, and few had diagnoses by 20 months. There was no significant association between having an older autistic sibling and exposure levels. In addition,

TABLE 3 Association Between Thimerosal Exposure and Autism Outcomes

Exposure Measure	Unadjusted Model Results (No Covariates)		Covariate Adjusted Model Results	
	1-U Difference in Exposure, OR (95% CLs)[a]	2-SD Difference in Exposure, OR (95% CLs)[b]	1-U Difference in Exposure, OR (95% CLs)[a]	2-SD Difference in Exposure, OR (95% CLs)[b]
Case-children with ASD ($n = 256$) vs controls ($n = 752$)				
Prenatal	1.007 (0.990, 1.025)	1.125 (0.846, 1.495)	1.007 (0.988, 1.026)	1.119 (0.827, 1.513)
Birth to 1 mo (28 d)	0.973 (0.898, 1.054)	0.894 (0.644, 1.240)	0.970 (0.889, 1.059)	0.883 (0.617, 1.264)
Birth to 7 mo (214 d)	0.992 (0.966, 1.020)	0.887 (0.582, 1.351)	0.967 (0.937, 0.999)[c]	0.597 (0.360, 0.990)[c]
Birth to 20 mo (609 d)	0.991 (0.965, 1.016)	0.862 (0.533, 1.336)	0.968 (0.938, 0.998)[c]	0.598 (0.317, 0.971)[c]
Case-children with AD ($n = 187$) vs controls ($n = 724$)				
Prenatal	1.010 (0.991, 1.030)	1.179 (0.862, 1.614)	1.011 (0.990, 1.032)	1.196 (0.855, 1.674)
Birth to 1 mo (28 d)	1.010 (0.927, 1.100)	1.040 (0.732, 1.478)	1.029 (0.935, 1.132)	1.123 (0.759, 1.661)
Birth to 7 mo (214 d)	0.991 (0.962, 1.022)	0.875 (0.545, 1.404)	0.958 (0.924, 0.994)[c]	0.516 (0.290, 0.916)[c]
Birth to 20 mo (609 d)	0.992 (0.964, 1.021)	0.884 (0.520, 1.449)	0.962 (0.928, 0.996)[c]	0.544 (0.265, 0.938)[c]
Case-children with ASD with regression ($n = 49$) vs controls ($n = 652$)				
Prenatal	1.031 (0.993, 1.072)	1.656 (0.885, 3.095)	1.039 (0.997, 1.083)	1.860 (0.945, 3.660)
Birth to 1 mo (28 d)	0.938 (0.794, 1.108)	0.769 (0.390, 1.519)	0.901 (0.761, 1.067)	0.653 (0.327, 1.303)
Birth to 7 mo (214 d)	0.936 (0.880, 0.994)[c]	0.355 (0.138, 0.915)[c]	0.906 (0.848, 0.968)[c]	0.214 (0.076, 0.600)[c]
Birth to 20 mo (609 d)	0.953 (0.900, 1.009)	0.473 (0.154, 1.170)	0.925 (0.869, 0.985)[c]	0.297 (0.081, 0.764)[c]

Covariates for ASD models: birth weight, maternal age, birth order, breastfeeding duration, family income, maternal health care–seeking behavior (Kotelchuck inadequacy of prenatal care, use of cholesterol screening, use of Papanicolaou test screening), maternal exposures during pregnancy with study child (alcohol use, folic acid use, viral infection, lead exposure), and early childhood health conditions (anemia between 6 and 30 months of age; pica before 3 years of age). CLs indicates confidence limits. Covariates for AD models: birth weight, maternal age, birth order, breastfeeding duration, family income, maternal health care–seeking behavior (Kotelchuck inadequacy of prenatal care, use of cholesterol screening, use of Papanicolaou test screening), maternal exposures during pregnancy with study child (folic acid use), and early childhood health conditions (anemia between 6 and 30 months of age; pica before 3 years of age). Covariates for ASD with regression models: birth weight, maternal age, family income, maternal education level, maternal exposures during pregnancy with study child (alcohol use).

[a] OR for autism associated with a 1 U increase in exposure. For prenatal exposure, 1 U = 1 μg of ethylmercury. For postnatal exposure, 1 U = 1 μg of ethylmercury per 1 kg of body weight at time of vaccine or immunoglobulin receipt.

[b] OR for autism associated with an increase in exposure equal to 2 SD units of the exposure measure. For the measure of prenatal exposure, 2 SDs = 16.34 μg. Two SDs of the birth-to-1-month measure is 4.08 μg/kg U. Similarly, 2 SDs of birth-to-7-month and birth-to-20-month exposures are 15.56 μg/kg and 17.82 μg/kg.

[c] 95% CLs for the OR does not include 1.000.

there was no substantive difference in the association between thimerosal exposure and risk for ASD among children with an older autistic sibling compared with children without an older autistic sibling, nor did we find that excluding children with older autistic siblings qualitatively changed our results.

Sensitivity analyses that assessed the effects of potentially influential observations and potential sources of bias are presented in the technical report.[11] For example, results from fitting models separately to data from the 2 largest MCOs showed that the exposure estimates in both were similar to the overall results. We found no evidence that the results were sensitive to extreme exposure amounts, extreme residual values, or were being driven by a few unusual individuals. We further determined that modeling exposure measures as linear terms was appropriate. Use of postnatal exposure variables that were not divided by the child's weight at the time of vaccine receipt did not change our findings. Exclusion of low birth weight children from the analyses resulted in only a slight attenuation of exposure effects toward 0.

Our study's primary limitations are those inherent in observational studies. Specifically, although we were able to control for many potential confounders, there is no way of knowing whether a critical confounder was omitted, and the relatively low response rates suggest a potential for selection bias to influence the results. However, analysis of ethylmercury exposure levels of the entire selected sample, as assessed through the use of computerized MCO records, indicated no significant differences among participant case-children, nonparticipant case-children, participant controls, and nonparticipant controls in cumulative exposure amounts at ages 1, 7, or 20 months, suggesting that self-selection did not bias the results.[11] In addition, all study children were MCO members for their first 2 years of life, and were members of the same MCOs 6 to 13 years later, at the time of sample selection. Although unlikely, if there were a relationship between a family's decision to leave or remain in the MCO and exposure level that differed according to case/control status, then the results could be biased.

Reporting bias can also be a concern with case-control studies, particularly because of differential recall of exposures by case-children compared with

controls. For measures of prenatal exposure, we used information obtained from the maternal interview on vaccination and immunoglobulin exposures during pregnancy. However, we attempted to minimize the effects of recall bias by also using information recorded in maternal medical charts.

ASDs are behaviorally defined and therefore difficult to diagnose definitively. Among the strengths of our study was the use of state-of-the-art assessment tools to validate the ASD diagnoses in children's medical charts and the use of the SCQ assessment tool to exclude children with potentially undiagnosed ASDs from the control group. Additional strengths were that measures of childhood exposure to ethylmercury from TCIs were derived from computerized and medical chart data sources and were therefore not susceptible to recall bias, and the collection of extensive information regarding potential confounding factors.

Given that a large-scale prospective randomized trial is not ethically feasible, no single study can definitively establish or disprove the hypothesis that thimerosal exposure increases the risk of ASDs. Our study adds to the growing base of epidemiologic studies that have been conducted to investigate the hypothesis. In 2004 the immunization safety review committee of the Institute of Medicine[20] published a review of the research evidence concerning relationships between thimerosal-containing vaccines and ASDs. The committee discussed the strengths and limitations of each study reviewed and concluded that the evidence available at that time did not demonstrate a link between thimerosal-containing vaccines and ASDs. Subsequently, 2 ecological studies have found that the prevalence of ASDs continued to increase after the removal of thimerosal from childhood vaccines that began in 1999,[21,22] and 2 studies of prenatal exposure via maternal receipt of thimerosal-containing immunoglobulin preparations during pregnancy did not find associations with ASDs.[23,24]

CONCLUSION

The results of our study of MCO members do not support the hypothesis that ethylmercury exposure from TCIs administered prenatally or during infancy is related to increased risk of ASDs.

ACKNOWLEDGMENTS

This work was supported by a contract from the CDC to America's Health Insurance Plans and via America's Health Insurance Plans subcontracts to Abt Associates Inc; Department of Population Medicine, Harvard Pilgrim Health Care Institute, Harvard Medical School; Southern California Kaiser Permanente, and Center for Vaccine Research, University of California Los Angeles; and Division of Research, Kaiser Permanente Northern California.

We thank clinical assessors Meg Manning, Seton Lindsay, Rachel Hundley, Mary Goyer Shapiro, Thomas Crawford, Liza Stevens, Anh Weber, Susan Bassett, Candace Wollard Bivona, Stephany Cox, Pegeen Cronin, James Earhart, Angela Geissbuhler, Elizabeth Lizaola, and Nuri Reyes; Natacha Akshoomoff, PhD (quality control clinician) from the School of Medicine, University of California San Diego; Ellen Hanson (clinical quality control manager), Cathleen Yoshida (programmer), Roxana Odouli (project manager), medical chart abstractors Darmell Brown, Martha Estrada, Jessica Locke, Sandy Bauska, Margarita Magallon, and Cat Magallon, and clinic coordinator Victoria M. Heffernan from the Division of Research, Kaiser Permanente Northern California; Tracy A. Lieu (principal investigator), Xian-Jie Yu (programmer/analyst), and Rupak Datta (recruiter and medical chart abstractor) from the Department of Population Medicine, Harvard Pilgrim Health Care Institute, Harvard Medical School; Joanne L. Mitchell (medical coordinator) and Deborah Samet (coordinator) from Harvard Vanguard Medical Associates, Developmental Consultation Service (Somerville, MA); Emily London (coordinator), Dorothy Yungman (autism management program coordinator), Linda Ford (recruitment), Tynesha Brown (recruitment and clinic coordinator), Daisy Gonzalez (recruitment and clinic management), Norma Kirk (recruitment and clinic management), Zendi Solano (medical chart abstractor), Oliver DelaCruz (medical chart abstractor), Jerri McIlhagga (medical chart abstractor), Dotty Carmichael (administrative support), Monica Marshall (administrative support), and Kathryn Lee (clinic coordinator) from the UCLA Center for Vaccine Research and Southern California Kaiser Permanente; Douglas Frazier (provision of information on thimerosal content of immunoglobulin preparations) from the US Food and Drug Administration (Bethesda, MD); Robert Chen, James Baggs, Fred Murphy, John Iskander, and Marshalyn Yeargin-Allsopp (for administrative and technical support) from the CDC; Kevin Fahey (for administrative and technical support) from America's Health Insurance Plans (Washington, DC); Stephen Kennedy (project quality assurance advisor), Patty Connor (field director), Carter Smith (design phase support), Gerrie Stewart (design phase support), Amanda Parsad (data analysis), Laura Simpson (data analysis), Julie Williams (data analysis), Yeqin He (data analysis), Bulbul Kaul (data analysis), Melanie Brown-Lyons (data management), Brenda Rodriguez (survey management), and Michael Harnett (survey management) from Abt Associates Inc; and external expert consultants David S. Baskin (Department of Neurosurgery, Methodist Neurological Institute); Sallie Bernard

(SafeMinds); Philip W. Davidson (Strong Center for Developmental Disabilities Golisano Children's Hospital, Strong University of Rochester School of Medicine and Dentistry); Irving Gottesman (Departments of Psychiatry and Psychology, University of Minnesota Medical School); Catherine Lord (Autism and Communication Disorders Center, Center for Human Growth and Development, University of Michigan); Thomas Saari (professor of pediatrics [Emeritus], Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Wisconsin School of Medicine and Public Health); Penelope H. Dennehy, MD (director of pediatric infectious diseases, Hasbro Children's Hospital, and vice chair for academic affairs, Department of Pediatrics, and professor of pediatrics, Warren Alpert Medical School of Brown University); and Andy Shih (Autism Speaks).

REFERENCES

1. Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics*. 2001;107(5):1147–1154
2. Goldfrank L, Flonenbaum N, Lewin N, et al. *Goldfrank's Toxicologic Emergencies. 7th ed. New York, NY: McGraw-Hill*; 2002
3. Joint statement of the American Academy of Pediatrics (AAP) and the United States Public Health Service (USPHS). *Pediatrics*. 1999;104(3 pt 1):568–569
4. Verstraeten T, Davis RL, DeStefano F, et al. Safety of thimerosal-containing vaccines: a two-phased study of computerized health maintenance organization databases. *Pediatrics*. 2003;112(5):1039–1048
5. Thompson WW, Price C, Goodson B, et al. Early thimerosal exposure and neuropsychological outcomes at 7 to 10 years. *N Engl J Med*. 2007;357(13):1281–1292
6. Tozzi AE, Bisiacchi P, Tarantino V, et al. Neuropsychological performance 10 years after immunization in infancy with thimerosal-containing vaccines. *Pediatrics*. 2009;123(2):475–482
7. Chen RT, DeStefano F, Davis RL, et al. The Vaccine Safety Datalink: immunization research in health maintenance organizations in the USA. *Bull World Health Organ*. 2000;78(2):186–194
8. Chen RT, Glasser JW, Rhodes PH, et al. Vaccine Safety Datalink project: a new tool for improving vaccine safety monitoring in the United States. The Vaccine Safety Datalink Team. *Pediatrics*. 1997;99(6):765–773
9. DeStefano F. The Vaccine Safety Datalink project. *Pharmacoepidemiol Drug Saf*. 2001;10(5):403–406
10. Price C, Robertson A, Goodson B. *Thimerosal and Autism*. Technical report. Vol I. Bethesda, MD: Abt Associates Inc; 2009
11. Price C, Robertson A, Goodson B. *Thimerosal and Autism*. Technical report. Vol II. Bethesda, MD: Abt Associates Inc; 2009
12. Rutter M, LeCouteur A, Lord C. *Autism Diagnostic Interview—Revised*. Los Angeles, CA: Western Psychological Services; 2003
13. Lord C, Rutter M, DiLavor PC, Risi S. *Autism Diagnostic Observation Schedule*. Los Angeles, CA: Western Psychological Services; 2003
14. Rutter M, Bailey A, Lord C. *SCQ: The Social Communication Questionnaire*. Manual. Los Angeles, CA: Western Psychological Services; 2003
15. *Physicians' Desk Reference*. 49th ed. Montvale, NJ: Medical Economics Data Production Co; 1995
16. Thimerosal in vaccines: an interim report to clinicians. American Academy of Pediatrics. Committee on Infectious Diseases and Committee on Environmental Health. *Pediatrics*. 1999;104(3 pt 1):570–574
17. *Physicians' Desk Reference*. 53rd ed. Montvale, NJ: Medical Economics Data Production Co; 1999
18. Kleinbaum DG, Klein M. *Logistic Regression: A Self-learning Text*. New York, NY: Springer-Verlag; 2002
19. Hosmer DW, Lemeshow S. *Applied Logistic Regression*. New York, NY: Wiley-Interscience; 2000
20. Institute of Medicine. *Immunization Safety Review: Vaccines and Autism*. Washington, DC: National Academies; 2004
21. Fombonne E, Zakarian R, Bennett A, et al. Pervasive developmental disorders in Montreal, Quebec, Canada: prevalence and links with immunizations. *Pediatrics*. 2006; 118(1). Available at: http://www.pediatrics.org/cgi/content/full/118/1/e139
22. Schechter R, Grether JK. Continuing increases in autism reported to California's developmental services system: mercury in retrograde. *Arch Gen Psychiatry*. 2008; 65(1):19–24
23. Croen LA, Matevia M, Yoshida CK, et al. Maternal Rh D status, anti-D immune globulin exposure during pregnancy, and risk of autism spectrum disorders. *Am J Obstet Gynecol*. 2008;199(3):234.e1–e6
24. Miles JH, Takahashi TN. Lack of association between Rh status, Rh immune globulin in pregnancy and autism. *Am J Med Genet A*. 2007;143A(13):1397–1407

Prenatal and Infant Exposure to Thimerosal From Vaccines and Immunoglobulins and Risk of Autism

Cristofer S. Price, William W. Thompson, Barbara Goodson, Eric S. Weintraub, Lisa A. Croen, Virginia L. Hinrichsen, Michael Marcy, Anne Robertson, Eileen Eriksen, Edwin Lewis, Pilar Bernal, David Shay, Robert L. Davis and Frank DeStefano

Pediatrics 2010;126;656; originally published online September 13, 2010;
DOI: 10.1542/peds.2010-0309

PEDIATRICS®
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Prenatal and Infant Exposure to Thimerosal From Vaccines and Immunoglobulins and Risk of Autism

Cristofer S. Price, William W. Thompson, Barbara Goodson, Eric S. Weintraub, Lisa A. Croen, Virginia L. Hinrichsen, Michael Marcy, Anne Robertson, Eileen Eriksen, Edwin Lewis, Pilar Bernal, David Shay, Robert L. Davis and Frank DeStefano

Pediatrics 2010;126;656; originally published online September 13, 2010;
DOI: 10.1542/peds.2010-0309