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Thimerosal exposure & increasing trends of premature puberty in the vaccine safety datalink

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***Background & objectives*: The US Agency for Toxic Substances and Disease Registry (ATSDR) reports that mercury (Hg) is a known endocrine disruptor and it adversely affects the steroid synthesis pathway in animals and humans, and may interact to enhance the risk for a child developing premature puberty. An association between premature puberty and exposure to Hg from thimerosal-containing vaccines (TCVs) was evaluated in computerized medical records within the Vaccine Safety Datalink (VSD).**

***Methods*: A total of 278,624 subjects were identified in birth cohorts from 1990-1996. The birth cohort prevalence rates of medically diagnosed International Classification of Disease, 9th revision (ICD-9) premature puberty 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.**

***Results*: Significantly increased ($P<0.0001$) rate ratios were observed for premature puberty for a 100 µg difference in Hg exposure from TCVs in the birth-7 months (rate ratio=5.58) and birth-13 months (rate ratio=6.45) of age exposure windows. By contrast, none of the control outcomes had significantly increased rate ratios with Hg exposure from TCVs.**

***Interpretation & conclusions*: 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 done to evaluate the relationship between Hg exposure and premature puberty.**

Key words Endocrine - mercury - merthiolate - precocious - thimerosal - vaccines

The US Department of Health and Human Services and the National Institute of Child Health and Development (NICHD) of the National Institutes of Health (NIH) estimate the historical incidence of premature puberty in the general population to be about one in 10,000 children[1]. Premature puberty is characterized by sexual development before the age of eight in girls, and age 10 in boys. While the early onset of puberty may seem fairly benign, in fact, it can cause problems when hormones trigger changes in the growth

pattern, essentially halting growth before the child has reached normal adult height. Further, children with this condition look noticeably different than their peers, and may feel rejected by their friends and socially isolated. Adults may expect these children to act more maturely simply because they look so much older. Many of these children, especially boys, are much more aggressive than others of their own age, leading to behaviour problems both at home and at school.

During the past decade, possible advancement in timing of puberty has been reported in the US. Potential explanations for this increasing trend such as ethnic, geographical, and socio-economic backgrounds appear to provide equivocal explanations for the earlier onset of puberty seen in the US. Recently, attention has been paid to the possible role of endocrine-disrupting chemicals from the environment on the timing of puberty[2]. A recent review published on the possible relationships between today's epidemics in children and environmental pollution called for immediate research on the relationship between environmental endocrine disrupters and premature puberty[3].

The US Agency for Toxic Substances and Disease Registry (ATSDR) reports that mercury (Hg) is a known endocrine disruptor and it adversely affects the steroid synthesis pathway in animals and humans. It has been purposed that Hg exposure and sex steroids may interact to enhance the risk for a child developing premature puberty[4].

In the last few decades, vaccines have helped to accomplish striking reductions of infectious diseases worldwide[5]. From the 1930s through the early 2000s, many routinely administered childhood vaccines in the United States contained thimerosal[6]. Thimerosal is an organic Hg-containing compound having 49.55 per cent Hg by weight, and is initially metabolized to ethylmercury compounds and thiosalicylate[7].

The American Academy of Pediatrics and the US Public Health Service in 1999[8] 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 yr of age were made available in thimerosal-free or thimerosal-reduced formulations in the US[9]. Exposures to thimerosal through paediatric 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[10].

The purpose of the present study was to epidemiologically evaluate the potential endocrine effects of infant Hg exposure from thimerosal-containing 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) was examined.

Material & 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 study was conducted based upon a retrospective ecological assessment of premature puberty diagnoses. Pre-existing HMO administered databases collected for the Vaccine Safety Datalink (VSD) project were evaluated for associations between premature puberty and cumulative thimerosal exposure.

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[11-13]. 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 four years of follow up for each member of the cohort.

Only those individuals who had a recorded oral polio vaccine within the first three months after birth were examined. A three 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 two 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 three months of their birth date and were born before January 1, 1997 were used as the denominator or population at risk for this study. Table I summarizes the demographic information for the population examined.

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 II summarizes the specific medically diagnosed condition International Classification of Disease, 9th revision (ICD-9)[14] codes examined in the present study, including specific diagnostic control disorder ICD-9 codes selected *a priori* as not having biologically plausible links to Hg exposure. Table III summarizes additional demographic information among those diagnosed with premature puberty examined in the present study

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 who were born in that same year. Because of concern that the cohorts from 1995-1996 had only 4-6 yr 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 yr. 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.

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 premature puberty showed little variation in point rate ratio estimates or statistical significance even when not imputing additional cases for limited follow-up time.

Determining exposure: Because the study protocol did not permit to match data across vaccine files,

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

Gender:	%
Male	51
Female	49
Birth cohort:	%
1990	1
1991	15
1992	16
1993	16
1994	17
1995	18
1996	17
Race[*]:	%
White	60
Black	9
Hispanic	17
Asian	13
Other	1
Birth characteristics[*]:	Mean (standard error)
Gestational age (wk)	39.3 (0.01)
Birth weight (g)	3,422 (1.5)
Maternal age (yr)	29.0 (0.02)

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

Table II. Medical diagnoses examined in the vaccine safety datalink (VSD) database

ICD-9 Codes	Diagnosis	N	% Male	Median age at initial diagnosis (yr)	Adjusted[*] overall prevalence rate
259.1	Premature puberty	819	7.0	4.5	39.5 / 10,000
	Control disorders[**]				
486	Pneumonia	33,648	53.3	3.1	13.2 / 100
759.9	Congenital anomalies	1,643	52.0	2.5	63.2 / 1,000
783.40, 783.41, 783.42, 783.43	Failure to thrive	4,754	56.0	1.7	18.5 / 1,000

[*] Based upon the age of diagnosis and length of follow up time in the VSD for each birth cohort
[**] Outcomes selected *a priori* as not having biologically plausible association with Hg exposure

Table III. Demographic information for cohort of patients diagnosed with premature puberty (n = 819)

Birth cohort:	%
1990	1
1991	24
1992	24
1993	17
1994	12
1995	12
1996	10
Race[*]:	**%**
White	51
Black	24
Hispanic	12
Asian	10
Other	3
Birth characteristics[*]:	Mean (standard error)
Gestational age (wk)	39.1 (0.10)
Birth weight (g)	3,278 (29.8)
Maternal age (yr)	29.5 (0.25)

[*]Not for total cohort-only those with birth file (n = 502)

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 [14], were assumed for the following routine childhood vaccines under study: Hib = 25 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.

The vaccine datasets were subset in a similar way to the population datasets in that an individual had to have the specific vaccine within three 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. 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.

Statistical analysis: 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 and counting 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 ratio. Hg dose was modeled as a continuous variable and rate ratio estimates and 95 per cent confidence intervals (CI) 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 $P<0.05$ was considered statistically significant.

Results

Table IV presents the rate ratios and 95 per cent confidence intervals for each diagnosis assuming a 100 µg increase in Hg 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 premature puberty following additional Hg exposure from thimerosal-containing childhood vaccines. The increased rate ratios for premature puberty ranged from a low of 5.58 for a 100 µg increase in Hg exposure in the birth to 7 month period to a high of 6.45 for a 100 µg increase

Table IV. Rate ratios (95% confidence intervals) for a 100 µg difference in Hg 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)
Premature puberty[*]	5.58 (2.79-11.16)	6.45 (3.39-12.30)
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)

[*]$P<0.0001$

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 observed with increasing Hg exposure from thimerosal-containing vaccines.

Discussion

The overall results of the present study showed a significant association between Hg exposure from thimerosal-containing vaccines and premature puberty. There were significantly increased rate ratios for premature puberty following increasing Hg exposure from thimerosal-containing vaccines administered in the first 7 and 13 months of life. Further, it was observed that the overall median age of puberty among those diagnosed with premature puberty in the present study (4.5 yr) was significantly reduced in comparison to the lower end of the normal reference ranges for puberty in girls (≥ 8 yr) and boys (≥ 10 yr) in the US[2]. The present study found an adjusted overall prevalence rate of premature puberty occurring in about one in 250 children. This represents a significant (about 40-fold) increase in the diagnosed rate of premature puberty of about one in 10,000 children from previous NIH estimates[1].

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 which allows for a consistent and specific physician diagnosed disease status to be examined among the patients. 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. 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). As a result of Hg dosing beginning from around the time of birth, since only children receiving vaccines by age 3 months were examined, and the US routine childhood vaccine schedule during the 1990s was to administer hepatitis B vaccine on the day of birth, virtually all the outcomes examined were diagnosed well after starting exposure to Hg from thimerosal-containing vaccines. Fourth, the methods of ensuring capture of Hg exposure from thimerosal-containing vaccines and outcomes appear to have yielded results consistent with previous studies[15]. 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. Finally, another significant strength of the present study stems from the trends in birth cohort Hg exposure and outcomes. It was observed there were increasing/decreasing trends in exposures and outcomes across the birth cohort years examined, and that for premature puberty 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 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 our 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 significant sources of Hg such as fish consumption or environmental exposure 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 endocrine 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 endocrine 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[11-13]. Further, 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 premature puberty, but not be present for the control conditions examined.

The study was also limited to a maximum of four 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. 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 premature puberty. Specifically, investigators reported that there are several social and medical attributes associated with avoidance or delay of vaccination and an increased risk of 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[16]. 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 premature puberty.

The timing of puberty can be influenced by neurotransmitters and neuropeptides that originate in the hypothalamus, in addition to peripheral or gonadal signals. Signals linked to the environment such as exposure to endocrine disrupters may impinge on the hypothalamic signaling network directly or through peripheral signals, and as a result, may occasion an earlier onset of puberty[2]. It was previously observed in pink disease, known to be primarily caused by the use of mercuric chloride teething powers in infants, that altered states of adrenocorticol secretion, excessive production of androgen hormones, and pseudohermaphroditism in infancy and childhood were common occurrences[17]. Other studies have also described increased testosterone and other androgen levels in tissue culture, animals, and in humans following low-dose Hg exposure[18-20]. It was observed *in vitro* that Hg exposure significantly altered the androgen synthesis pathway by inhibiting hydroxysteroid sulphotransferase (HST) activity, thus reducing the conversion of dehydroepiandrosterone (DHEA) to dehydroepiandrosterone–sulphate (DHEA-S)[21-24]. In addition, aromatase activity, a key steroid synthesis enzyme that catalyses the conversion of androgens to estrogens, was inhibited by Hg exposure[25].

Another study reported that Hg could significant induce increased cellular steroid synthesis by binding to specific receptor sites on ovarian cellular membranes[26]. A recent study revealed that blood inorganic Hg levels were associated with significant alterations in circulating luteinizing hormone (LH) levels in the blood[27]. These combined occurrences may tend to significantly adversely affect circulating hormonal levels, and if they occur during early childhood, may significantly increase the risk of an individual developing premature puberty.

The biological plausibility of the present study is further supported by findings on the distribution of Hg following thimerosal administration[28-31]. It was observed that thimerosal administration to animals significantly increased Hg levels in generation and regulation sites of steroid synthesis, and a significant fraction of the Hg in these tissues was present as inorganic Hg. In addition, infant monkeys following injection of doses of thimerosal comparable to the dosing schedule (weight- and age-adjusted) US children received during the 1990s resulted in significant accumulation of Hg in the animal tissues[31]. Moreover, much of the accumulated Hg in the animal tissues was in the inorganic form, and the half-life of the inorganic Hg was too long to estimate a value from the available data (no significant measurable decline was detectable by 120 days). Similarly, other studies have described that inorganic Hg may persist for many years in other human tissues following Hg exposure[32].

In conclusion, the results of the present study show an association between increased Hg exposure from thimerosal-containing vaccines and premature puberty. The observed effects were consistent with the known human endocrine disrupting effects of Hg exposure. 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 endocrine function.

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