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REVIEW ARTICLE

An Assessment of Thimerosal Use in Childhood Vaccines

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ABSTRACT. *Background.* **On July 7, 1999, the American Academy of Pediatrics and the US Public Health Service issued a joint statement calling for removal of thimerosal, a mercury-containing preservative, from vaccines. This action was prompted in part by a risk assessment from the Food and Drug Administration that is presented here.**

Methods. **The risk assessment consisted of hazard identification, dose-response assessment, exposure assessment, and risk characterization. The literature was reviewed to identify known toxicity of thimerosal, ethylmercury (a metabolite of thimerosal) and methylmercury (a similar organic mercury compound) and to determine the doses at which toxicity occurs. Maximal potential exposure to mercury from vaccines was calculated for children at 6 months old and 2 years, under the US childhood immunization schedule, and compared with the limits for mercury exposure developed by the Environmental Protection Agency (EPA), the Agency for Toxic Substance and Disease Registry, the Food and Drug Administration, and the World Health Organization.**

Results. **Delayed-type hypersensitivity reactions from thimerosal exposure are well-recognized. Identified acute toxicity from inadvertent high-dose exposure to thimerosal includes neurotoxicity and nephrotoxicity. Limited data on toxicity from low-dose exposures to ethylmercury are available, but toxicity may be similar to that of methylmercury. Chronic, low-dose methylmercury exposure may cause subtle neurologic abnormalities. Depending on the immunization schedule, vaccine formulation, and infant weight, cumulative exposure of infants to mercury from thimerosal during the first 6 months of life may exceed EPA guidelines.**

Conclusion. **Our review revealed no evidence of harm caused by doses of thimerosal in vaccines, except for local hypersensitivity reactions. However, some infants may be exposed to cumulative levels of mercury during the first 6 months of life that exceed EPA recommendations. Exposure of infants to mercury in vaccines can be reduced or eliminated by using products formulated without thimerosal as a preservative.** *Pediatrics* **2001;107: 1147–1154;** ***thimerosal, thiomersal, merthiolate, vaccine, immunization, mercury, methylmercury, ethylmercury, adverse event, risk assessment.***

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The views in this article are those of the authors and are not intended to represent those of the Food and Drug Administration or the US Public Health Service.

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ABBREVIATIONS. FDA, Food and Drug Administration; USPHS, US Public Health Service; AAP, American Academy of Pediatrics; Td, diphtheria and tetanus toxoids; DTP, diphtheria and tetanus toxoids and whole cell pertussis vaccine; VAERS, Vaccine Adverse Event Reporting System; DTaP, diphtheria and tetanus toxoids and acellular pertussis vaccine; Hib, *Haemophilus influenzae* type b vaccine; EPA, Environmental Protection Agency; ATSDR, Agency for Toxic Substance and Disease Registry; WHO, World Health Organization; ACIP, Advisory Committee on Immunization Practices.

Thimerosal, an organic mercurial compound in use since the 1930s, is a preservative in over 30 US-licensed and currently marketed vaccines in concentrations of 0.003% to 0.01%. An analysis by the Food and Drug Administration (FDA) of the "adverse effects on health of children and other sensitive populations from exposure to . . . mercury" conducted under the FDA Modernization Act of 1997[1] was followed by a joint statement issued by the US Public Health Service (USPHS) Agencies and the American Academy of Pediatrics (AAP),[2] and an interim report to clinicians by the AAP[3] in July 1999, recommending that thimerosal be reduced or eliminated from vaccines. This paper reports the results of the FDA risk assessment of thimerosal in childhood vaccines.

FDA regulations require that preservatives be present in multidose vials of vaccines, with the exception of certain live viral vaccines, to prevent bacterial and fungal contamination.[4] Preservatives are not required for products formulated in single-dose vials. Multidose vials are preferred by some physicians and health clinics because they are often less expensive per vaccine dose and require less storage space along the cold chain. As a preservative, thimerosal may be added at the end of the production process to the bulk or final container, or it may be added to the diluent of a lyophilized vaccine. In addition to its prominent role as a preservative, thimerosal is used as an inactivating agent in the manufacture of certain vaccines (eg, whole cell pertussis vaccines and some acellular pertussis products) and as a bacteriostatic agent during the production process of other vaccines (eg, influenza vaccines).[5] Uses other than as a preservative, however, contribute little to the final concentration of thimerosal in vaccines (at most 2–3 μg thimerosal/

mL), with limits of detection of <0.2 μg thimerosal/mL.[6]

The benefits of adding preservatives to multidose preparations were apparent in the early 20th century after several episodes of bacterial contamination of biological products caused illness and death in recipients. In 1916, 4 children in South Carolina died and over 60 others experienced severe local and systemic symptoms after receipt of a typhoid vaccine contaminated with *Staphylococcus aureus*.[7] In 1928 in Bundaberg, Australia, *Staphylococcal* contamination of a multidose vial of a diphtheria toxin-antitoxin mixture formulated without a preservative caused the death of 12 of 21 children who received an injection from this vial. A report by the investigating committee published shortly after this incident recommended that biological products issued in containers for repeated use should include antiseptics to inhibit bacterial growth.[7] Although the specific events leading to the US regulatory requirement for preservatives in multidose vials were not found in our review, these and similar incidents most likely played a role. However, preservative use in multidose vials has not always prevented bacterial contamination. Several published reports of pyogenic infections after receipt of diphtheria and tetanus toxoids (Td) and diphtheria and tetanus toxoids and whole cell pertussis (DTP) vaccine containing thimerosal as a preservative emphasize the need for proper handling of multidose vials to avoid microbial contamination after opening.[8,9]

Formal FDA review of thimerosal use in biological products, including vaccines, last occurred in 1976. This review evaluated exposure to thimerosal from biological products using the 1974 AAP *Red Book* immunization schedule and concluded that, with the exception of long-term immune globulin replacement therapy, "no dangerous quantity of mercury is likely to be received from biological products in a lifetime."[10] Thimerosal is no longer used as a preservative in US-licensed immune globulin products, such as intravenous immune globulin, hepatitis B immune globulin, and varicella immune globulin, with the exception of a few immune globulin preparations for intramuscular administration and some Rho (D) immune globulins. Reassessment of the risks from thimerosal in vaccines is appropriate in light of advances in the understanding of the human health effects of low-level exposure to mercury,[11–13] as well as the increased number of vaccines recommended for routine use in children.

METHODS

Our risk assessment of thimerosal in childhood vaccines, adapted from the paradigm outlined by the National Research Council,[14] consisted of hazard identification, dose-response assessment, exposure assessment, and risk characterization. The risk assessment focused on infants and young children because of their small body size, developing brain, and exposure to vaccines containing thimerosal.

We reviewed the medical literature to identify the known risks of thimerosal and related organic mercury compounds by querying Medline and Toxline databases, using the terms "thimerosal," "thiomersal," "merthiolate," "mercury," "ethylmercury," "methylmercury," "immunization," "vaccine," and "preservative." Additional articles were obtained from the reference lists acquired during the initial search and from colleagues. The Vaccine Adverse Event Reporting System (VAERS), a national passive surveillance system maintained by the FDA and the Centers for Disease Control and Prevention,[15] was queried for reports of adverse events associated with thimerosal. We examined dose-response relationships and exposure limits recommended by various agencies for methylmercury, a related organic mercurial compound. To simplify the comparison between ethyl- and methylmercury, we calculated the amount of mercury by weight for both compounds. We tabulated the mercury content of all US-licensed vaccines, determined the range of exposures to mercury that a child could receive under the recommended US childhood immunization schedule, and characterized the potential risk to infants. Given the limitations of available data pertaining to thimerosal toxicity, we did not attempt a quantitative risk characterization.

RESULTS

Hazard Identification

To identify hazards of thimerosal, we reviewed reports of toxicity in animals and humans. Because no controlled studies have been conducted to examine low-dose thimerosal toxicity in humans, the reported toxicity of methylmercury, a related organic mercury compound, was evaluated.

Animal Studies

Limited animal studies have examined the toxicity of thimerosal or ethylmercury. Low doses of thimerosal equivalent to ethylmercury doses of either 1 or 6 μg/kg/d in adult squirrel monkeys were converted to inorganic mercury, with high levels detected in the kidney and lower levels found in the brain.[16] Histopathological changes were not observed in either the kidney or brain.

Before the marketing of thimerosal as a preservative in 1931, high-dose toxicity studies were conducted in rabbits, rats, mice, dogs, and guinea pigs.[17] Rabbits, rats, and mice received intravenous injections of 1% solution with observation periods limited to 7 days; the use of control animals was not reported. The maximum tolerated doses were reported as 20 mg/kg (rabbits) and 45 mg/kg (rats). For rabbits, the pathology of fatal cases was described as "essentially that of mercurial poisoning, including kidney and intestinal lesions." Four dogs received 2 mg/kg of 1% solution every third day for 12 doses. Autopsies performed 7 days after completion found "only minor microscopic tissue changes." Immediately after intraperitoneal injections of 1/1000 (0.1%) solution, guinea pigs demonstrated evidence of severe pain. "Fairly pronounced" congestion and hemorrhage in the visceral, parietal, and omental peritoneum was observed when animals were killed and examined 1 to 2 days after injection. The authors reported that "no abnormal pain responses" were seen in guinea pigs injected with dilutions of 1/4000 and 1/8000.[17]

In a carcinogenicity and toxicity study of preservatives in vaccines published in 1971, Fischer rats were subcutaneously injected twice-weekly with thimerosal at doses ranging from 30 to 1000 μg/kg for 1 year.[18] Control rats were either untreated (negative control), or treated with nickel, which is known to induce local inflammatory reactions (positive control). Animals were weighed weekly and autopsied

at either 12 or 18 months after initial injection. All animals with spontaneous deaths, moribund, or with gross organ pathology had organs examined histologically. The thimerosal-treated rats had a dose-dependent increase in the incidence of bronchopneumonia, compared with rats receiving other preservatives or controls, with 60% of the thimerosal-treated animals demonstrating unspecified histopathologic changes at the highest dose, compared with 13% of untreated controls. The death rate for the thimerosal-treated animals paralleled that of other preservatives and controls leading the authors to conclude "the damage was slight, continuous, and perhaps cumulative." In addition, animals receiving thimerosal at the highest dose levels over the 12 month-period demonstrated on average a 10% (range: 5%–14%) retardation of weight gain when compared with controls. Histopathology of the brain and kidney in thimerosal-treated animals was not reported. Quantitative data were compiled only for the highest dose levels; at lower doses the retardation of weight gains was reported to be "less significant."[18]

Human Studies

Allergy to thimerosal is well-described in the clinical literature, primarily in the form of delayed-type hypersensitivity.[19] Some authors postulate that the thiosalicylate component is the major determinant of allergic reactions.[20] The clinical importance of the high prevalence of thimerosal sensitivity detected by patch testing remains controversial. Some investigators feel that it is of little significance,[21,22] while others suggest it is important enough to require removal of thimerosal from pharmaceutical products.[19,23,24]

Our search did not locate any clinical studies formally evaluating the safety of thimerosal before its initial marketing. The earliest report of thimerosal use in humans was found in a 1931 article by Powell and Jamieson.[17] In this report of clinical use by another investigator, 22 individuals received 1% solution of thimerosal intravenously for unspecified therapeutic reasons. Participants received up to 10 mg thimerosal/kg with no reported toxic effects, although 2 participants demonstrated phlebitis or sloughing of skin after local infiltration. Of note, this study was not specifically designed to examine toxicity; 7 of 22 participants were observed for only 1 day, the specific clinical assessments were not described, and no laboratory studies were reported.

Clinical cases of accidental and intentional acute poisonings with very high doses of thimerosal, while rare, point to the severest forms of toxicity. Several cases of acute mercury poisoning from thimerosal-containing products were found in the medical literature. These reports included the administration of immune globulin[26] (gamma globulin) and hepatitis B immune globulin,[26] choramphenicol formulated with 1000 times the proper dose of thimerosal as a preservative,[27] thimerosal ear irrigation in a child with tympanostomy tubes,[28] thimerosal treatment of omphaloceles in infants,[29] and a suicide attempt with thimerosal.[30] Total doses of thimerosal administered in these reports of acute toxicity ranged from ~3 mg/kg to several hundred mg/kg. These studies reported local necrosis, acute hemolysis, disseminated intravascular coagulation, acute renal tubular necrosis, and central nervous system injury including obtundation, coma, and death.

Methylmercury Toxicity Studies

We did not find any reports of toxicity after low-dose exposure to thimerosal in humans in the medical literature. However, available data suggest that the toxicity of ethylmercury, the thimerosal metabolite, and methylmercury may be similar. We found only 1 animal study directly comparing the toxicity ethyl- versus methylmercury. Magos et al[31] studied adult male and female rats administered 5 daily doses of equimolar concentrations of ethyl- or methylmercury by gavage (8.0 or 9.6 mg/kg). Tissue distribution, and the extent and severity of histologic changes in the brain and kidney were assessed. Neurotoxicity of ethyl- and methylmercury was similar, with higher levels of inorganic mercury observed in the brains of ethylmercury-treated rats. Renal damage was greater in rats receiving ethylmercury. In humans, high-dose exposure to ethylmercury has resulted in toxicity similar to that of high-dose exposure to methylmercury.[32] Because high-dose exposure to ethylmercury from thimerosal results in toxicity comparable to that observed after high-dose exposure to methylmercury, and because of the chemical similarity of the 2 compounds, it appears reasonable to consider toxicity of low doses of methylmercury and ethylmercury to be similar.[33,34]

Much of what is known about methylmercury toxicity comes from poisoning episodes in Japan[35] and Iraq,[36] as well as studies of populations with dietary exposure, primarily in the Seychelles[37] and Faroe Islands.[38] The toxicity of methylmercury was first recognized during the late 1950s and early 1960s with the consumption of contaminated fish in Minamata, Japan.[35] Epidemics of methylmercury poisoning also occurred in Iraq during the 1970s when seed grain treated with a methylmercury fungicide entered the food chain as bread.[36] Maternal methylmercury exposure in these epidemics was associated with neurologic abnormalities, such as delays in motor function, among children exposed in utero.

Additional data from low-dose exposure to methylmercury derived from studies of populations exposed in their diet are conflicting.[37,38] Studies from the Faroe Islands reported that subtle cognitive deficits (eg, performance on attention, language, and memory tests), detectable by sophisticated neuropsychometric testing, were associated with methylmercury levels previously thought to be safe.[38] Studies in the Seychelles, evaluating more global developmental outcomes, did not reveal any correlation between abnormalities and mercury levels.[37]

VAERS

To identify any events reported as attributable to thimerosal in vaccines, we queried approximately VAERS 90 000 reports from 1990–1998 by searching text fields for "thimerosal," "thiomersal," "merthiolate," and "mercury." A total of 45 reports were

identified. Twenty-eight reports involved hepatitis B vaccine, 10 concerned influenza vaccine, 3 concerned Td, and 1 each involved diphtheria and tetanus toxoids and acellular pertussis (DTaP), combination DTP and *Haemophilus influenzae* type b (DTP-Hib), and concurrent but separate administration of DTP and Hib. The types of events attributed by the reporter to thimerosal included injection-site reactions in 13 reports, rash in 9, urticaria in 8, edema in 5, and flu-like syndrome and joint aches in 4. One report involved each of the following events: anaphylaxis, "severe allergic reaction" (not otherwise specified), wheezing, stridor, and malaise/agitation. Only 1 report required hospitalization (for angioneurotic edema); most others reported doctor or emergency department visits. Of the 5 reports of edema, 2 reports concerned facial edema, 1 involved angioneurotic edema, 1 mentioned eyelid swelling and 1 report involved peripheral edema. One report involved a patient with both urticaria and wheezing; the time of onset after vaccination was not specified. Of note, 1 report described an individual who experienced anaphylaxis after hepatitis B vaccine. When rechallenged with a similar but thimerosal-free product, anaphylaxis occurred again, implying thimerosal was not the causative agent. VAERS has several limitations, including lack of consistent diagnostic criteria, data acquired from a diverse group of voluntary reporters, underreporting, and the difficulty in determining whether a vaccine caused the adverse event reported.[15] A cause-and-effect relationship between the reported adverse events and thimerosal in vaccines cannot be established because of these limitations.

Summary of Hazard Identification

The only well-established hazard of thimerosal at doses found in vaccines is delayed-type hypersensitivity reactions. At very high doses, the identified hazards of thimerosal are neurotoxicity and nephrotoxicity. Methylmercury, a similar organic mercurial, has been associated in some studies with subtle neurodevelopmental abnormalities at low doses. Although the data are limited, similar toxicological profiles between ethylmercury and methylmercury suggest that neurotoxicity may also occur at low doses of thimerosal; however, such effects have not been reported.

Dose-Response Assessment

Guidelines for Safe Exposure to Methylmercury

Guidelines for safe exposure to methylmercury, based on dose-response analysis of exposures resulting in overt toxicity, were used to determine if the mercury dose from vaccines approaches a level of concern. The US Environmental Protection Agency (EPA),[12] the US Agency for Toxic Substances and Disease Registry (ATSDR),[39] the FDA,[40] and the World Health Organization (WHO)[41] have developed recommendations for limits of exposure to methylmercury in the diet. These range from 0.1 μg/kg body weight/day (EPA) to 0.47 μg/kg body weight/day (WHO)[a] and include varying safety margins. The range of recommendations is attributable to differing emphasis placed on various primary data sources and the different purposes for these recommendations. All guidelines, however, fall within the same order of magnitude. A complete discussion of how each agency reached its recommendations and the intended purpose is beyond the scope of this risk assessment. The interested reader is referred to a recent review.[13] Application of these guidelines to a female infant at the 5th, 50th, and 95th percentile of weight between birth and 26 weeks,[42] the period during which most infant vaccines are given, resulted in calculated recommended limits of mercury exposure shown in Table 1. This assessment assumed that the toxicity and pharmacokinetics of ethylmercury are the same as methylmercury, that effects of low-dose oral exposure are the same as bolus intramuscular injections, and that the susceptibility of the infant to toxicity from organic mercurials is the same as that of the fetus. Calculations also assumed limited or no excretion in newborns.

TABLE 1. Calculated Exposure Limits for Mercury, Using Various Agency Guidelines for Exposure to Methylmercury, in Infants ≤6 Months of Age by Percentile Body Weight

Agency	Percentile Body Weight		
	5th	50th	95th
EPA	65 μg	89 μg	106 μg
ATSDR	194 μg	266 μg	319 μg
FDA	259 μg	354 μg	425 μg
WHO	305 μg	417 μg	501 μg

- Calculated Exposure Limit = dose/kg body weight/week X average weight × 26 weeks × 0.932 (mercury molecular weight/methylmercury molecular weight); eg, EPA calculated exposure limit = 0.7 μg/kg body weight/week × 26 weeks × (2.36 kg + 5.25 kg)/2 × 0.932 = 65 μg.
- Assumes average of 5th, 50th, and 95th% weight for females at birth (2.36 kg, 3.23 kg, 3.81 kg) and 6 months (5.25 kg, 7.21 kg, 8.73 kg) = 3.81 kg, 5.22 kg, 6.27 kg. Females were selected because their smaller body weight makes them more susceptible than males.
- Recommended limits on methylmercury exposure: EPA: 0.1 μg/kg body weight/day; ATSDR: 0.3 μg/kg body weight/day; FDA: 0.4 μg/kg body weight/day; WHO 3.3 μg/kg body weight/week. For calculations, daily limits multiplied by 7 to obtain weekly limits.

Exposure Assessment

An exposure assessment was undertaken of the mercury content of vaccines included in the recommended US childhood immunization schedule.[43] At the time of this review, childhood vaccines that might contain thimerosal as a preservative included single-antigen hepatitis B vaccines; some DTaP vaccines; all DTP vaccines; and some Hib vaccines. The total amount of mercury by weight was calculated for each vaccine in the infant schedule. For formulations containing thimerosal as a preservative, hepatitis B vaccine contains ~12.5 μg mercury per 0.5-mL dose, DTaP or DTP ~25 μg mercury, and Hib vaccine ~25 μg mercury. Depending on the particular

[a]The WHO guideline is expressed as 3.3 μg/kg body weight/wk and has been converted to a daily dose for purpose of comparison.

vaccine formulation and schedule, an infant may receive a total mercury dose from vaccines as much as 187.5 μg during the first 6 months of life. In special populations, influenza vaccine may be administered at 6 months of age, which would increase the total dose to ~200 μg (Table 2). Thus, comparison with Table 1 shows that some infants may receive doses of mercury from vaccines that are in excess of EPA guidelines, but not the ATSDR, FDA, or WHO guidelines.

At the time of this risk assessment, vaccine formulations not containing thimerosal as a preservative were available for Hib (ActHIB [Aventis Pasteur] and HibTITER [Wyeth Lederle Vaccines] in single-dose vials), DTaP (Infanrix [Glaxo Smithkline]), and a combination of Hib-hepatitis B vaccine (COMVAX [Merck]). COMVAX is licensed for use in infants ≥6 weeks of age, born to mothers with low risk of hepatitis B. Vaccines that use thimerosal during the production process, but not as a preservative, contain <3 μg thimerosal/mL and, therefore, are not considered in this exposure assessment. Under special circumstances the Advisory Committee on Immunization Practices (ACIP)[45] and the AAP[46] allow for accelerated schedules for infants, such as infants at risk of exposure to pertussis and for travelers. Administering vaccines containing thimerosal as a preservative to these infants would result in exposure to more mercury per kilogram body weight over a shorter period of time.

Subsequent to our review, 2 single-dose formulations of preservative-free hepatitis B vaccine were approved by the FDA: on August 27, 1999, for Recombivax-HB (Merck)[44] and on March 28, 2000, for Engerix-B (Glaxo Smithkline). A second preservative-free DTaP vaccine (Tripedia-Aventis Pasteur) was approved on March 7, 2001. With the currently available US-licensed vaccines, cumulative infant exposure to mercury from vaccines is less than EPA recommended limits, under most circumstances.

Estimates of thimerosal exposure from vaccines among 85 000 children who receive health care in a large health maintenance organization in California indicate that ~10% of infants received >112 μg ethylmercury from vaccines during the first 6 months of life.[47] In addition, certain infants may be exposed to high levels of mercury from the diet or environment. These exposures should be added to those from vaccines in assessing the total exposure of infants to mercury. By the second year of life the larger body size of even the smallest children results in a calculated exposure which is less than the EPA, ATSDR, FDA, and WHO guidelines (Table 3).

TABLE 2. Exposure to Mercury From Vaccines in US Infants (≤6 Months) at the Time of Review (1999)

Vaccine	Minimum Mercury Dose	Maximum Mercury Dose
DTaP × 3	0 μg	75 μg
Hib × 3	0 μg	75 μg
Hepatitis B × 3	0 μg	37.5 μg
Hib-Hepatitis B × 2	0 μg	Not applicable
[Influenza]* (selected populations)	[12.5 μg]	[12.5 μg]
Total	[12.5 μg]	187.5 μg [200 μg]

* Brackets denote dose of mercury if influenza vaccine is administered.
Thimerosal is 49.6% mercury by weight; eg, 0.005% thimerosal concentration is equivalent to 50 μg thimerosal/1.0 mL or 25 μg thimerosal/0.5 mL and results in approximately 12.5 μg mercury/0.5-mL dose.
Note: These calculations do not include mercury exposures from sources other than vaccines.

TABLE 3. Exposure to Mercury From Vaccines in US Children (<2 Years) at the Time of Review (1999)

Vaccine	Minimum Mercury Dose	Maximum Mercury Dose
DTaP × 4	0 μg	100 μg
Hib × 4	0 μg	100 μg
Hepatitis B × 3	0 μg	37.5 μg
Hib-Hepatitis B × 3	0 μg	Not applicable
[Influenza] ×3* (selected populations)	[37.5 μg]	[37.5 μg]
Total	[37.5 μg]	237.5 μg[275 μg]

* Brackets denote dose of mercury if influenza vaccine is administered.
Thimerosal is 49.6% mercury by weight; eg, 0.005% thimerosal concentration is equivalent to 50 μg thimerosal/1.0 mL or 25 μg thimerosal/0.5 mL and results in approximately 12.5 μg mercury/0.5-mL dose.
Note: These calculations do not include mercury exposures from sources other than vaccines.

No human data are available regarding neurotoxicity from thimerosal-containing vaccines. However, 1 recent study measured the change in total mercury blood levels in a small number of infants after hepatitis B vaccination. After 1 dose of hepatitis B vaccine (~12.5 μg of mercury) given within 3 days of birth, mean mercury blood levels increased from 0.54 to 7.36 μg/L (range: 1.3–23.6) in 15 preterm infants with a mean body weight of 748 g; and from 0.04 to 2.24 μg/L (range: 1.4- 2.9) in 5 term infants with a mean body weight of 3.59 kg.[48] This study suggested that a birth dose of hepatitis B vaccine may measurably increase infant mercury blood levels. These levels are not generally considered acutely toxic; however, the long-term effects on neurodevelopment from this level of exposure have not been studied.

DISCUSSION

Risk Characterization

No evidence of harm has been demonstrated at doses of thimerosal found in vaccines, except for local hypersensitivity reactions. Available clinical data, however, do not address the potential for subtle effects in infants. A prelicensure study of intentionally administered high-dose thimerosal,[17] cited as demonstrating its safety,[10] may not be directly relevant to the issue of thimerosal in childhood vaccines. This study was performed over 60 years ago when different safety standards existed; the study was not designed to look for chronic toxicity, did not include pharmacokinetics, and did not enroll infants. Case reports of neurotoxicity and renal toxicity from thimerosal in humans were found only at doses >100 times that found in vaccines. Our analysis concluded that the use of thimerosal as a preservative in vac-

cines might result in intake of mercury during the first 6 months of life that exceeds the EPA, but not the ATSDR, FDA, or WHO guidelines for methylmercury intake. The clinical significance of this conclusion is not currently known. The EPA guidelines contain as much as a 10-fold safety factor. Such guidelines are meant to be starting points for evaluation of mercury exposure, and should not be viewed as absolute levels above which toxicity can be expected to occur.[13]

Precisely identifying the risk from thimerosal in vaccines is problematic because of gaps in knowledge of its toxicity. This risk assessment extrapolates the toxicity from methylmercury exposure to that of ethylmercury from thimerosal in vaccines. This extrapolation has several limitations. The comparative toxicity of ethyl- and methylmercury has not been well-characterized. Moreover, the metabolism and elimination of ethylmercury compared with methylmercury, and the effect of intermittent intramuscular doses of thimerosal from vaccines compared with chronic low-dose oral exposure to methylmercury, has not been studied. Several of the guidelines for methylmercury exposure are based on studies of fetal outcomes after in utero exposures from maternal ingestion of methylmercury-contaminated food. The susceptibility of the infant compared with the fetus to adverse effects from organic mercurials is not known. Although acknowledging the limitations of available data and the uncertainties inherent in our risk assessment, we cannot exclude the possibility of subtle neurodevelopmental abnormalities from the cumulative exposure to thimerosal in vaccines.

Options Regarding Thimerosal as a Preservative in Childhood Vaccines

Three general options exist regarding the use of thimerosal as a preservative in childhood vaccines: maintaining current vaccine formulations, eliminating thimerosal from vaccines, or reducing exposure to thimerosal. Reduction in exposure to thimerosal from vaccines is merited given the goal of reducing human exposure to mercury from all sources, the feasibility of removing thimerosal as a preservative in vaccines, and the potential risk to infants.

Complete elimination of thimerosal from all vaccines in the near future is not likely. Reformulation of vaccines that include thimerosal in the production process will require additional product characterization, and perhaps clinical studies, to establish safety, purity, potency, stability, and efficacy.[5] For some vaccines, removal of thimerosal may alter the antigenic structure and thus the immune response. If a new preservative is to replace thimerosal, the safety and efficacy of the alternative must be established.[49]

Several approaches are available to reduce exposure of children to thimerosal. Clinicians may select existing products not containing thimerosal. Reformulation of vaccines in single-dose vials may eliminate the need for a preservative. For some vaccines, such as the recently approved single antigen hepatitis B vaccine (Recombivax), reformulation in single-dose vials could be accomplished rapidly because the vaccine was already formulated and stored in bulk without thimerosal as a preservative. Although transition to single-dose vials may be an option in the United States, multidose vials containing thimerosal remain, at present, an important component of immunization programs in developing countries because of their reduced cost and storage requirements. In such settings, the WHO has determined that the benefits of vaccination and the risk of microbial contamination of multidose vials outweigh the theoretical risks of thimerosal in vaccines.[50,51]

New vaccines, including combination products, formulated without thimerosal as a preservative are under development by vaccine manufacturers and review by FDA. If licensed, these vaccines would greatly expand options available to clinicians. Another possibility to reduce thimerosal exposure is to reformulate vaccines with reduced amounts of thimerosal that still have a preservative effect. In the long-term, preservative-free products formulated in single-dose vials, substitution of alternative preservatives, or implementation of new vaccine technologies such as combination, mucosal, trancutaneous, and DNA vaccines may further reduce or eliminate the need for thimerosal as a preservative in childhood vaccines.

Actions Taken to Date

On July 1, 1999, the FDA sent a letter to manufacturers of vaccines requesting their plans to remove thimerosal from US-licensed vaccines, or alternatively, an explanation for continued use of thimerosal as a vaccine preservative.[52] In July 1999, the AAP and the USPHS issued a joint statement[2] and the AAP released an interim report to clinicians[3] recommending that thimerosal be removed from vaccines as soon as possible, while maintaining efforts to ensure high vaccination levels. The joint statement included a commitment by the FDA to expedite the review of manufacturers' proposals to remove thimerosal as a preservative from vaccines. One recommendation arising from these reports included deferral of hepatitis B vaccination until 2 to 6 months of age for infants born to low-risk mothers. With the approval of a single-antigen thimerosal-free hepatitis B vaccine in August 1999, the ACIP recommended that the birth dose of hepatitis B vaccine be resumed, and that infants under 2 months of age be given preference for thimerosal-free products where supplies are limited. In November 1999, the ACIP reaffirmed these recommendations.[53] Additional proposals by manufacturers to remove thimerosal as a preservative from vaccines are under review by the FDA. In August 1999 the Centers for Disease Control and Prevention and the National Vaccine Advisory Committee sponsored an open public forum on Thimerosal in Vaccines, with representatives from USPHS agencies, other US government agencies, academia, industry, and the international vaccine community, to examine relevant issues.

Research Needs

Data are lacking regarding the biotransformation and pharmacokinetics of thimerosal and its derivatives after intramuscular injection in humans and

animal models. Moreover, insufficient information is available to adequately assess the potential for neurodevelopmental, renal, immunologic, and reproductive toxicity of thimerosal. Limited data exist on the mercury exposure of infants from vaccines, and no observational studies have been done in humans to assess the effect of thimerosal exposure on neurodevelopment, renal, and immunologic function. Thimerosal is unlikely to be eliminated from all vaccines in the near future, and studies are needed to address these gaps to provide a more precise characterization of the potential risk from thimerosal in vaccines.

CONCLUSION

Our review revealed no evidence of harm caused by doses of thimerosal found in vaccines, except for local hypersensitivity reactions. At the time of our review, vaccines containing thimerosal as a preservative could expose infants to cumulative mercury at levels that exceed EPA recommendations during the first 6 months of life. The clinical significance of this conclusion is not currently known; EPA guidelines contain as much as a 10-fold safety factor and such guidelines are meant to be starting points for the evaluation of mercury exposure. However, reducing exposure to thimerosal from vaccines is merited given the goal of reducing human exposure to mercury from all sources, the feasibility of removing thimerosal as a vaccine preservative, and the desirability of ensuring public confidence in the safety of vaccines.

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ABSTRACT

Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med*. 2001;344:403–409.

Background. Ear infections are a common cause of illness during the first 2 years of life. New conjugate vaccines may be able to prevent a substantial portion of cases of acute otitis media caused by *Streptococcus pneumoniae.*

Methods. We enrolled 1662 infants in a randomized, double-blind efficacy trial of a heptavalent pneumococcal polysaccharide conjugate vaccine in which the carrier protein is the nontoxic diphtheria-toxin analog CRM197. The children received either the study vaccine or a hepatitis B vaccine as a control at 2, 4, 6, and 12 months of age. The clinical diagnosis of acute otitis media was based on predefined criteria, and the bacteriologic diagnosis was based on a culture of middle-ear fluid obtained by myringotomy.

Results. Of the children who were enrolled, 95.1% completed the trial . . . There were 2596 episodes of acute otitis media during the follow-up period between 6.5 and 24 months of age. The vaccine reduced the number of episodes of acute otitis media from any cause by 6% (95% confidence interval, −4% to 16% [the negative number indicates a possible increase in the number of episodes]), culture-confirmed pneumococcal episodes by 34% (95% confidence interval, 21% to 45%), and the number of episodes due to the serotypes contained in the vaccine by 57% (95% confidence interval, 44% to 67%). The number of episodes attributed to serotypes that are cross-reactive with those in the vaccine was reduced by 51%, whereas the number of episodes due to all other serotypes increased by 33%.

Conclusions. The heptavalent pneumococcal polysaccharide–CRM197 conjugate vaccine is safe and efficacious in the prevention of acute otitis media caused by the serotypes included in the vaccine.

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