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Pediatrics 2008;121:e208-e214
DOI: 10.1542/peds.2006-3363

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Michael E. Pichichero, MDa, Angela Gentile, MDb, Norberto Giglio, MDc, Veronica Umido, MDb, Thomas Clarkson, PhDd, Elsa Cernichiari, MSc, Grazyna Zareba, PhDc, Carlos Gotelli, PhDd, Mariano Gotelli, PhDd, Lihan Yan, MSe, John Treanor, MDa

aDepartment of Microbiology/Immunology, Pediatrics, and Medicine, University of Rochester, Rochester, New York; bDepartment of Epidemiology, R. Gutierrez Children’s Hospital, Buenos Aires, Argentina; cDepartment of Environmental Medicine, University of Rochester, Rochester, New York; dCenter of Toxicology Research, Buenos Aires, Argentina; eEMMES Corp, Rockville, Maryland

The authors have indicated they have no financial relationships relevant to this article to disclose.

ABSTRACT

OBJECTIVES. Thimerosal is a mercurial preservative that was widely used in multidose vaccine vials in the United States and Europe until 2001 and continues to be used in many countries throughout the world. We conducted a pharmacokinetic study to assess blood levels and elimination of ethyl mercury after vaccination of infants with thimerosal-containing vaccines.

METHODS. Blood, stool, and urine samples were obtained before vaccination and 12 hours to 30 days after vaccination from 216 healthy children: 72 newborns (group 1), 72 infants aged 2 months (group 2), and 72 infants aged 6 months (group 3). Total mercury levels were measured by atomic absorption. Blood mercury pharmacokinetics were calculated by pooling the data on the group and were based on a 1-compartment first-order pharmacokinetics model.

RESULTS. For groups 1, 2, and 3, respectively, (1) mean ± SD weights were 3.4 ± 0.4, 5.1 ± 0.6, and 7.7 ± 1.1 kg; (2) maximal mean ± SD blood mercury levels were 5.0 ± 1.3, 3.6 ± 1.5, and 2.8 ± 0.9 ng/mL occurring at 0.5 to 1 day after vaccination; (3) maximal mean ± SD stool mercury levels were 19.1 ± 11.8, 37.0 ± 27.4, and 44.3 ± 23.9 ng/g occurring on day 5 after vaccination for all groups; and (4) urine mercury levels were mostly nondetectable. The blood mercury half-life was calculated to be 3.7 days and returned to prevaccination levels by day 30.

CONCLUSIONS. The blood half-life of intramuscular ethyl mercury from thimerosal in vaccines in infants is substantially shorter than that of oral methyl mercury in adults. Increased mercury levels were detected in stools after vaccination, suggesting that the gastrointestinal tract is involved in ethyl mercury elimination. Because of the differing pharmacokinetics of ethyl and methyl mercury, exposure guidelines based on oral methyl mercury in adults may not be accurate for risk assessments in children who receive thimerosal-containing vaccines.

Thimerosal has been used as a preservative in biologicals and vaccines since the 1930s because it is very effective in killing bacteria in vaccines and in preventing bacterial contamination, particularly in opened multidose containers. Before 1999, all whole-cell diphtheria-tetanus-pertussis vaccines in the United States and 2 diphtheria-tetanus-acellular pertussis vaccines contained thimerosal, as did 2 Haemophilus influenzae type b (Hib) and hepatitis B virus (HBV) vaccines.

The antibacterial activity of thimerosal (sodium ethyl mercury thiosalicylate) is attributed to the ethyl mercury cation that dissociates from the thimerosal molecule. By 1999, expanding recommendations for infant vaccination meant that US children who received a complete series of vaccines that contained thimerosal potentially received up to 187.5 μg of ethyl mercury during the first 6 months of life.1,2,3 This cumulative exposure could exceed the US Environmental Protection Agency’s recommended safe intake level, estimated in 1997 to be no more than 0.1 μg of mercury per kg of body weight per day.4 This observation led to a recommendation by the American...
Academy of Pediatrics that thimerosal be removed from all vaccines that are administered to infants in the United States.

The US Environmental Protection Agency exposure guidelines, as well as those of other agencies, are generally based on information about the toxicity of oral methyl mercury, the type of exposure associated with consumption of fish. After oral exposure to methyl mercury, ~95% of that ingested is absorbed into the bloodstream (reviewed in\textsuperscript{3}). Methyl mercury distributes to all tissues in the body, including passage across the blood-brain barrier. After distribution is completed in ~1.5 days, ~5% of the ingested dose remains in the blood compartment. Methyl mercury is actively secreted into bile as a complex with reduced glutathione and is in part reabsorbed back into the portal blood supply to form an enterohepatic cycle.\textsuperscript{6} Unabsorbed methyl mercury in the gut is converted by intestinal microflora to inorganic mercury that is poorly absorbed and passes directly into the feces. Urinary excretion is negligible. The overall elimination from the body can be described by an average half-life of ~70 days. The half-life in blood is shorter, ~44 days on average.

Methyl mercury (CH\textsubscript{3}Hg\textsuperscript{+}) and ethyl mercury (CH\textsubscript{3}CH\textsubscript{2}Hg\textsuperscript{+}) have similar chemical structures, and these 2 short-chain alkyl mercurials have generally been assumed to possess similar toxicologic properties; however, much less information is available for ethyl mercury, especially for humans. Animal data indicate that, similar to methyl mercury, ethyl mercury readily transports to all tissues but that it is has a shorter half-life.\textsuperscript{7} Little is known about possible enterohepatic cycling and fecal excretion of ethyl mercury.

In a previous pilot study,\textsuperscript{8} we demonstrated that blood mercury levels were low after administration of vaccines that contained thimerosal to term infants and young children, that mercury was detected in the stool after vaccination, and that the blood half-life of mercury after vaccination was ~7 days (95% confidence interval [CI]: 4–10 days), significantly shorter than that reported for oral methyl mercury in adults; however, that study consisted of only 21 subjects. The primary objective of this study was to confirm the short half-life of mercury in blood in a larger cohort of children who received routine immunizations that contained thimerosal and to evaluate further the excretion of mercury in these children by examining mercury levels in stool and urine.

**METHODS**

The study was conducted as a prospective observational study at the R. Gutierrez Children’s Hospital (Buenos Aires, Argentina) from February 2003 to February 2004. Healthy infants with gestational ages of >32 weeks were recruited from 3 age cohorts (newborns, 2-month-olds, and 6-month-olds), which differed in body weight and cumulative exposure to thimerosal-containing vaccines. All children received age-appropriate vaccines as routinely administered in Argentina (described next), and all vaccines were administered by the investigators at the time of the enrollment visit. Because children in the study received all of their vaccinations at the Gutierrez Hospital, accurate records of previous vaccines were available for the 2-month and 6-month cohorts. In each of the 3 age cohorts, samples of blood, urine, and stool were obtained before vaccination and at 1 time interval after vaccine, as described next. Each individual child contributed 1 prevaccination and 1 postvaccination sample.

The newborn age group received the birth dose of BCG and HBV vaccine, which contained either 12.5 \( \mu \)g (Hepavax AGB; LG Chemicals, Iksan-City, Chun Buk, Korea) or 32.5 \( \mu \)g (Euvax B, Hepavax-Gene; LG Chemicals, Iksan-City, Chun Buk, Korea) of mercury. These children all had samples taken before vaccination (via cord blood) and were randomly assigned to have samples taken at 1 of the following time points after vaccination: 12 ± 3 hours, 24 ± 6 hours, 48 ± 8 hours, 5 ± 1 days, 11 ± 2 days, or 30 ± 3 days.

The 2-month-old group received the first dose of diphtheria–tetanus–whole-cell pertussis (DTwP) and Hib combined, the first dose of oral polio vaccine, and a second dose of the HBV vaccine. This resulted in a total dose of between 37.5 and 57.5 \( \mu \)g of mercury and a cumulative dose of from between 50 and 90 \( \mu \)g. These children all had samples taken before administration of the vaccine and were randomly assigned to have samples taken at 1 of the following time points after vaccination: 1 day ± 8 hours, 3 ± 1 days, 5 ± 1 days, 11 ± 2 days, 21 ± 3 days, and 30 ± 3 days.

The 6-month-old group received the third dose of DTwP, Hib combined, and HBV vaccines for total dose at 6 months of between 37.5 and 57.5 \( \mu \)g of mercury and a cumulative dose of between 112.5 and 162.5 \( \mu \)g. Sampling in the 6-month-old group was identical to that in the 2-month-old group.

All children were in good health as determined by normal gestational history, medical history, and physical assessment. Children with contraindications to routine vaccinations were excluded from participation. This study was approved by the human subjects review boards of the University of Rochester and the R. Gutierrez Children’s Hospital.

**Laboratory Assessments**

**Cold-Vapor Atomic Fluorescence Spectrophotometry**

Mercury levels in blood and urine were determined by cold-vapor atomic fluorescence spectrophotometry (CVAFS) using the Millennium Merlin/Galahad system (PA 10.035; P Analytical Ltd, Orpington, Kent, United Kingdom). The limit of detection in blood was 0.01 ng/mL, depending on sample volume, or ~100-fold lower than the method used in the pilot study.\textsuperscript{4} CVAFS determinations measure total mercury, including ethyl or methyl mercury, and inorganic forms. The accuracy of the method for blood was assessed by using blood Seronorm 201605 (Seronorm, SERO, Billingstad, Norway) and for urine using urine Seronorm 20125 as the reference material. Total blood mercury determinations were performed in Rochester, NY, and all samples were coded and assayed by the laboratory without awareness.
of either the cohort or the order of sampling (before or after vaccination).

Cold-Vapor Atomic Absorption
Mercury levels in stools were determined by cold-vapor atomic absorption.9 Stool samples that were positive for mercury were differentiated into total and inorganic mercury, and the difference between the 2 was assumed to be organic mercury. Stool mercury determinations were performed in Buenos Aires, Argentina; all samples were coded and assayed in a blinded manner. Results are reported in nanograms of total mercury per gram of stool dry weight.

Each lot of vaccine administered to the children in the study was also tested in Argentina for total mercury levels by using cold-vapor absorption. The organic mercury present in the vaccines was further specified as ethyl or methyl mercury as described next.

Speciation of Ethyl and Methyl Mercury
In samples of vaccines from the trial and in a subgroup of blood samples of sufficient volume, organic mercury was further specified as ethyl or methyl mercury by gas chromatograph atomic fluorescence mercury speciation. Mercury speciation testing was performed in the laboratory of Dr Milena Horvat in Slovenia; all samples were coded and assayed in a blinded manner.

Urinary γ-glutamyl Transpeptidase and Creatinine Levels
Levels of γ-glutamyl transpeptidase (GGT) and creatinine were also measured in urine samples collected during the study. GGT levels were determined by enzyme immunoassay.10 Urine creatinine levels were determined by using colorimetric analysis. Urine GGT levels were adjusted by measurement of urine creatinine to account for differing urine concentrations. These assays were performed in Argentina.

Statistical Methods
The primary objective of this study was to obtain an estimate of blood mercury levels at each time point after vaccination. A sample size of 10 produces a 95% CI equal to the sample mean ± 72% of the SD. This study targeted enrollment of 12 children per group (a total of 216 children) to allow for 20% loss in follow-up. Descriptive statistics were generated for levels of total blood, urine, and stool mercury at each time point.

Each child in this study had samples taken at a maximum of 2 time points, once before vaccination, and once at a randomly assigned time point after vaccination. We estimated the pharmacokinetics of mercury using a model that averages all of the samples obtained from the population rather than evaluating multiple samples from the same individual, to avoid multiple blood draws in these infants. Half-life estimates of blood mercury level were based on a 1-compartment first-order pharmacokinetics model taking into account the dosage of thimerosal received and weight and age effects on volume and clearance. Specifically, the estimation process consisted of the following joint model being fit simultaneously:

\[
\text{Prevaccination blood mercury} = C_0 + \text{error} \\
\text{Postvaccination blood mercury} = C_0 + \frac{\text{dose}}{V} \times \exp(-\alpha \times \text{time}) + \text{error}
\]

where \(C_0\) is the baseline level, \(\alpha\) is the elimination rate constant, \(\frac{\text{dose}}{V}\) and half-life is estimated as \(\ln(2)/\alpha\); \(\text{CL} = CL_0 / (\text{weight})^{0.75} \times (1 + \text{age}^8)\) is the clearance (allometric scaling of weight and dependence on age are considered in the estimation), and \(V = V_0 \times (\text{weight})\) is the volume of distribution; the percentage of mercury in the vaccine doses being initially distributed to body (assume 8% of the body weight is blood) can then be estimated as \(8%/V_0\).

Analyses were performed by using SAS 9.1.3 (SAS Institute Inc, Cary, NC). Half-life was estimated accordingly on the basis of the estimated parameter values, and the CIs were calculated by using the Delta method function in SAS. A nonlinear model (PROC NLIMIXED in SAS) was used in the model fitting. For reduction of bias, a few outlier data points that were detected from a robust regression model (PROC ROBUSTREG in SAS) were excluded in the kinetic model.

RESULTS
Seventy-two newborns, 72 infants who were 2 months of age, and 72 infants who were 6 months of age were enrolled in the study (N = 216). A summary of infant weights is shown in Table 1. Study children were an appropriate weight for gestational age at birth, and the newborns lost an expected amount of weight in the first few days of life, with subsequent weight gain. Similarly, the 2- and 6-month-olds were at age-appropriate weights when enrolled and showed appropriate weight gain during subsequent study visits.

Some children were unable to contribute an adequate blood sample for mercury determination at either the enrollment visit or the follow-up visit. The numbers of blood, stool, and urine mercury determinations that were available from children at each visit are also shown in Table 1. Both prevaccination and postvaccination blood determinations were available from 128 (59.3%) of the children, including 40 newborns, 50 infants aged 2 months, and 38 infants aged 6 months. Adequate amounts of blood for mercury determination were not available from either the prevaccination or the postvaccination samples from 17 children (7 newborns, 3 infants aged 2 months, and 7 infants aged 6 months), who were not included in the analysis.

Mercury concentrations in blood, stool, and urine for the 3 study groups are presented in Figs 1 to 3, respectively. The highest levels of mercury in blood were detected in the first samples obtained after vaccination (ie, at 12 hours after vaccination in the newborn group and at 24 hours after vaccination in the 2- and 6-month-old groups). Regardless of age group, mercury levels were relatively low, with the highest level detected being 8.0 ng/mL in a newborn 12 hours after vaccination.
receiving the birth dose of HBV vaccine that contained 32.5 μg of mercury. Blood mercury levels fell rapidly and had largely returned to baseline levels by day 11 after vaccination.

Mercury was detected in virtually all stool samples tested (Fig 2) and increased significantly after vaccination in all 3 groups. Mercury was detected in stools throughout the sampling time points. All of the mercury in stool samples was inorganic mercury.

Mercury was nearly undetectable in urine in all samples (Fig 3). Because mercury is known to be toxic to renal tissues, we measured urine GGT levels, a sensitive indicator of damage to the proximal renal tubules, to assess the potential renal toxicity of thimerosal. There was no evidence of clinically significant elevations of urine GGT and no connection between blood mercury and urine GGT (data not shown).

Fish is not a commonly consumed food in Argentina, and in preliminary studies, we did not detect any mercury in a sample of 10 randomly selected umbilical cord blood samples from the R. Gutierrez hospital (data not shown); however, blood mercury levels ranging from 0.3 to 5.0 ng/mL were detected in prevaccination samples from all 3 age groups in this study, including newborns, in which we detected blood mercury levels as high as 2.6 ng/mL before vaccination. Therefore, speciation of organic mercury into ethyl and methyl mercury by gas chromatography atomic fluorescence was performed on 23 blood samples that had sufficient remaining volume for testing. These included 5 postvaccination samples from newborns (1 collected at 48 hours and 4 collected at day 5), 9 postvaccination samples from 2-month-olds (3 collected at 24 hours, 4 collected on day 3, and 2 collected on day 5), and 9 postvaccination samples from 6-month-olds (5 collected at 24 hours and 4 collected at day 3). No prevaccination samples were available for speciation.

Some methyl mercury was detected in all of the samples tested, ranging from 1% to 50% of the total organic mercury in samples with both methyl and ethyl mercury, and in 2 samples (a newborn 48 hours after vaccination and a 2-month-old 5 days after vaccination), only methyl mercury was detected. The mean concentration of methyl mercury in the postvaccination blood of newborns was $0.39 \pm 0.44$ ng/mL (minimum: 0.067 ng/mL; maximum: 1.06 ng/mL). In the 2-month-old group, the mean concentration of methyl mercury in the blood was $0.26 \pm 0.30$ ng/mL (minimum: 0.37 ng/mL; maximum: 0.79 ng/mL). In the 6-month-old group, the mean concentration of methyl mercury in the blood was $0.10 \pm 0.07$ ng/mL (minimum: 0.02 μg/mL; maximum: 0.23 ng/mL).

We also measured mercury levels in the administered vaccines and found that the stated amounts from the manufacturers were accurate and that the mercury in the vaccines was exclusively ethyl mercury. The presence of methyl mercury in the blood samples therefore suggests that sources of mercury other than thimerosal contributed to the total mercury measurements.

A half-life for blood mercury was estimated using a 1-compartment model as described in “Methods.” The half-life of blood mercury was estimated to be 3.7 days for newborns, 2.0 days for 2-month-olds, and 2.2 days for 6-month-olds (Table 2). We also calculated the percentage of mercury that was distributed to the blood compartment at the time of vaccination. Assuming that 8% of the child’s body weight is blood, the estimate was 3.2% (95% CI: 3.0%–3.5%), lower than the 5% blood distribution for methyl mercury. A 2-compartment model was also explored for evidence of a multiepoxi-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Children’s Weights and Mercury Sampling</th>
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<tr>
<td>Age Group</td>
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<tr>
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nential elimination pattern; however, because of the significant and variable background of blood mercury in our data set, a 2-compartment model did not provide a better fit to the data.

The statistical model used in this analysis controls for prevaccination blood mercury levels in the group as a whole (ie, the $C_0$ term, or baseline level of mercury) because not all of the children had both prevaccination and postvaccination samples available. However, when the analysis was restricted to only those children with both prevaccine and postvaccine measurements and the
DISCUSSION

In this study, we evaluated the time course of mercury in the blood of infants after parenteral exposure to ethyl mercury, the chemical species responsible for the preservative action of thimerosal. Although considerable information is available regarding oral exposures to methyl mercury, relatively little is known about the pharmacokinetics of ethyl mercury, particularly when administered intramuscularly.

We observed that blood mercury levels after intramuscular administration of thimerosal-containing vaccines to newborn, 2-month-old, and 6-month-old infants were at their highest level shortly after vaccination and returned to prevaccination levels within a few weeks. Prevaccination levels of blood mercury in 6-month-olds were not higher than those in 2-month-olds, suggesting that exposure to thimerosal-containing vaccines does not result in an accumulation of mercury in blood as might have been predicted if the blood half-life were similar to that of methyl mercury. Using a model that accounted for baseline mercury levels, ethyl mercury dosage, and timing of vaccination, we estimated the blood half-life of mercury after administration of thimerosal to be 3.7 days, which did not vary significantly by age group.

We also observed that the highest levels in samples that were taken from children shortly after vaccination were <8 ng/mL. The importance of blood levels of ethyl mercury for assessing toxicity is unknown, but blood levels have been shown to be a predictor of toxicity for methyl mercury exposure.11,12 The low levels of mercury detected in this study suggests relatively low risk for toxicity from this exposure.

Our estimate of mercury blood half-life after intramuscular ethyl mercury is consistent with a recent comparison of the pharmacokinetics of mercury after administration of oral methyl or intramuscular ethyl mercury to infant rhesus macaques.7 That study showed significant differences between the elimination half-lives of methyl and ethyl mercury, with a much shorter half-life for ethyl mercury. Although the detailed study in rhesus macaques was more consistent with a 2-compartment rather than a 1-compartment model, the finding of an initial elimination half-life of 2.1 days and terminal elimination half-life of 8.6 days both are within range of our estimate of a blood half-life of 2.9 to 4.1 days. The significant difference in blood half-life of intramuscular ethyl and oral methyl mercury suggests that exposure guidelines based on oral methyl mercury may not be appropriate for use in risk assessments of thimerosal in vaccines.

There was an increase in stool mercury levels shortly after vaccination, which slowly fell afterward. This pattern would be consistent with an enterohepatic excre-
tion pathway similar to that described for methyl mercury; however, because these observations were made on spot (as opposed to 24-hour) collections only, it is not possible to draw conclusions regarding what proportion of the administered mercury was eliminated in the stool and the kinetics of the elimination process. There was no evidence of significant urinary excretion of mercury in this study, similar to previous observations with methyl mercury.

This study has several limitations. First, both methyl and ethyl mercury were present in the blood of some study children, suggesting that our measurements of mercury represent a mixture of organic species with different sources of exposure and different intrinsic half-lives. This measurement may therefore overestimate the actual amount of blood mercury contributed specifically by ethyl mercury from vaccines.

Second, our measurements are unable to determine the fate of the mercury after it leaves the blood, because our sampling was limited to blood, urine, and stool, and we did not collect 24-hour samples; therefore the data do not allow any conclusions about the proportion of administered ethyl mercury that is ultimately excreted in stools or the time course of that excretion, only that some excretion seems to occur by the gastrointestinal route and that the kidneys do not seem to play an important role. The available data are compatible with a 1-compartment model with a short half-life or with a 2-compartment model with a rapid initial distribution phase followed by a more prolonged terminal elimination phase in which blood ethyl mercury levels are at a level that cannot be distinguished from background. In either case, the results of our studies and those in monkeys1 suggest that the kinetics of mercury in blood after intramuscular thimerosal are significantly different from that after oral exposures to methyl mercury.

**CONCLUSIONS**

Our study was not designed to assess the toxicity of thimerosal but to provide data that may be useful in assessing the risks related to ethyl mercury exposure of infants who receive thimerosal at dosages consistent with standard vaccination regimens. Concerns have been raised that administration of vaccines that contain thimerosal but to provide data that may be useful in assessing the risks related to ethyl mercury exposure of infants who receive thimerosal at dosages consistent with standard vaccination regimens. Concerns have been raised that administration of vaccines that contain thimerosal could cause an increased risk for pervasive developmental disorders, including autism, although epidemiologic studies have generally concluded that there is no evidence for such an association.13–15 Our results suggest that a new risk assessment regarding exposure to thimerosal used as a preservative should be conducted in light of the demonstrated short half-life of ethyl mercury after vaccination. Additional studies to assess the full range of thimerosal elimination kinetics are under way in preterm infants who receive a birth dose of HBV vaccine.

**ACKNOWLEDGMENTS**

This work was supported by contracts NO1 AI25249 and NO1 AI25460 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health.

We acknowledge the efforts of Dr Melena Horvat, who performed the speciation of mercury; Margaret Langdon and Alfredo Lo Balbo, PhD, for assistance with mercury determinations; Doreen Francis for assistance with regulatory filings; Paola Czerniuk, MD, and Ana Carro, MD, for assistance with recruitment and enrollment; and Melinda Tibbals for study coordination and data management.

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