TOXICOLOGICAL SCIENCES **120(2)**, 499–506 (2011)
doi:10.1093/toxsci/kfr009
Advance Access publication January 20, 2011

Toxicokinetics of Mercury after Long-Term Repeated Exposure to Thimerosal-Containing Vaccine

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Received October 10, 2010; accepted January 11, 2011

The preservative thimerosal contains ethyl mercury (EtHg). Concerns over possible toxicity have re-emerged recently due to its presence in (swine and other) flu vaccines. We examined the potential accumulation of mercury in adults given repeated injections of a thimerosal-preserved vaccine for many years. Fifteen female patients were recruited from an outpatient clinic running a clinical trial with repeated injections (1 ml every 3–4 weeks) of a staphylococcus toxoid vaccine containing 0.01% thimerosal to treat chronic fatigue syndrome. Fifteen untreated female patients with the same diagnoses served as controls. Blood samples were taken before injecting the vaccine, 1 day later, about 2 weeks later, and just before the next injection. In the 15 controls, samples were taken twice. Blood was analyzed for total mercury and EtHg. The toxicokinetics were assessed for each patient separately as well as with a population-based pharmacokinetic model. Total mercury in blood increased on Day 1 in all treated patients (median: 0.33, range: 0.17–1.3 μg/l), as did EtHg (median: 0.14 μg/l, range: 0.06–0.43 μg/l). After a few weeks, levels were back to normal and similar to those in controls. Levels of methyl mercury (MeHg; from fish consumption) were much higher than those of EtHg. After exclusion of an outlier, the mean half-life in a population-based model was 5.6 (95% CI: 4.8–6.3) days. The results indicate that mercury from thimerosal is not accumulated in blood in adults. This is in accordance with short half-lives and rapid metabolism of EtHg to inorganic mercury.

Key Words: **Mercury; ethyl mercury; methyl mercury; thimerosal; toxicokinetics; half-life.**

Mercury exists in three major forms: mercury vapor, inorganic divalent mercury, and organic mercury (Clarkson, 2002; Fig. 1). Important human exposure sources are mercury vapor inhaled after release from dental amalgam and methyl mercury (MeHg) from fish. Another organic mercury compound is ethyl mercury (EtHg); this is present in the preservative thimerosal found in vaccines and some other pharmaceutical products. The issue of thimerosal safety has recently been revived owing to its presence in influenza vaccines (U.S. FDA, 2009).

Mercury vapor and MeHg are both neurotoxic (Nierenberg *et al*., 1998; WHO, 1990, 1991), and MeHg is known to cause adverse effects on the developing brain at exposure *in utero*. MeHg is distributed to most tissue and mainly eliminated in feces after demethylation, with a half-life of approximately 2 months, following a single compartment model and first-order kinetics (Clarkson, 2002).

EtHg has been used as a preservative since the 1930s, for example, in vaccines, immunoglobulins, eye drops, and cosmetic products. An EtHg radical attached to the sulfur group of thiosalicylate gives the product thimerosal. The kinetics and toxicity of EtHg are less well known than those of MeHg, and so safety assessments have generally used data for MeHg (Clements, 2004; Richer *et al*., 2002).

The use of thimerosal in vaccines led to concerns over possible risks of toxicity (e.g., autism) some years ago (Ball *et al*., 2001; CDC, 1999; Clarkson *et al*., 2003; Geier and Geier, 2003; IOM, 2001, 2004), but systematic studies have failed to indicate an association between vaccination and autism in children (Clements, 2004; Hviid *et al*., 2003; Stehr-Green *et al*., 2003; Verstraeten *et al*., 2003). Although recent follow-up studies provided little support for effects of previous thimerosal exposure on neuropsychological performance or on risk of autism (Hertz-Picciotto *et al*., 2010; Thompson *et al*., 2007; Tozzi *et al*., 2009), the controversy continues (Geier *et al*., 2007). These concerns have led to a large decrease in the use of thimerosal as a preservative in vaccines administered to children and infants.

The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

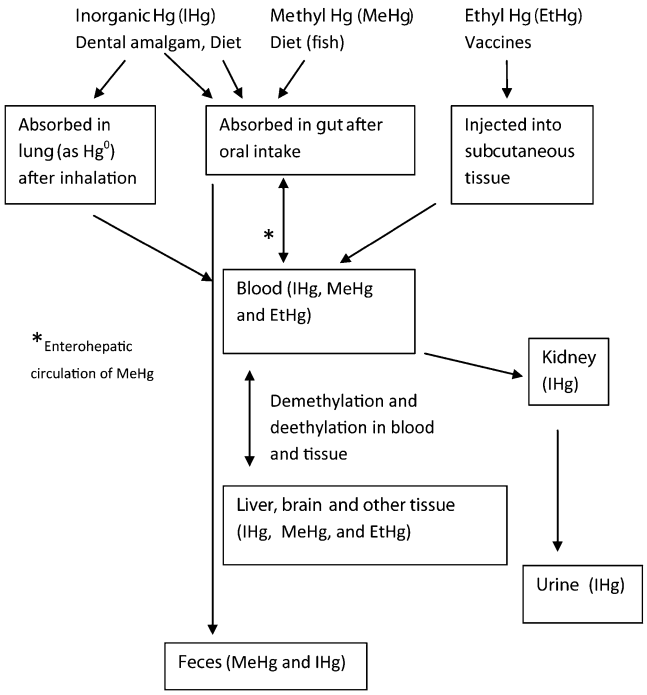

FIG. 1 Simplified outline of the sources and metabolism of different mercury species.

Although MeHg and EtHg are closely related chemically and cause similar types of damage to the brain in toxic doses, EtHg seems to have a shorter half-life and is more rapidly metabolized into inorganic mercury. This will result in a lower body burden of EtHg, both at steady state and when some time has elapsed after a single dose (Magos, 2003). The same is true for EtHg levels in the brain (Aschner and Ceccatelli, 2010). There is, however, need for data on the toxicokinetics of EtHg in man (Clarkson *et al.*, 2003). Previous studies in humans have been performed in infants (see below), and these were based on determinations of total mercury only and not EtHg. Magos (2003) noted that scarce data indicated a half-life of 18 days in adults, but no empirical data from adults have been reported.

Mercury levels in infants have been examined after intramuscular vaccination. One study was performed in 33 infants aged 2–6 months, with the total mercury concentration in blood measured on a single occasion 3–28 days after a dose of vaccine (Pichichero *et al.*, 2002). The concentrations measured in blood were generally low, and the half-life was estimated to be 7 (95% confidence interval [CI]: 4–10) days. No prevaccine blood tests were made, however, and only total mercury in blood was analyzed. A recent larger study of infants analyzed prevaccine blood concentrations of total mercury along with postvaccine concentrations up to 30 days after vaccination, though only one postvaccine mercury determination was made per infant (Pichichero *et al.*, 2008). The half-life of total mercury in blood was estimated at 3.7 (95% CI: 2.9–4.5) days. A similar study in premature and low–birth weight infants indicated a half-life of 6.3 (95% CI: 3.9–8.8) days (Pichichero *et al.*, 2009). These studies used determinations of total mercury. To our knowledge, no similar study in adults has been reported and no toxicokinetic study on EtHg.

Vaccines preserved with thimerosal usually contain 0.001–0.01% thimerosal. A single 0.5 ml dose of vaccine with 0.01% thimerosal contains 50 μg thimerosal or approximately 25 μg of mercury. With time, alternative preservatives in vaccines have replaced thimerosal, but its use was still reported recently in some immunoglobulin preparations, antivenins, skin test antigens, and ophthalmic and nasal products; and several influenza vaccines in the United States still contain thimerosal (U.S. FDA, 2009).

The present study took advantage of an ongoing clinical trial using repeated injections of a staphylococcus toxoid vaccine preserved with thimerosal. The aim of the present study was to investigate the EtHg kinetics in adults. More specifically, we wished to estimate the fraction of the dose deposited in blood and the half-life of EtHg in blood after sc administration of EtHg. In addition, we tested the hypothesis that EtHg was accumulated in these patients after several years of treatment. MeHg levels were also determined in order to assess the contribution to total mercury levels.

SUBJECTS AND METHODS

Subjects. The subjects were recruited from an outpatient clinic running a clinical trial with repeated injections of the staphylococcus toxoid vaccine Staphypan Berna (Swiss Serum and Vaccine Institute, Berne) in the treatment of patients suffering from chronic fatigue syndrome and fibromyalgia (Andersson *et al.*, 1998; Zachrisson *et al.*, 2002). The patients received sc injections with 1 ml of the vaccine every 3–4 weeks. According to the manufacturer, the vaccine contains 0.01% thimerosal, 50% of which is mercury by weight.

Fifteen female patients with chronic fatigue syndrome or fibromyalgia, treated with the vaccine for at least 1 year, and 15 untreated female patients with the same diagnoses, frequency matched for age, were recruited from the outpatient clinic. Their height, weight, number of amalgam surfaces, and fish consumption in the past 3 months and during the study period were recorded using a questionnaire. As shown in Table 1, the patients were well matched for age and body mass index and roughly matched with respect to fish consumption and number of dental amalgam surfaces. No patients had eaten freshwater fish in the past 3 months. The ethics committee of the University of Gothenburg approved the study, and the patients provided signed informed consent.

Blood sampling. Based on the assumption in the literature, of a half-life in adults of about 18 days (Magos, 2003), venous blood samples were taken from the vaccine-treated group just before injecting the vaccine (Day 0), 1 day later (median: 23 h, range: 20–28 h), about 2 weeks later (11–16 days), and just before the next injection (after 19–29 days). In the 15 nontreated patients (controls), blood samples were taken on two different occasions 14–63 days apart. The blood samples (two tubes of 7 ml each) were drawn in mercury-free vials (Venoject) and frozen (within an hour, first at −20°C, and then at -80°C) until analyzed (within 6 months).

Because the main study showed a much shorter half-life than expected (see below), we made a separate additional study in two male subjects, aged 41–76 years, who had received vaccine injections monthly for 4 and 40 years. In these subjects, a blood sample was taken before injection and then four times in the next 3 days (16, 24, 48, and 72 h).

TABLE 1
Background Characteristics of 15 Female Patients Treated with a Thimerosal-Preserved Vaccine, Two Men Treated with the Same Vaccine, and 15 Untreated Female Controls

Group	*N*	Age, mean (range)	Body mass index, mean (range)	Fish meals per week, never/≤ 1 per week/> 1 per week	Number of amalgam surfaces, median (range)
Treated	15	56 (31–73)	29 (20–41)	3/10/2	12 (0–39)
Controls	15	53 (30–77)	28 (23–39)	0/13/2	8 (0–48)
Treated	2	41/76	25/29	0/0/2	5/14

Mercury analyses. Total mercury in blood was determined in acid-digested samples by cold vapor atomic fluorescence spectrometry (Sandborgh-Englund *et al*., 1998a) at the Department of Occupational and Environmental Medicine, Lund University Hospital. The detection limit (three times the SD of the blanks) was 0.19 μg/l. The analytical accuracy was checked by analysis of external reference samples with satisfactory results. All determinations were made in duplicate preparations. The method imprecision, calculated as the coefficient of variation for duplicate measurements, was 4.1%.

EtHg and MeHg were determined by a method based on acid leaching (H_2SO_4/KBr/$CuSO_4$) followed by extraction of MeHg and EtHg bromides into an organic solvent (CH_2Cl_2), back extraction into Milli-Q water, propylation with sodium tetrapropylborate ($NaPr_4B$), room temperature precollection on Tenax, isothermal gas chromatographic separation, pyrolysis, and cold vapor atomic fluorescence spectrometric detection of mercury (Gibicar *et al*., 2007). Blank values for MeHg and EtHg were very low and reproducible over the whole period of the study. Estimated limits of detection (LOD) calculated as three times the SD of the blanks were only about 0.01 ng/g for both species. However, inhomogeneity of blood samples is another source of variability. We therefore calculated the LOD as three times the SD of repeated determinations of the blood samples with the lowest samples concentrations of EtHg (< 0.05 ng/g) and MeHg (< 0.5 ng/g). Then, the LOD was 0.03 ng/g for EtHg and 0.08 ng/g for MeHg. The repeatability of aqueous propylation was investigated by spiking Milli-Q water with 100 pg MeHg and EtHg, by addition of aqueous standard solutions and performing 12 consecutive derivatizations. Relative standard deviations (RSDs) of the measurements were 2% for MeHg and 5% for EtHg. To check the repeatability in blood samples, two samples with variable MeHg and EtHg concentrations were used (Sample 1: 0.06 ng EtHg/g and 0.36 ng MeHg/g and Sample 2: 1.5 ng EtHg/g and 0. 18 ng MeHg/g). The RSD for EtHg from four replicate analyses was calculated to be 5 and 10% for Sample 2 and Sample 1, respectively. The RSD for MeHg was calculated to be 7 and 12% for Sample 1 and Sample 2, respectively. Recoveries were between 87 and 96% for EtHg and between 90 and 110% for MeHg. Recovery factors were therefore not applied in the final calculations. All blood samples were analyzed in duplicate. Due to lack of reference materials certified for EtHg and MeHg in blood, the reference material IAEA MA-A-1/TM (Copepod Homogenate), obtained from the International Atomic Energy Agency, was employed as a compromise to validate the developed method. This reference material contains both mercury species, MeHg and EtHg, although not certified. In a previous study, MeHg and EtHg were analyzed by three different independent analytical methods (Horvat, 1991) and good agreement was obtained. These values were re-established during method validation used in present study (Gibicar *et al*., 2007). Additional quality control steps were also undertaken in each set of analysis. These included replicate spike recovery of the blood samples and multiple blank measurements (three or more) in each set of analysis. EtHg and MeHg were analyzed in all treated patients, but only in seven of the untreated ones.

Data analysis. Descriptive analyses were performed, and differences in levels of total mercury or EtHg in blood on the various days of sampling in the treated group were tested using the Kruskal-Wallis and Wilcoxon signed rank tests. In addition, the relative increase of EtHg and total Hg was assessed by a mixed effects model (Mixed procedure; SAS v9.1; SAS Institute, Cary, NC). The differences between the treated and untreated patients were tested with the Wilcoxon rank sum test. The associations between log-transformed blood mercury levels and fish consumption, number of amalgam surfaces, and age were assessed using multiple linear regression. Blood volumes used when calculating the fraction of the dose deposited in blood were obtained using a nomogram (Ciba-Geigy, 1984).

As a second step, the half-life of EtHg and total mercury in blood in the treated group was calculated for each patient separately, assuming first-order kinetics, a single compartment model, and maximum concentration (*C*max) 1 day after injection. For EtHg, we assumed no exposure source other than thimerosal in the vaccine. For total mercury, there is a continuous (varying) exposure from diet. Half-lives were estimated using nonlinear regression (NLIN procedure; SAS v9.1).

Then, EtHg concentration time data were evaluated using a population model–based approach in NONMEM VI software (Globomax LLC, Ellicott City, MD) under a Compaq Visual Fortran v.6.6 compiler. NONMEM allows the estimation of typical population pharmacokinetic parameters and their respective inter- and intra-individual variability in combination with the estimation of residual random variability. First-order conditional method with interaction between individual and random effects was used for estimation of pharmacokinetic data. The log-likelihood ratio, the precision of estimated parameters, and diagnostic plots, including visual predictive checks, were used to discriminate between models. Census (Wilkins, 2005), Xpose (Jonsson and Karlsson, 1999), and PsN (Lindbom *et al*., 2005) were used for handling model and output files, model evaluation, and goodness of fit assessment. R v. 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria) and Spotfire v. 3 (Tibco Software, CA, USA) were used for plotting and data visualization. Several structural models including one- or two-compartment models, with fixed or estimated absorption, were tested during model development. The final structural model consisted of a sc dosing compartment with fixed (instantaneous) absorption to the central compartment and was parameterized in terms of apparent clearance (CL/F) and apparent central volume of distribution (V/F). Between-subject variability could only be estimated for V/F, assuming an exponential model and lognormal distribution. Residual variability accounting for within-subject variability was accounted by an additive residual model. Random effects models were revaluated throughout the modeling process.

RESULTS

Mercury Levels

A summary of the mercury levels is shown in Table 2, and a full list of all values can be obtained from the authors. Levels of total mercury increased on Day 1 compared with Day 0 in all treated patients (median: 0.33, range: 0.17–1.3 μg/l). The corresponding results for EtHg showed a median increase of 0.14 (range 0.06–0.43) μg/l. The increase in total mercury was significantly larger than the increase in EtHg ($p < 0.001$). The

TABLE 2
Concentrations of Total Mercury (Tot-Hg), EtHg, and MeHg in Blood (in μg/l) on Four Occasions in 15 Patients Treated with a Thimerosal-Preserved Vaccine and in 15 Untreated Controls

Group	Sample 1, Day 0, median (range)	Sample 2, Day 1, median (range)	Sample 3, Days 11–16, median (range)	Sample 4, Days 19–29, median (range)
Treated				
Tot-Hg	1.4 (0.38–7.2)	1.7 (0.65–7.6)	1.3 (0.41–7.1)	1.3 (0.46–7.0)
EtHg	0.03 (< LOD to 0.04)	0.17 (0.10–0.48)	0.03 (< LOD to 0.49)	0.05 (< LOD to 0.11)
MeHg	1.2 (0.17–8.7)	1.3 (0.19–7.4)	1.2 (0.32–7.9)	1.2 (0.25–7.2)
Controls				
Tot-Hg	1.4 (0.65–5.3)		1.6 (0.52–6.1)[a]	
EtHg[b]	< LOD			
MeHg[b]	1.1 (0.58–3.1)			

[a]Days 14–63.
[b]$N = 8$.

median individual ratio of Δtotal Hg/ΔEtHg was 2.0 (95% CI: 1.6–2.9). An analysis using a mixed effects model showed a similar result.

The mean pre-injection level of EtHg on Day 0 was 11% of the level on Day 1. After 2–4 weeks, mean levels were back to normal and were similar to those of the untreated patients (Fig. 2). In the nontreated patients, the median difference between the two sampling occasions was −0.02 g/l (range: −0.17 to 0.43) for total mercury, and the EtHg levels were below the detection limit.

According to the manufacturer, the mercury concentration in the vaccine was 50 μg/ml. On the first day after injection, the median increase of total mercury in blood corresponded to 3.0% of the mercury dose (range: 1.7–22%), whereas the median EtHg concentration in blood on that day represented only 1.2% of the dose (range: 0.6–4.5%).

Blood levels of MeHg were generally much higher than those of EtHg, even on Day 1 after vaccine injection. The median fraction of MeHg on Day 0 was 92% of total mercury in the treated patients and 96% in the untreated group. In the treated patients on Day 1, 74% (median) was MeHg and 9% (median) was EtHg. After a month, before the next injection, the median fractions of MeHg and EtHg in the treated patients were 90% and 1%, respectively. As could be expected, MeHg and total mercury levels increased with self-reported fish consumption (Fig. 3). There was no association with number of amalgam surfaces, even when age and fish consumption were taken into account.

In the two subjects who underwent frequent blood sampling in the first 3 days after injection, *C*max for total Hg was found 24 h after injection in one and 40 h after injection in the other (Table 3). For EtHg, *C*max was found on the first sampling occasion (at 16 h) in both subjects.

One of the patients had a concentration time profile different from the others. As shown in Figure 4, the increase in EtHg after injection was larger and more persistent than in the others. This patient had the highest level of MeHg on Day 0, seven times higher than the average. A close look at the data showed a substantial decrease of MeHg from Day 0 to Day 1 and Day 11, which is not compatible with the normal half-life of MeHg (about 2 months; e.g., a decrease of 1–2% per day at ceased exposure). We therefore suspect analytical problems and may have underestimated MeHg and overestimated EtHg in this patient.

Individual Half-Lives for EtHg

When calculating half-lives for each patient ($N = 15$) separately for EtHg in blood, the median half-life was 4.7 (95% CI: 3.3–8.0) days. In some patients, the data could be fitted to a two-compartment model, but this model was not significantly better than the one-compartment model. The concentration time profiles were similar for all patients except for the above-mentioned outlier as shown in Figure 4. This patient was omitted in the results for the population model–based approach

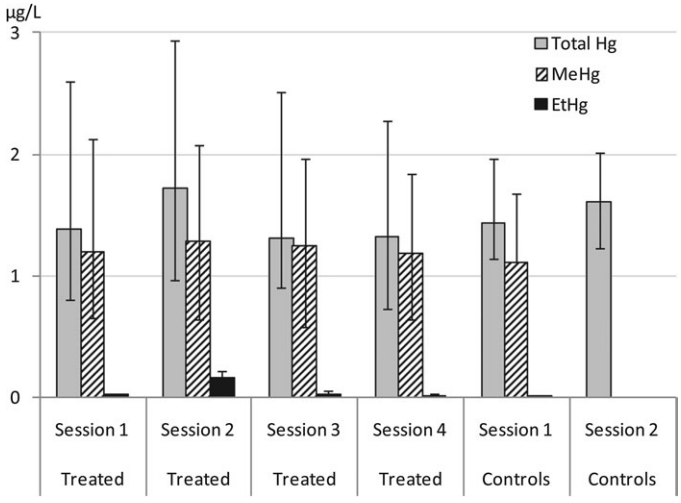

FIG. 2 Total mercury (gray), MeHg (striped), and EtHg (black) in blood in 15 patients treated with a thimerosal-preserved vaccine and in 15 untreated controls.

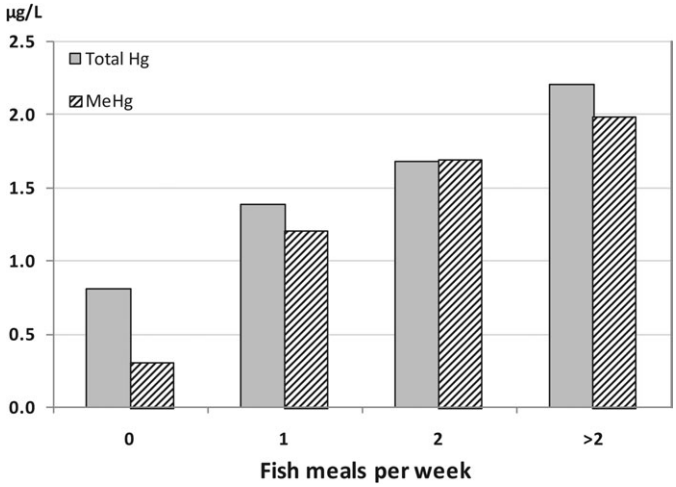

FIG. 3 Total mercury (gray) and MeHg (striped) in blood, stratified by self-reported fish consumption (meals per week) in 23 women (15 patients treated with a thimerosal-preserved vaccine and 8 untreated controls).

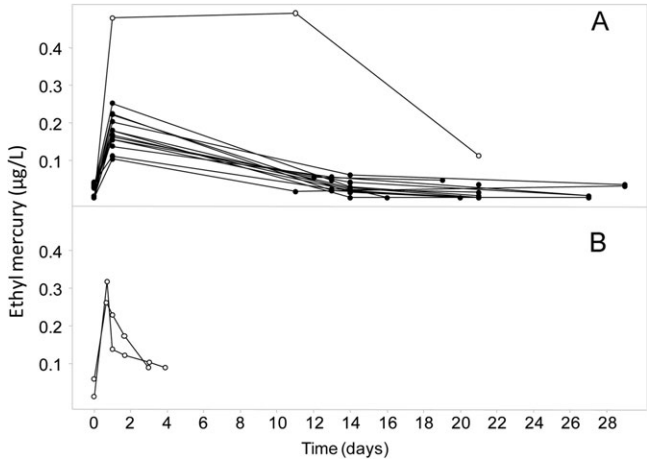

FIG. 4 EtHg concentration time profile in 15 patients after sc injection of a thimerosal-preserved vaccine. The open circles (Panels A and B) represent the subjects omitted from population pharmacokinetic analysis. Panel B shows data from two subjects from an additional separate study.

given below. If included, the final parameters changed somewhat and their precision decreased.

MeHg levels varied somewhat, and in some patients, increased over the study period, and this affected also total mercury levels. In five patients, total mercury levels were lower than pre-injection levels as early as on the third sampling occasion, implying a short half-life. In two patients, the total mercury levels increased slightly from the second to the third sampling. The estimated median half-life of total mercury for all 15 patients was similar to that of EtHg (data not shown), but due to daily variability, the estimates were too imprecise to be useful.

Population-Based Pharmacokinetic Model for EtHg

In the population model, a one-compartment model with fixed absorption from the sc dosing compartment was found to capture the data ($N = 14$) adequately. Between-subject variability could only be estimated for V/F under the additive residual error model. Using the slope-intercept residual model prevented the between-subject variability to be estimated on any of the pharmacokinetic parameters. None of the goodness of fit criteria including the visual predictive check (available from the authors) offered any conclusive guidance on model selection. Finally, it was decided that between-subject variability on V/F was a more valuable parameter to estimate for the purposes of this study. Population estimates from the final model are presented in Table 3 with relative standard errors (RSEs), residual- and between-subject variability where appropriate. The mean terminal half-life computed from the individually obtained estimates was 5.6 (95% CI 4.8-6.3) days.

Inclusion of the two subjects sampled frequently for 3 days in the population model–based calculations provided some support for a two-compartment model with fixed absorption from the sc dosing compartment. The two-compartment model parameters were, however, very imprecise, and the main interest of this study was the terminal half-life of EtHg. Therefore, we did not include these two subjects in the calculations but merely use them to illustrate *C*max.

TABLE 3
Concentrations of Total Mercury (Tot-Hg), EtHg, and MeHg in Blood (in µg/l) on Four Occasions in Two Men Treated with a Thimerosal-Preserved Vaccine Just after Blood Sample 1 on Day 0

	Sample 1, Day 0	Sample 2, 16 h	Sample 3, 24 h	Sample 4, 40 h	Sample 5, 72 h
Tot-Hg	4.3/3.5	4.4/3.9	4.5/3.8	4.3/4.0	4.1/3.7
EtHg	0.06/< LOD	0.26/0.32	0.23/0.14	0.17/0.12	0.09/0.10
MeHg	4.7/3.5	4.1/4.1	4.7/4.1	5.1/3.8	4.6/4.2

Note. < LOD = below detection limit.

DISCUSSION

In this study, we found that patients who had received sc vaccine with thimerosal monthly for several years had similar levels of total mercury as the nontreated group; this indicates that mercury from thimerosal is not accumulated in adults. Further evidence for nonaccumulation is provided by the short half-lives found in our patients. The decline was faster than expected, given the available half-life estimates of 18 days in adults (Magos, 2003; Pichichero *et al.*, 2002). It is, however, in agreement with recent studies in infants receiving intramuscular injections of vaccine containing thimerosal (Pichichero *et al.*, 2008, 2009). The levels of total mercury in blood and the fraction of organic mercury were similar to those found in previous studies in Sweden (Berglund *et al.*, 2005) or in the

United States (Mahaffey *et al*., 2004, 2009), but lower than in coastal Asian countries (Kim and Lee, 2010).

Rapid Decomposition of EtHg to Inorganic Mercury

The fact that the increase of EtHg in blood 1 day after vaccine treatment was only about half of the increase of total mercury indicates that EtHg is rapidly metabolized to inorganic mercury *in vivo*. Therefore, it is likely that there is also an early rapid EtHg elimination phase, as indicated by the results for the additional two subjects. We considered the possibility that our findings were due to decomposition in the vaccine before it was administered, but speciation of mercury in vaccine specimens showed that virtually all the mercury content was EtHg. However, some decomposition may have occurred because analyses of other vaccine brands have shown that not all the mercury in these brands was EtHg (Horvat, unpublished data). We also considered the possibility that EtHg decomposed to inorganic mercury during storage, but a separate experiment with whole blood spiked with EtHg and analyzed immediately, after 6 h, and after 24 hours showed no decrease in EtHg content.

Our findings should be taken into consideration when assessing the safety of thimerosal in adults receiving a moderate dose of EtHg from vaccines or other pharmaceutical products. There is no tendency toward accumulation in blood even after years of repeated vaccine injections. In addition, as pointed out by Magos (2003), in contrast to MeHg, EtHg does not have any facilitated transport over the blood-brain barrier. Moreover, it is not favored by its size in penetrating the barrier by passive diffusion. EtHg is also less stable than the smaller MeHg, and therefore, it is metabolized faster to inorganic mercury. Owing to less penetration to the brain and a faster clearance, the toxicity of EtHg is considered lower than that of MeHg (Aschner and Ceccatelli, 2010; Magos, 2003). The present study could not, however, assess the fraction of inorganic mercury in the brain, which may have increased due to the slow half-life of this fraction. A study in monkeys indicated that whereas total mercury was cleared more rapidly from the brain after exposure to EtHg than after MeHg, this was not the case for the inorganic fraction of mercury, probably due to a faster dealkylation of EtHg compared with MeHg (Burbacher *et al*., 2005).

Because EtHg is metabolized to inorganic Hg, the kinetics of the latter compound will determine the final elimination of total mercury. The elimination of inorganic mercury from blood has been described by a two-compartment model with half-lives of about 2 and 20 days, respectively (Barregard *et al*., 1992; Sallsten *et al*., 1993; Sandborgh-Englund *et al*., 1998b). The occurrence of a fraction with a half-life on the order of weeks raises the possibility of a slight increase of inorganic Hg in blood and other tissue after repeated vaccination. This could not be shown in our patients, however. The reason for this may be that a dose of 50-μg mercury every 4 weeks (less than 2 μg/day) is small in comparison with the daily intake of MeHg from fish consumption, and in some cases of inorganic mercury vapor from dental amalgam.

Strengths and Limitations of the Study

The main strength of the present study is the unique sample of adult subjects undergoing long-term treatment with thimerosal-containing vaccines administered sc. Assessment of the toxicokinetics of EtHg requires valid analyses of low levels of total Hg, MeHg, and EtHg in blood, and we used laboratories with long-term experience in such analyses. Quality assurance, including the analyses of external reference samples, indicates good precision and accuracy. Nevertheless, small deviations from true levels are possible, as shown by the fact that in some cases (Table 4), MeHg concentrations were slightly higher than total Hg, which is not logical. It may be due to inhomogeneity of samples, and inherent uncertainty of the analytical methods, in particular, when the fraction MeHg/total Hg is close to 100%. Also, the choice of external reference material (IAEA MA-A-1/TM, Copepod Homogenate) was not optimal because the matrix is quite different from that of blood.

Due to the fast decline with a shorter half-life than expected, the timing of our blood sampling in the main group was not optimal. Nevertheless, the population-based pharmacokinetic model resulted in a relatively narrow estimate of the half-life. The point estimates from the population-based approach and the regression analyses of individual curves were similar, although the latter had a wider CI, as could be expected. The fact that pre-injection levels on Day 0 were about 10% of levels on Day 1, fits well with a half-life of about 5 days considering that the previous injection had been given 3–4 weeks earlier, and indicates that if there is an additional slow compartment, its size is insignificant.

A limitation of the study is the fact that these subjects were not healthy; they suffered from a long-term chronic fatigue syndrome. Major somatic disease had, however, been excluded, and medication was limited. Another limitation is the fact that, obviously, brain mercury levels could not be measured.

TABLE 4
Parameter Estimates and Their 95% CIs for the Final One-Compartment Model, Including Fixed Absorption to the Central Compartment (Ka = 100). BSV = Between-Subject Variability Expressed as RSD in %

Parameter	Estimate (95% CI)	BSV (95% CI)
CL/F (clearance in L/day)	34 (29–38)	
V/F (volume of distribution in L)	266 (224–308)	26 (10–36)
Half-life (days)[a]	5.6	
Additive residual error	0.02 (0.016–0.022)	

[a]Secondary parameter calculated from individual estimates (half-life = ln2 × V/CL).

Conclusions

In summary, this first study of the mercury kinetics in adults receiving a thimerosal-containing vaccine monthly for more than a year indicates that the half-life in blood is short with no accumulation of mercury.

FUNDING

This work was supported by The Sahlgrenska University Hospital (ALF 44601).

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance from professor Carl-Gerhard Gottfries and Bjorn Regland with the recruitment of patients for the study. Nusa Horvat and Martina Logar are acknowledged for their technical assistance in speciation analysis of mercury.

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