December 10, 2013

Gary S. Goldman, Ph.D.
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Dear Andrea M. Boccelli,

In a letter dated March 1, 2012 you wrote to Ms. Dannemann, Director of the National Coalition of Organized Women (NCOW), and indicated that the only agenda of the American Journal of Obstetrics and Gynecology (AJOG) is "to ensure access to information that will improve maternal outcomes and the lives of women." You continued to elaborate that the "articles by Dr. Pedro Moro were reviewed favorably by three very credible reviewers who assigned favorable scores." By contrast, "The paper by Dr. Gary Goldman was in fact reviewed by one reviewer who is the Journal's Advisor on infectious diseases and one of the most respected people in the United States in this field." The "Goldman article" scores were "so low that the chances of publication were slim, it was not sent for subsequent review."

Since that time, the Goldman manuscript was sent to another peer-reviewed journal, accepted, and now appears as an Open Access article with the National Library of Medicine (on www.Pubmed.gov).[1] Please see the attached graph showing 23 consecutive influenza seasons (from 1990/91 to 2012/13) showing a spike that occurred during the 2009/10 two-dose pandemic (H1N1)/Seasonal (TIV) vaccine season (based on reports accessed on 12/09/13 in VAERS database). After 20 years of just 0 to 6 reports annually, suddenly there was a spike of 158 fetal deaths occurring in one year! Yet, your editors agreed with Dr. Moro's conclusion: "No unusual pattern!" Interestingly, but not unexpected, VAERS case reports of Guillain-Barré also spiked during that same pandemic season—an illness associated with thimerosal-containing vaccines, since the mercury content creates a potential for neurological and auto-immune disorders.

A study[2] by Brown and Austin using a different methodology, but supporting the same conclusion as Goldman, found that "Babies in utero are particularly at risk of higher Hg exposure than adults (on a dose/weight basis through maternal Hg transfer via the placenta), and are more susceptible to adverse effects from mercury and its biologically active compounds." The study, concluded, in part, "Data demonstrated that Hg exposures, particularly during the first trimester of pregnancy, at well-established dose/weight ratios produced severe damage to humans including death."

It is unfortunate that the reviewer(s) did not take the time to request the actual survey data obtained by NCOW. By suggesting that vaccination during pregnancy is safe (in the first trimester), this contributed to poor maternal outcomes and the unnecessary deaths of many fetuses. Here are three representative samples taken from those contained in the NCOW survey:

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[1] Goldman GS. Comparison of VAERS fetal-loss reports during three consecutive influenza seasons: Was there a synergistic fetal toxicity associated with the two-vaccine 2009/10 season? *Hum Exp Toxicol* 2013 May;32(5):464-475. http://het.sagepub.com/content/32/5/464.long

[2] Brown IA, Austin DW. Maternal transfer of mercury to the developing embryo/fetus: is there a safe level? *Toxicological & Environmental Chemistry* 2012 Sept;94(8):1610-1627.

**Case 1**: The mother reports: I had an ultrasound at 14 weeks on October 29, 2009 reporting all was well with myself and the baby. On that same day I received both the flu shot and the H1N1 shot. Two weeks later I went for a routine check-up and the doctor could not find the heartbeat. I had another ultrasound showing no heartbeat or activity. After reviewing the scan my OB said the baby died 12 days prior which would have been two days after the influenza shots were administered. I had a DNC and asked my doctor if that shot could've played a role in this "miscarriage." He said it was "impossible."

**Case 2:** A women received an [H1N1] vaccine and within hours contracted pain and birthed a still born baby the next morning. She went to her physician to obtain the H1N1 shot and that same night started experiencing cramps. She noticed that the baby started kicking more than usual. She didn't think anything of it at first, just thought that she was getting sick or something; but the next morning she still had cramps so she went to the hospital and had a stillborn (natural birth) at 17 weeks old and 7 inches long. The mother and child were healthy with no complications prior to the vaccine.

**Case 3:** On October 29, 2009, my baby was doing well at 13 weeks, 6 days. The nurse said I NEEDED to get the vaccines for the safety of my baby. I got both influenza shots. Two weeks later I had another appointment and my baby was dead. According to the ultrasound the baby had died 10-12 days prior…that is 2-4 days after the influenza shots.

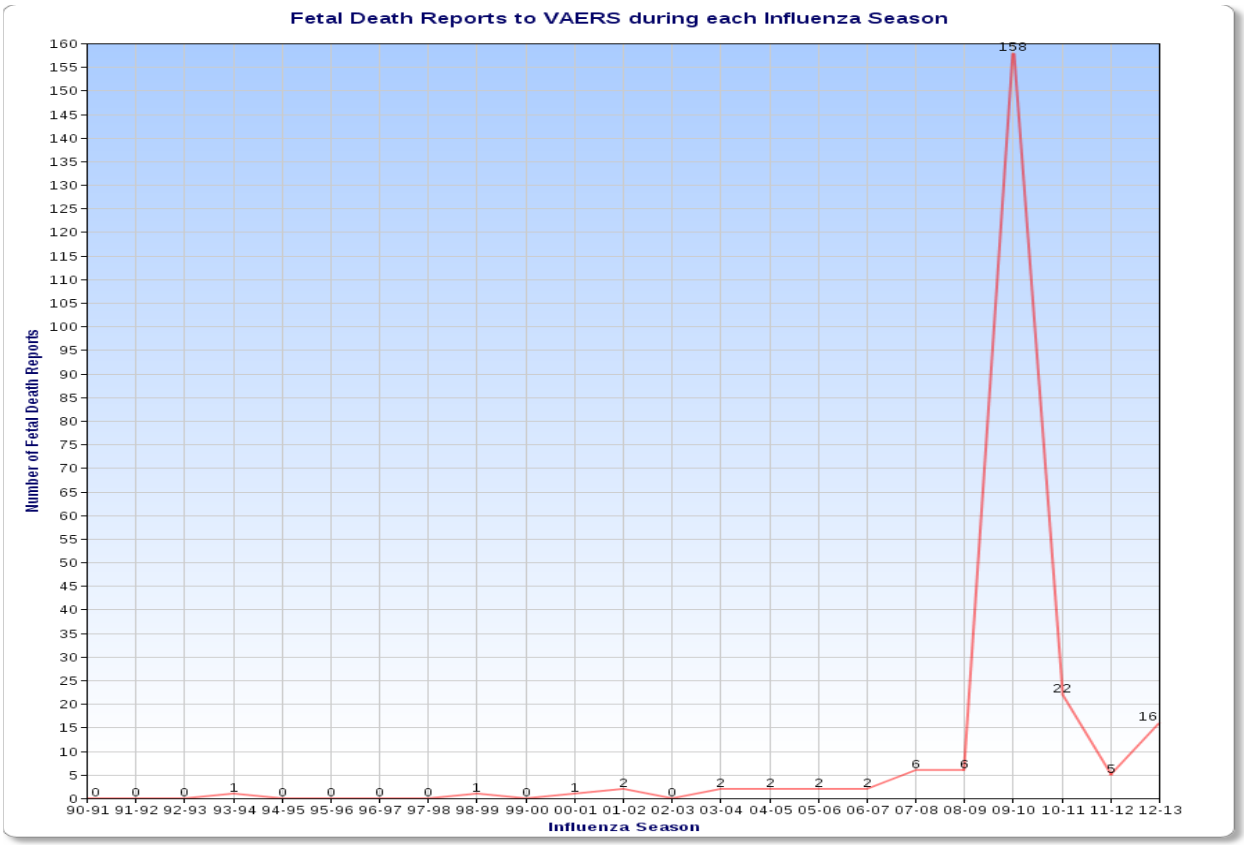
NCOW has another 50 similar case reports.

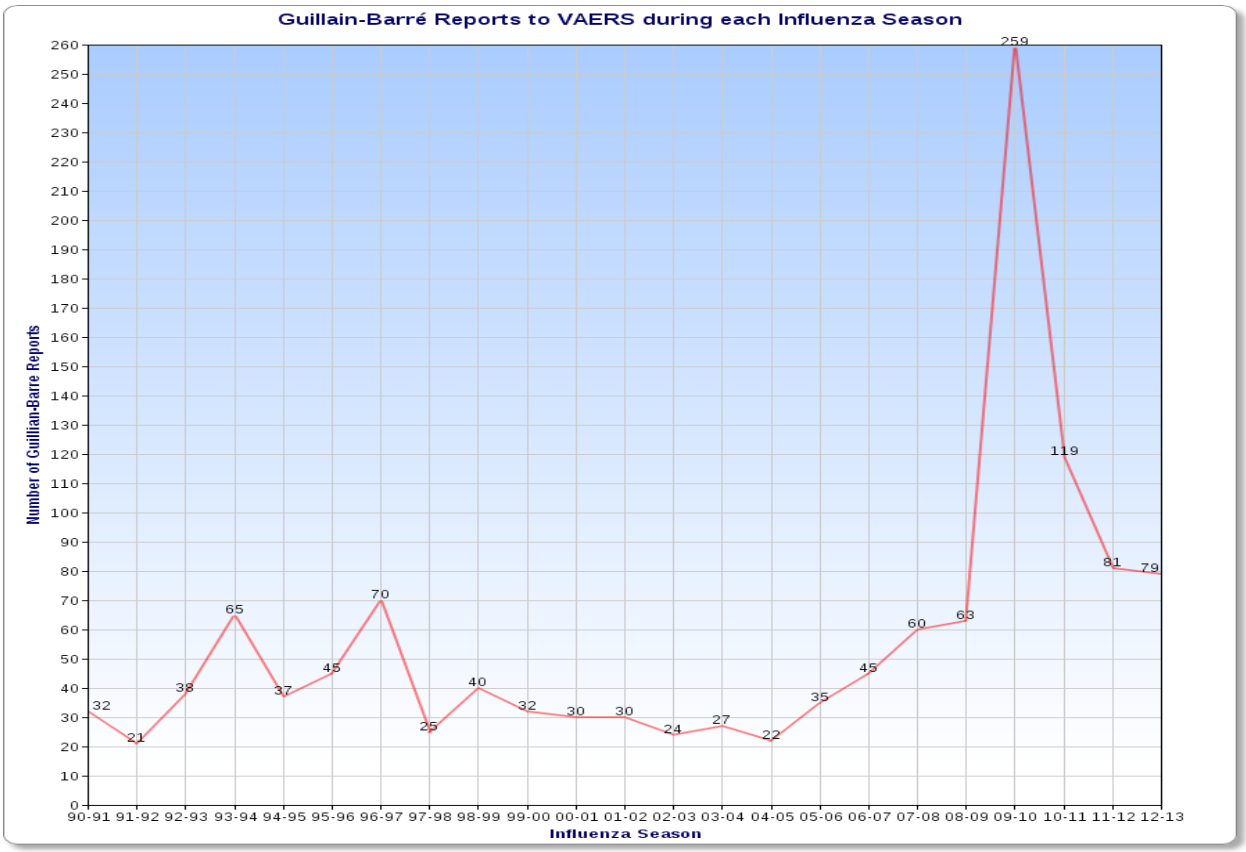
In conclusion, pregnant women who experienced fetal loss in close proximity to influenza vaccination, were misled by their physicians who encouraged them with patently false information that the influenza vaccine was "perfectly safe" (especially during the first trimester of pregnancy) or that "only 1 in a million pregnant women" experience fetal loss based on ascertainment unadjusted fetal death reports to VAERS (largely among woman vaccinated in the 2nd and 3rd trimesters of pregnancy). Studies such as Moro et al, published by AJOG and supported by the CDC, misled the medical community to assess the pandemic influenza vaccine as safe. In reality, this false propaganda instilled distrust and outrage among the many women experiencing fetal loss. Presently, it is now possible to locate unbiased research (Goldman's study and the Brown and Austin study) that is reasonable and explains the biological mechanism causing the large spike in fetal death reports that occurred during the 2009/10 pandemic influenza season that resulted in an estimated 1 fetal death in 1,695 pregnant woman vaccinated. AJOG's failure to rectify the falsehoods concerning vaccination during pregnancy has contributed to additional dangerous proposals to vaccinate pregnant women—leading to future generations of damaged children.

Sincerely,

Gary S. Goldman, Ph.D.
Computer Scientist

Cc: Moon H. Kim, MD, Co-Editor, AJOG
Thomas J. Garite, MD, Co-Editor, AJOG
Pamela Poppalardo, Publishing Director, Elsevier
Paul Taylor, Senior Publishing Editor, Life Sciences, Elsevier


Fetal Death Reports to VAERS during each Influenza Season
Number of Fetal Death Reports
Influenza Season
90-91 91-92 92-93 93-94 94-95 95-96 96-97 97-98 98-99 99-00 00-01 01-02 02-03 03-04 04-05 05-06 06-07 07-08 08-09 09-10 10-11 11-12 12-13
0 0 0 1 0 0 0 0 1 0 1 2 0 2 2 2 2 6 6 158 22 5 16


Guillain-Barré Reports to VAERS during each Influenza Season
Number of Guillian-Barre Reports
Influenza Season
90-91 91-92 92-93 93-94 94-95 95-96 96-97 97-98 98-99 99-00 00-01 01-02 02-03 03-04 04-05 05-06 06-07 07-08 08-09 09-10 10-11 11-12 12-13
32 21 38 65 37 45 70 25 40 32 30 30 24 27 22 35 45 60 63 259 119 81 79

# Comparison of VAERS fetal-loss reports during three consecutive influenza seasons: Was there a synergistic fetal toxicity associated with the two-vaccine 2009/2010 season?

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# Comparison of VAERS fetal-loss reports during three consecutive influenza seasons: Was there a synergistic fetal toxicity associated with the two-vaccine 2009/2010 season?

**GS Goldman**

## Abstract

The aim of this study was to compare the number of inactivated-influenza vaccine–related spontaneous abortion and stillbirth (SB) reports in the Vaccine Adverse Event Reporting System (VAERS) database during three consecutive flu seasons beginning 2008/2009 and assess the relative fetal death reports associated with the two-vaccine 2009/2010 season. The VAERS database was searched for reports of fetal demise following administration of the influenza vaccine/vaccines to pregnant women. Utilization of an independent surveillance survey and VAERS, two-source capture–recapture analysis estimated the reporting completeness in the 2009/2010 flu season. Capture–recapture demonstrated that the VAERS database captured about 13.2% of the total 1321 (95% confidence interval (CI): 815–2795) estimated reports, yielding an ascertainment-corrected rate of 590 fetal-loss reports per million pregnant women vaccinated (or 1 per 1695). The unadjusted fetal-loss report rates for the three consecutive influenza seasons beginning 2008/2009 were 6.8 (95% CI: 0.1–13.1), 77.8 (95% CI: 66.3–89.4), and 12.6 (95% CI: 7.2–18.0) cases per million pregnant women vaccinated, respectively. The observed reporting bias was too low to explain the magnitude increase in fetal-demise reporting rates in the VAERS database relative to the reported annual trends. Thus, a synergistic fetal toxicity likely resulted from the administration of both the pandemic (A-H1N1) and seasonal influenza vaccines during the 2009/2010 season.

## Keywords

Human toxicology, immunization, influenza vaccine, spontaneous abortion, stillbirth, Thimerosal

## Introduction

Since 1997, the Advisory Committee on Immunization Practices (ACIP) has recommended the routine vaccination of pregnant women with trivalent inactivated influenza vaccine (TIV) after the first trimester of pregnancy. This recommendation was expanded in 2004 to include all trimesters of pregnancy.[1]

All previously published studies of pregnant women who were administered with TIV have reported this vaccine as safe during all stages of pregnancy.[2–4] Christian et al. explained the reason for this record of safety: 'The inflammatory response elicited by TIV is substantially milder and more transient than seen in infectious illness.'[5]

Two frequently cited peer-reviewed reports on the safety of influenza vaccination during pregnancy did not reveal any adverse outcomes among 56 women[6] and 180 women.[7] Both these studies, which used 'no Thimerosal' influenza vaccines, had insufficient statistical power to adequately detect and assess complications due to the small sample size. A third follow-up safety study (conducted among 2291

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pregnant women) cited by ACIP did not find increased childhood mortality associated with exposure to TIV in pregnancy.[3] However, fetal losses were not included in the analysis.

Based on the prior record of safety of TIV and the fact that the pandemic A-H1N1 vaccine shared the same licensure and manufacturing processes as the seasonal TIV, the ACIP recommended for the 2009/2010 influenza season that pregnant women receive the pandemic inactivated A-H1N1-virus vaccine in addition to the seasonal TIV (both produced by five approved vaccine manufacturers) during any trimester of pregnancy.

However, the safety and effectiveness of the pandemic (monovalent influenza) A-H1N1 vaccine had neither been previously established in pregnant women nor the combination of two different influenza vaccines ever tested in pregnant women. The A-H1N1 vaccine inserts from the various manufacturers contained this caution: "It is also not known whether these vaccines can cause fetal harm when administered to pregnant women or can affect reproduction capacity."

In October 2010, Moro et al. summarized that during 19 influenza seasons (1990/1991 through 2008/2009), there were a total of 17 spontaneous abortion (SAB) and 6 stillbirth (SB) reports following TIV in the Vaccine Adverse Event Reporting System (VAERS) database for an overall mean of 1.21 (23/19) fetal loss reports per year. This study's stated rate of fetal-loss reporting was 1.9 per 1 million (or 23/11,800,000) vaccinated pregnant women.[8]

In a second study published 8 months following the first, Moro et al. noted 121 SAB and 19 SB reports or a total of 140 fetal-loss reports to VAERS during the first 5 months of the 2009/2010 influenza season.[9] This equates to greater than 57 reports per million (>140/2,437,113) vaccinated pregnant women. The ratio of the 140 fetal-loss reports during the incomplete 2009/2010 season to the 1.21 reports/year representing the mean of the 19 prior seasons, yields a 116-fold (140/1.21) increase in fetal-loss reports (SAB and SB) in the VAERS database. Moro et al. attributed this dramatic increase, in part, to reporting bias, citing a "Weber-like effect."[9] The Weber effect is a temporal reporting pattern whereby the number of reported adverse events (AEs) for a new drug increases during the first 2 years of marketing and then subsequently declines, presumably reflecting decreased enthusiasm for reporting as AEs become well known.

Despite the statistically significant rate ratio (RR) of 29.4 (95% confidence interval (CI): 19.0–45.8) for 2009/2010 fetal-loss report rate (57 reports/1 million) to the mean rate of 1.9 reports/1 million (over the previous 19 influenza seasons), the second Moro et al. study concluded, " . . . H1N1 vaccination in pregnant women did not identify any concerning patterns of maternal or fetal outcomes."[9]

Was the increase in fetal-loss outcomes during the two-vaccine 2009/2010 influenza season merely the result of reporting bias or was there a synergistic toxicity associated with the two-dose 2009/2010 influenza season?

## Methodology

Fetal-loss reports in the VAERS database for the two-vaccine 2009/2010 influenza season were compared with those reports from the immediately prior (2008/2009) and subsequent (2010/2011) single-vaccine seasons. The incidence of fetal-loss reports per 1 million pregnant women vaccinated was estimated for each season with 95% CIs computed based on the Poisson distribution. The RR of the fetal-loss report rate and CIs for the two-dose 2009/2010 influenza season to the fetal-loss report rate in the adjacent seasons were similarly estimated.

### *Independent survey of fetal loss related to 2009/2010 A-H1N1 vaccine*

An independent survey was conducted by the National Coalition of Organized Women (NCOW) via the Internet to serve as a second surveillance source for pregnant women suffering A-H1N1 fetal loss during the two-vaccine 2009/2010 influenza season. Eileen Dannemann, director of NCOW, oversaw this study and the data collected are summarized in the Results section. In response to a public service announcement delivered via several websites on the Internet, respondents contacted one of two study coordinators via phone or e-mail address. The respondents provided relevant details including (a) type of influenza vaccine received, (b) date of vaccination, (c) type of vaccine, (d) date of onset of symptom/symptoms, (e) date of SAB or miscarriage, (f) geographic location, (g) whether or not the AE was reported to VAERS, and (h) other miscellaneous comments.

Capture–recapture analysis was used to determine the reporting completeness of fetal-loss reporting using two ascertainment sources: (1) the NCOW survey and (2) the VAERS database. Ascertainment-corrected fetal-loss report rates are computed by applying two-source capture–recapture methods to

**Table 1.** Comparison of fetal losses reported to VAERS for three consecutive influenza seasons, 2008/2009, 2009/2010, and 2010/2011.

| | TIV 2008/2009 season | Additional monovalent A-H1N1 vaccine 2009/2010 season | TIV 2010/2011 season[a] |
|---|---|---|---|
| A. No. of pregnancies[b] | 5,200,000 | 5,200,000 | 5,200,000 |
| B. Approx. percentage vaccinated | 11.3%[17] | 43%[c] | 32%[18] |
| C. No. of pregnant women vaccinated (A•B) | 587,600 | 2,236,000 | 1,664,000 |
| D. No. of fetal losses from VAERS | 4 | 174[d] (152 A-H1N1 only + 18 A-H1N1 and TIV + 4 TIV only) | 21 |
| E. Incidence of reported fetal losses per 1 million pregnant women vaccinated (D/C) | 6.8 (95% CI: 0.1–13.1)[e] | 77.8 (95% CI: 66.3–89.4)[f] | 12.6 (95% CI: 7.2–18.0) |
| F. RR of 2009/2010 season to adjacent flu season | 11.4 (95% CI: 4.2–30.8) | | 6.2 (95% CI: 3.9–9.7) |

VAERS: Vaccine Adverse Event Reporting System; RR: rate ratio; CI: confidence interval; TIV: trivalent inactivated influenza vaccine.
[a]The 2009 A-H1N1 strain, along with two seasonal strains (A/Perth/16/2009 (H3N2)-like, and B/Brisbane/60/2008-like antigens) comprised the seasonal TIV in 2010/11, obviating the need for two separate vaccines.
[b]Number of annual pregnancies minus number of elective annual abortions = 6,408,000–1,210,000 is about 5,200,000.[16]
[c]National Health Family Survey (NHFS) reports 43% of pregnant women received the 2009 H1N1 vaccine (unpublished data from the Centers for Disease Control and Prevention (CDC)). This same figure is cited in the Moro et al. manuscript.[9]
[d]Shimabukuro reported 170 cases from VAERS, but did not include the entire influenza season. Shimabukuro T. Influenza Vaccine Safety Monitoring Update: Advisory Committee on Immunization Practices. Immunization Safety Office at the Division of Healthcare Quality Promotion, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC). Presented on October 28, 2010. Slide #20 reports 149 SAB and 21 SB = 170 (unpublished CDC data).
[e]Moro et al. determined 5.5 million, but the denominator of the rate calculation included elected abortions.[9]
[f]Moro et al. determined 57.0 per million; however, the numerator of the rate calculation included case reports for only a partial influenza season and the denominator of the rate calculation included annual elective abortions.[8]

the number of reported fetal-loss incidents.[10–12] The estimator $N^*$ of the total fetal-loss incidents is given by $N^* = [(b + 1)(c + 1)/(a + 1)] - 1$, where $a$ is the number of fetal-loss incidents reported by both ascertainment sources, and $b$ and $c$ denote the number of fetal-loss incidents reported by the NCOW survey and VAERS ascertainment sources, respectively. When $a > 6$, there is 95% confidence that the theoretical bias is negligible; however, this does not account for any bias that might result from source dependencies or heterogeneity of the population within an ascertainment source.[13,14]

Since the distribution of the capture–recapture estimate is skewed in practice, to avoid misleading results associated with standard error estimates of result uncertainty, goodness-of-fit–based CIs were utilized.[15]

## *Number of annual pregnancies and percentage of vaccinated pregnant women*

The number of pregnancies given in Table 1 for each of the three consecutive influenza seasons was derived from Ventura et al. and was presumed to remain relatively constant at about 5,200,000.[16] While this same reference was used by Moro et al.,[8] his figure of 6,408,000 pregnancies per year included about 1,210,000 elective annual abortions.

The 11.3% (for 2008/2009) and 43% (for 2009/2010) uptake percentages for pregnant women vaccinated shown in Table 1 were taken from the National Health Interview Survey (NHIS)[17] and an unpublished National Health Family Survey (NHFS), respectively. These percentages are cited by Moro et al.[8,9] A recent 2012 Centers for Disease Control and Prevention (CDC) report confirms the 43% uptake percentage during the 2009/2010 influenza season by reporting coverage among pregnant women as 47.1% for seasonal and 40.4% for A-H1N1 vaccine (mean 43.75%).[18] The 32% uptake percentage for pregnant women vaccinated in the 2010/2011 influenza season was reported by the CDC (and does not include the percentage of women vaccinated prior to or after pregnancy).[19]

## *Qualitative and quantitative assessment of trends in fetal-loss reports*

The VAERS reports were examined for evidence of temporal or location clustering. In addition, the rate of fetal-loss reported per million population by state was assessed to determine any trends in reporting

**Table 2.** Comparison of mean time from vaccination to fetal demise and mean gestational age at fetal demise for VAERS reports and the NCOW survey, 2009/2010 influenza season.

| | VAERS reports, $n = 174$ | | NCOW survey, $n = 67$ | |
|---|---|---|---|---|
| Description | Mean | Number of respondents | Mean | Number of respondents |
| Elapsed time from vaccination to fetal demise | 11.8 days (range 0–66) | 166 (95%) | 7.6 days (range 1–75) | 57 (85%) |
| Gestational age at fetal demise | 13.4 weeks (range 4–39) | 113 (65%) | 12.8 weeks (range 1–39) | 56 (84%) |

VAERS: Vaccine Adverse Event Reporting System; NCOW: National Coalition of Organized Women.

rates by state adjusted by population for the 2009/2010 influenza season. Also, fetal-loss reports due to seasonal TIV vaccine and percentages of female reports to total VAERS reports were compared for each of the three consecutive influenza seasons as well as two prior seasons in an attempt to discern and quantify any historical reporting trends or anomalies for the seasonal influenza vaccine adverse reports.

### *Quantitative estimate of factor of increased reporting potentially due to Weber-like effect*

If no Weber-like effect existed, that is there was no increased or enhanced AE reporting associated with the newly marketed pandemic A-H1N1 vaccine during the 2009/2010 influenza season, we would expect the number of VAERS reports resulting from administration of the seasonal TIV and pandemic A-H1N1 vaccine to be approximately equal. In other words, the ratio of AE reports for A-H1N1 to seasonal TIV would be 1:1. Any increase in the number of VAERS reports associated with A-H1N1 over the seasonal TIV would yield a ratio or factor greater than one – representing the possible effect of a Weber-like reporting bias. Such a Weber or Weber-like reporting bias would expect to be generally distributed among all VAERS reports – not only those describing pregnant women experiencing temporally related fetal loss but also those describing other AEs among nonpregnant females and males. VAERS reports of anaphylactic shock occurring the same day of administration of influenza vaccine served as a control to test the potential Weber-like reporting bias.

## Results

### *VAERS reports*

Although there was an approximate fourfold (43%/11.3%) increase in the percentage of pregnant women vaccinated in 2009/2010 compared with 2008/2009, there was a 43.5-fold increase in fetal-loss reports – from 4 in 2008/2009 to 174 in 2009/2010. The report RR of 11.4 (95% CI: 4.2–30.8) of the 2009/2010 rate of 77.8 fetal-loss reports/1 million pregnant women vaccinated to the 2008/2009 report rate of 6.8 fetal-loss reports/1 million pregnant women vaccinated is statistically significant (Table 1).

### *Summary of the independent NCOW survey*

The NCOW survey of fetal losses had a total of 72 respondents, 5 (7%) of which were excluded for the following reasons: 1 (1.4%) report of indirect H1N1 transmission to a child, which caused infection and miscarriage in a pregnant woman; 3 (4.2%) reports outside the United States (US); and 1 (1.4%) report with no adverse outcome. Of the 67 remaining instances, 62 (92.5%) and 5 (7.5%) reports of fetal demise were following A-H1N1 and seasonal TIV, respectively,

A comparison of the mean elapsed time from administration of influenza vaccine to fetal demise and mean gestational age at time of fetal demise is given in Table 2 for those of the 174 VAERS cases and 67 NCOW survey respondents that provided sufficient information. There was no statistically significant difference in the distribution of fetal loss by trimester between the VAERS reports and NCOW survey respondents ($\chi^2 = 1.69$; $p = 0.43$; Table 3).

### *Ascertainment-corrected reports for the two-vaccine 2009/2010 influenza season*

Applying capture–recapture using 67 case reports from the NCOW survey, 174 case reports from VAERS, and 8 cases shared by both ascertainment sources, yields an overall reporting completeness for the two ascertainment sources of 17.6% based on an estimated ascertainment-corrected 1321 (95% CI: 815–2795) fetal-loss reports. Thus, the 174 VAERS fetal loss case reports represent 13.2% (174/1321) of

**Table 3.** Comparison of trimester of fetal demise for VAERS reports and the NCOW survey, 2009/2010 influenza season.

| Trimester | | VAERS reports, *n* (%) | NCOW reports, *n* (%) |
|---|---|---|---|
| First | (0–13 weeks) | 74 (65.5) | 40 (71.4) |
| Second | (14–27 weeks) | 26 (23.0) | 13 (23.2) |
| Third | (>27 weeks) | 13 (11.5) | 3 (5.4) |
| Total reports | | 113[a] (100.0) | 56[b] (100.0) |

VAERS: Vaccine Adverse Event Reporting System; NCOW: National Coalition of Organized Women.
[a]113 (65%) of 174 total reports contained gestational date information; 62 (35%) did not.
[b]56 (84%) of 67 total reports contained gestational information; 11 (16%) did not.

the total estimated fetal loss reports in the US population. The ascertainment-corrected rate of 590 fetal-loss reports per 1 million pregnant women vaccinated (or 1 per 1695) is 7.6-fold higher than the uncorrected VAERS rate of 77.8 (95% CI: 66.3–89.4).

## *Qualitative and quantitative assessments of trends in fetal-loss reports*

Through an inspection of the lot numbers and demographics of the individual 174 fetal-loss reports in VAERS for the two-dose 2009/2010 influenza season, there appeared no clustering of the reports. Only a few 'states' provide evidence of increased fetal-loss reports during that season. The three 'states' with the highest reporting rates were District of Columbia (five cases), Vermont (three cases), and Montana (three cases), with 8.3, 3.2, and 3.0 fetal-loss reports per million population, respectively. The three states with the lowest fetal-loss report rates were Texas (five cases), New York (three cases), and New Jersey (one case) with rates of 0.198, 0.154, and 0.114 reports of fetal loss per million population, respectively. The highest number of fetal-loss reports, 20, was from California, yielding a rate of 0.536 fetal-loss reports per million population.

Presuming no significant uptake variability among the states based on the agreement of CDC's 2010 ten-state estimate (46.6%)[20] and Moro's 2011 reporting for the entire country (43%),[9] the state-to-state reporting of fetal loss following A-H1N1 vaccination appears highly variable (Table 4). In fact, nine states (Connecticut, Delaware, Idaho, Louisiana, New Hampshire, New Mexico, North Dakota, Oklahoma, and Wyoming) representing a combined population of 20.5 million reported no influenza-vaccine–related fetal losses. Eleven states reported only one case: Alabama, Alaska, Hawaii, Mississippi, New Jersey, Puerto Rico, Rhode Island, South Carolina, South Dakota, Utah, and West Virginia. Ten states reported two cases: Arizona, Arkansas, Iowa, Kentucky, Maine, Minnesota, Nebraska, Oregon, Tennessee, and Vermont (Table 4).

The ages of the women in the fetal-loss reports indicated a reporting bias associated with older pregnant women (mean age 32 years) as has been previously observed.[8,9]

The percentage of females filing VAERS AE reports in 2009/2010 was similar to that of the previous 2008/2009 season, with 63.9% (12,061 reports/18,866 total reports) and 61.8% (3529 reports/5707 total reports), respectively. The RR of 1.03 (95% CI: 0.996–1.07) was not statistically significant. In the 2010/2011 season, the reporting percentage for females was 66.4% (6372 female reports/9602 total reports). Despite the increase in females filing AE reports, there were no unusual trends in the percentage of female adverse reports over the three consecutive influenza seasons, 2008/2009 through 2010/2011 (Table 5).

Inspection of all influenza reports of males and females (shown in bold in Table 5, column 3) associated with the administration of all influenza vaccines over five consecutive influenza seasons reveals what appears to be an underlying linear increase for seasonal influenza-vaccine–related adverse reports from 3123 reports in 2006/2007 to 9602 in 2010/2011 having a constant increase of $1642 \pm 109$ reports/year ($r^2 = 0.99$; Table 5). Similarly, restricting the reports to females, there again appears to be a linear increase from 2048 reports in 2006/2007 to 6372 in 2010/2011, having a constant increase in $1086 \pm 111$ reports/year ($r^2 = 0.97$; Table 5, column 4).

## *Quantitative estimate of increased AE reporting attributed to a Weber-like effect*

The factor of increased reporting that might be potentially due to a Weber-like effect in the 2009/2010 influenza season is quantified by computing the ratio of 7734 females reporting AEs associated with A-H1N1 vaccine to the 4863 females reporting AEs associated with seasonal TIV (Table 5), yielding a 1.6-fold increase in the A-H1N1 AE reports. Based on this potential Weber-like effect, given 22 reports of fetal loss associated with TIV, we would have expected approximately 35 fetal-loss reports (actually

**Table 4.** Rate of fetal-loss reports by state for two-vaccine 2009/2010 influenza season.

| State | Population (in millions)[a] | No. of fetal-loss reports | Rate (fetal-loss reports/million population) |
|---|---|---|---|
| Alabama | 4.803 | 1 | 0.208 |
| Alaska | 0.721 | 1 | 1.387 |
| Arizona | 6.413 | 2 | 0.312 |
| Arkansas | 2.926 | 2 | 0.684 |
| California | 37.342 | 20 | 0.536 |
| Colorado | 4.939 | 5 | 1.012 |
| District of Columbia | 0.602 | 5 | 8.306 |
| Florida | 18.901 | 7 | 0.370 |
| Georgia | 9.727 | 5 | 0.514 |
| Hawaii | 1.367 | 1 | 0.732 |
| Illinois | 12.864 | 6 | 0.466 |
| Indiana | 6.501 | 6 | 0.923 |
| Iowa | 3.054 | 2 | 0.655 |
| Kansas | 2.864 | 4 | 1.397 |
| Kentucky | 4.351 | 2 | 0.460 |
| Maine | 1.333 | 2 | 1.500 |
| Maryland | 5.790 | 6 | 1.036 |
| Massachusetts | 6.560 | 13 | 1.982 |
| Michigan | 9.912 | 8 | 0.807 |
| Minnesota | 5.315 | 2 | 0.376 |
| Mississippi | 2.978 | 1 | 0.336 |
| Missouri | 6.011 | 5 | 0.832 |
| Montana | 0.994 | 3 | 3.018 |
| Nebraska | 1.832 | 2 | 1.092 |
| Nevada | 2.709 | 3 | 0.738 |
| New Jersey | 8.807 | 1 | 0.114 |
| New York | 19.421 | 3 | 0.154 |
| North Carolina | 9.566 | 8 | 0.836 |
| Ohio | 11.568 | 5 | 0.432 |
| Oregon | 3.849 | 2 | 0.520 |
| Pennsylvania | 12.735 | 6 | 0.471 |
| Puerto Rico | 3.989 | 1 | 0.251 |
| Rhode Island | 1.055 | 1 | 0.948 |
| South Carolina | 4.646 | 1 | 0.215 |
| South Dakota | 0.820 | 1 | 1.220 |
| Tennessee | 6.375 | 2 | 0.314 |
| Texas | 25.268 | 5 | 0.198 |
| Utah | 2.771 | 1 | 0.361 |
| Vermont | 0.630 | 2 | 3.175 |
| Virginia | 8.038 | 6 | 0.746 |
| Washington | 6.753 | 5 | 0.740 |
| West Virginia | 1.860 | 1 | 0.538 |
| Wisconsin | 5.698 | 6 | 1.053 |

[a]http://www.worldatlas.com/aatlas/populations/usapoptable.htm for the States; DC and Puerto Rico from www.cia.gov.
[b]Nine states reported no cases: Connecticut, Delaware, Idaho, Louisiana, New Hampshire, New Mexico, North Dakota, Oklahoma, and Wyoming.

$1.6 \times 22$ TIV $= 35.2$ reports) attributable to a 'Weber-like' effect associated with the A-H1N1 vaccines. Thus, the magnitude of the observed possible Weber-like effect explains neither the 170 fetal-loss reports in VAERS nor the nearly eightfold increase (170 A-H1N1 fetal-loss reports/22 TIV fetal-loss reports) that was found.

## *Use of an independent control AE group to isolate and independently estimate the potential size of a true Weber-like effect*

To further investigate the presence of a Weber-like effect, VAERS reports were searched for an obvious AE, anaphylactic shock (including anaphylactic and anaphylactoid reaction and shock), occurring on the day of administration of influenza vaccine – usually shortly after the dose is administered. A review of the VAERS database found 20 and 22 such reports during the single-dose 2008/2009 and 2010/2011 influenza seasons, respectively; whereas, 46 reports were found during the two-vaccine 2009/2010 season. Presuming no Weber effect bias and relatively equal uptake of the pandemic A-H1N1 vaccine and seasonal TIV in the 2009/2010 flu season, about 21 AE reports $((20 + 22)/2)$ should have been expected for each of the two (seasonal and pandemic) vaccination programs or a total of about 42 reports for the 2009/2010 influenza season. The difference of four reports $(46 - 42 = 4)$ between the actual and expected anaphylactic shock reports indicates a potential Weber-like, increase-in-reporting bias of less than 10% associated with the A-H1N1 vaccination program.

## *VAERS reports of fetal demise following administration of A-H1N1 vaccine and TIV*

A recently published CDC morbidity and mortality weekly report[18] indicated that 28.5% of pregnant women were administered with both A-H1N1 vaccine and TIV. Since approximately 43% of pregnant women received at least one influenza vaccine (Table 1), the majority of those vaccinated – 66% (28.5/43%) – received a dose of both types of inactivated influenza vaccines.

Since the TIV became available early in the 2009/2010 influenza season, it was initially administered first followed then by the subsequent administration of a pandemic A-H1N1 vaccine when those inactivated 2009 A-H1N1 influenza vaccines became available. This probably partially accounts for the high

**Table 5.** A comparison of United States VAERS reports during five consecutive influenza seasons, 2006/2006 through 2010/2011.

| Season and vaccine (July–June) | All VAERS reports | All influenza reports[a] | VAERS female influenza reports[b] | % of VAERS female influenza reports (100•B/A) | No. of fetal-loss reports to VAERS |
|---|---|---|---|---|---|
| 2006/2007 TIV | 20,502 | **3123** | **2048** | 65.6 | –[c] |
| 2007/2008 TIV | 26,117 | **4205** | **2654** | 63.1 | 4 |
| 2008/2009 TIV | 22,579 | **5707** | **3529** | 61.8 | 5[d] |
| 2009/2010 A-H1N1 | 32,877 | 12,300[e] | 7734[f] | 62.9 | 170[g] |
| 2009/2010 TIV | | **7671**[e] | **4863**[f] | 63.4 | 22[g] |
| 2010/2011 TIV | 23,416 | **9602** | **6372** | 66.4 | 21 |

Note: The bold figures show existing trends for the Trivalent Influenza Vaccine (TIV) over several years and should not be confused with the figures for the special 2-dose 2009/10 Influenza season which includes the unique, separate dose of A-H1N1. Also, linear regression analysis was run on the figures shown in bold to show statistical correlation and annual existing trends in TIV reports. VAERS: Vaccine Adverse Event Reporting System; TIV: trivalent inactivated influenza vaccine.

[a]All influenza adverse reports for TIV by year demonstrate linear correlation (figures in blue), $r^2 = 0.99$.

[b]Female influenza adverse reports for TIV by year demonstrate a linear correlation (figures in blue), $r^2 = 0.97$.

[c]Not Reviewed.

[d]Includes one live virus–related fetal death.

[e]For 2009/2010, the combined A-H1N1 and TIV influenza reports total 19,971; however, 1105 duplicate reports must be deducted due to patients reporting receipt of both TIV and A-H1N1, yielding 18,866.

[f]For 2009/2010, the combined A-H1N1 and TIV female influenza reports total 12,597; however, 536 duplicate reports must similarly be deducted, yielding 12,061.

[g]Figure includes 18 VAERS fetal-loss reports specifying receipt of both A-H1N1 vaccine and TIV.

percentage – 87.4% (152/174) – of VAERS reports that only reflect a SAB or SB after A-H1N1 inoculation and low percentage of 2.3% (4/174) of VAERS reports that reflect an incident of fetal demise after only a TIV inoculation.

## Discussion

Capture–recapture estimates can lead to inaccurate and sometimes misleading results if the underlying assumptions are not met.[21] In epidemiological investigations, ascertainment sources often display dependence and heterogeneity of capture probabilities.[22] The major question individuals ask regarding capture–recapture is 'Will capture–recapture give you the truth?' That is, will it provide an extremely accurate estimate of the fetal loss incidence rates? Simply answered, no – it will not. When capture–recapture techniques are not utilized, the estimates presented in most epidemiologic studies are extremely poor, missing 10–90% of the cases, with a high degree of variation.[10,11,23,24] Thus, often the disease incidence that is reported simply reflects the incomplete case ascertainment of the study and not the true incidence of the disease in the population. Therefore, the options are (a) not to use capture–recapture and report fetal loss from which the incidence rates are almost uninterpretable since such rates merely reflect the level of case ascertainment, (b) try to count every case of fetal loss, which is horrendously expensive and slow, or (c) utilize capture–recapture, which, depending on the degree to which the assumptions are satisfied, as a compromise, can be a reasonably accurate, quick, and inexpensive approach.

The estimated 13.2% reporting completeness of the VAERS fetal-loss case reporting is suggestive of a low fetal-loss reporting rate during the 2009/2010 influenza season rather than a high reporting completeness of AEs – such as might be caused by a Weber-like effect. Furthermore, the general level of reporting of fetal-loss reports was variable when adjusted by state population with 56% of states reporting 0–2 cases (mean 1 report/state) and 44% reporting >2 cases (mean 5.4 reports/state) with no clustering of reports. Moreover, the percentage of influenza vaccine–related reports to VAERS for females was similar for each of the consecutive influenza seasons. Finally, the fetal loss rate dramatically declined from 77.8 fetal-loss reports per million women vaccinated in the two-vaccine 2009/2010 season to 12.6 fetal-loss reports per million vaccinated in the following single-vaccine 2010/

2011 influenza season. All these results argue against a significant fetal-loss reporting bias associated with the two-vaccine 2009/2010 season.

Based on respondents' comments to the NCOW survey in the 2009/2010 season, it is likely that the ascertainment-corrected rate of 535 fetal losses per million pregnant women vaccinated represents a significant underestimate during the two-vaccine 2009/2010 influenza season since health care professionals explained to patients 'the benefits of influenza vaccination outweighed the risks.' Medical literature reporting the mean rate of '1.9 fetal losses per million pregnant women vaccinated' for the previous 19 single-vaccine influenza seasons based on counts of VAERS reports that were not adjusted for under-ascertainment,[8] likely contributed to this perception of safety. Because both patient and health care professionals relied on a historical profile that was incomplete with respect to assessing fetal-demise reporting, a possible link to fetal demise following administration of influenza vaccine/vaccines during 2009/2010 was rarely contemplated or was considered highly unlikely and thus, more often than not, not reported.

The ratio of the 12,300 AE reports associated with A-H1N1 vaccine to the 7691 due to TIV is 1.60, which is similar to the ratio of 1.59 using female AE reports (Table 5). If a Weber-like increase existed, a readily discernible AE, such as anaphylactic shock, should have generated at least a 1.6-fold increase in VAERS reports associated with the 'new' pandemic A-H1N1 vaccine; however, no such increase was found. This independent AE control group confirms that most of the observed 7.7-fold (170 A-H1N1 fetal-loss reports/22 TIV fetal-loss reports) increase in fetal-loss reports associated with the administration of the 2009 A-H1N1 vaccine appears to be attributable to some type of toxicity effect rather than a 'new vaccine' Weber-like reporting effect.

When one or more Thimerosal-containing vaccines, including some formulations of the seasonal TIV and pandemic monovalent A-H1N1 vaccines are administered to a pregnant woman, the fetus is also indirectly exposed to mercury. In the following paragraphs, several peer-reviewed publications highlight the concerns that this mercury exposure poses.

A study using rabbits injected with Thimerosal-containing radioactive mercury showed that from 1-h post-injection to 6 h, the level of radioactive mercury in the blood dropped over 75% while from 2 h post-injection to 6 h, there were significantly increased radioactivity levels in the fetal brain, liver, and kidney.[25] Thus, the rapid drop in blood mercury levels from Thimerosal injection is due to uptake by other organs of the body and not due to excretion.[26] Therefore, the implications by others of Thimerosal's safety based on shorter blood level half-lives[27] suffers from lack of a circumspect view regarding this process.

The linkage between Thimerosal and neurodevelopmental disorders is a concern because several studies have shown that children with autistic spectrum disorders (ASDs) have higher levels of mercury body burden than typically developing children.[28–33] In addition, there is a positive correlation between mercury body burden and severity of ASD symptoms.[34–36] Direct measurement of injury in the brains of children with ASD reinforce this finding; there is a significant dose-dependent positive correlation between oxidative stress markers (evidence of brain injury) and mercury levels in the brains of children with ASD.[37]

The amount of mercury that accumulates in any given fetus and the severity of its impact depend upon several factors in addition to the maternal mercury exposure due to injected Thimerosal-containing inactivated influenza vaccines. Dental amalgams in pregnant women contribute to increased mercury burden in the developing fetus and newborn.[38–40] Also, the maternal–fetal genetic background can modulate fetal exposure to mercury; thus, certain gene variants influence mercury toxicokinetics causing the variable susceptibility that is observed with respect to mercury toxicity.[41] This variation in genetic susceptibility, combined with factors of diet and antibiotic use, can synergistically enhance mercury toxicity[42] and effectively preclude establishment of a safe mercury dosing level for all individuals. Moreover, the 0.1 mcg/kg/day reference dose that the Environmental Protection Agency (EPA) established as safe based on oral ingestion of mercury is not applicable for injected Thimerosal via vaccination since injection bypasses the absorption protection provided by the gastrointestinal system (which is also apparently dependent on the manner in which the fish or other mercury-containing food is prepared).[43] thereby delivering more of the toxic dose of mercury administered into the body.

Finally, Thimerosal has been found to be toxic at very low levels. For example, Parran et al. examined the effects of Thimerosal on cell death in a human neuroblastoma cell line. Following 48 h of a single dose of 4.35 nanomolar Thimerosal (or about 0.87 mcg/kg of mercury) over 50% of cells were dead.[44]

Thus, it is biologically plausible that during the two-vaccine 2009/2010 influenza season, when pregnant women were administered two Thimerosal-containing

**Table 6.** Gestational age, mean weight, and multiple of the EPA's RfD using 50% exposure.

| Trimester | Gestational age in weeks | Mean weight (kg)[a] | Multiple of the EPA's RfD[b] based on 1 mcg of Hg in the vaccine dose[c] | Multiple of the EPA's RfD[b] based on 25 mcg of Hg in the vaccine dose[c] |
|---|---|---|---|---|
| First trimester | ≤8 | ≤0.001 | ≥5000 | ≥125,000 |
| | 9 | 0.002 | 2500 | 62,500 |
| | 10 | 0.004 | 1250 | 31,300 |
| | 11 | 0.007 | 710 | 17,900 |
| | 12 | 0.014 | 360 | 8900 |
| | 13 | 0.023 | 220 | 5400 |
| Second trimester | 14 | 0.043 | 120 | 2900 |
| | 15 | 0.070 | 70 | 1800 |
| | 16 | 0.100 | 50 | 1250 |
| | 27 | 0.875 | 5.7 | 140 |
| Third trimester | 28 | 1.01 | 5.0 | 124 |
| | 29 | 1.15 | 4.3 | 109 |
| | 30 | 1.32 | 3.8 | 95 |
| | 42 | 3.69 | 1.4 | 34 |

EPA: Environmental Protection Agency's; RfD: reference dose.
[a]Mean weights 8-16 weeks[45] and 27-42 weeks.[46]
[b]Oral RfD = 0.0001 mg/kg/day (or 0.1 mcg/kg/day) for ingested mercury presumably from 'methylmercury species.'[47]
[c]Multiple of EPA's RfD based on 50% exposure = (0.50 • V/W)/0.1 mcg/kg; where V = micrograms (mcg) of mercury (Hg) in the vaccine dose and W = mean weight of fetus in kilograms (kg).

influenza vaccines each delivering 50 mcg of Thimerosal (or 25 mcg of mercury per dose), the fetus' mercury dose exceeded the EPAs reference dose (0.1 mcg of mercury/kg/day). This overexposure could be a significant contributing factor to some of the reported SABs and SBs. Moreover, the mercury in injected Thimerosal-containing vaccine doses has been found to preferentially bioaccumulate in the fetal tissues.[25] Table 6 demonstrates that depending upon the gestational age, the safety level of mercury (as specified by the EPA's reference dose) may be exceeded by several thousand fold for an early developing fetus during the first trimester to a factor of just over 1 at full-term – even for a single reduced Thimerosal vaccine dose presuming only 50% of the mercury (0.5 mcg) bioaccumulates in the fetus (Table 6, fourth column labeled '1 mcg of Hg in the vaccine dose').

Recent studies have similarly described biologically plausible mechanisms associated with the synergistic toxicity associated with multiple vaccine doses administered to children aged <1 year.[48,49]

The bias in reporting of fetal loss by older women may be due, in part, to this cohort's previous experience with one or more normal pregnancies, free from maternal complications when they did not receive an influenza vaccine during pregnancy, and thus, having more birthing experience than younger, first-time pregnant women. Also, this cohort may have a higher body burden of mercury from the bioaccumulation of mercury from dental amalgams, diet, prior doses of Thimerosal-containing vaccines, and other drugs.

The Internet survey was self-administered, thus, the responses are subject to reporting error since pregnancy and vaccination status were not validated by a medical record review. There may also be selection bias since women without Internet access would be excluded from referencing the Public Service announcement (and the survey). Nevertheless, Internet panels have been useful as surveillance data sources for postseason evaluation of influenza vaccination among pregnant women.[19]

## Conclusion

The 1.8-fold increase in female AEs reports to VAERS following administration of pandemic A-H1N1 vaccine relative to seasonal TIV in the 2009/2010 influenza season is too small of a Weber-like increased reporting effect to account for the more than 40-fold increase in fetal-loss reports. Thus, the concomitant administration of the seasonal influenza and pandemic A-H1N1 vaccines during 2009/2010 suggests a synergistic toxicity and a statistically significant higher rate of fetal loss reporting relative to the single-dose seasons. When capture–recapture is applied to the two-vaccine 2009/2010

influenza season, the ascertainment-corrected reports yield an estimated rate of 590 fetal-loss reports per 1 million pregnant women vaccinated (or 1 per 1695). Without additional ascertainment sources, it was not possible to determine the reporting completeness of fetal losses associated with the 2008/2009 and 2010/2011 seasons.

The VAERS rates of 6.8 and 12.6 fetal-loss reports per million women vaccinated for those single-vaccine seasons may provide health care professionals with a sense that influenza vaccines administered during pregnancy are relatively safe, when, in reality, these rates merely reflect the low level of case ascertainment associated with VAERS and thus, grossly underestimate the true rates encountered in the US population. Just because a single vaccine has been tested and considered safe does not imply there will not be a synergistic fetal toxicity effect associated with the administration of two or more Thimerosal-containing vaccines to a pregnant women and/or a synergistic toxicity effect from the combination of the biologically active components contained in concomitantly administered vaccines.

In addition, because of the order of magnitude increase in fetal-loss report rates, from 6.8 fetal-loss reports per million pregnant women vaccinated in the single-dose 2008/2009 season to 77.8 in the two-dose 2009/2010 season, further long-term studies are needed to assess adverse outcomes in the surviving children. Additional research concerning potential synergistic risk factors associated with the administration of Thimerosal-containing vaccines is warranted, and the exposure-effect association should be verified in further toxicological and case–control studies.

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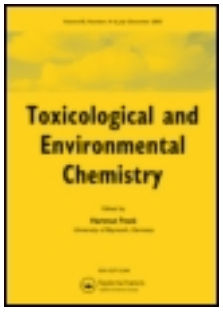


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# Maternal transfer of mercury to the developing embryo/fetus: is there a safe level?

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# Maternal transfer of mercury to the developing embryo/fetus: is there a safe level?

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Mercury (Hg) exposure is ubiquitous in modern society via vaccines, fish/crustacea, dental amalgam, food, water, and the atmosphere. This article examines Hg exposure in the context of primary exposure to pregnant women and secondary exposure experienced by their unborn babies. Babies *in utero* are particularly at risk of higher Hg exposure than adults (on a dose/weight basis through maternal Hg transfer via the placenta), and are more susceptible to adverse effects from mercury and its biologically active compounds. It is, therefore, critical that regulatory advisories around maximum safe Hg exposures account for pregnant women and secondary exposure that children *in utero* experience. This study focused on standardized embryonic and fetal Hg exposures via primary exposure to the pregnant mother of two common Hg sources (dietary fish and parenteral vaccines). Data demonstrated that Hg exposures, particularly during the first trimester of pregnancy, at well-established dose/weight ratios produced severe damage to humans including death. In light of research suggestive of a mercuric risk factor for childhood conditions such as tic disorders, cerebral palsy, and autism, it is essential that Hg advisories account for secondary prenatal human exposures.

**Keywords:** mercury; pregnancy; fetus; placental transfer; mercury in fish; vaccines



## Introduction

Mercury (Hg) and its various biologically and environmentally active compounds have a diverse global distribution and enter the environment, food chain, and drinking water from a number of dispersed sources which result in dangerous health associations for humans and adverse impacts on flora and fauna in a contaminated environment. The aim of this study, therefore, was to critically examine the most significant human Hg exposures in Australia; specifically those derived from consumption of freshwater fish (methylmercury) and vaccine preservatives (ethylmercury) in the context of maternal transfer of that Hg via the placenta to the developing child.

Mercury is the only naturally occurring element existing in liquid form at normal temperatures and pressures. It has a high vapor pressure (0.17 Pa at 20°C), readily evaporating at ambient temperatures with 30% of its mass evaporating at 20°C and >80% at 60°C (Hellings 2009). Mercury vapor is heavier than air, flows down-slope in

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highly contaminated areas when wind circulation is moderate or absent, and collects in low points. Mercury vapor condenses back to elemental (liquid) Hg under normal atmospheric conditions prevailing on earth thus completing the entire Hg cycle in the environment. Elemental Hg has a high density of $13.5\,kg\,L^{-1}$.

Mercury (in its common forms) is extensively distributed throughout the earth, occurring naturally in volcanic emissions from terrestrial and undersea volcanoes, of which the latter forms 85% of all active volcanoes on earth (Plimer 2009). Mercury occurs in ore bodies as several minerals but predominantly as cinnabar; a mercuric sulfide (HgS). Atmospheric transport of Hg is a global phenomenon and results in an average atmospheric concentration of $1.5–4\,ng\,m^{-3}$ (northern hemisphere) (Parsons and Percival 2005; Lippmann 2009).

Anthropogenic uses of Hg arise from the mining and reworking of naturally occurring Hg deposits or, indirectly, from the processing of materials in which the metal occurs naturally in trace amounts (e.g., coal burning). Mercury is extensively used in various industries as a primary material including automotive (e.g., sensors and switches for antiskid safety systems), chemical and petrochemical (e.g., as catalysts and process materials), electronics (e.g., in industrial, street, high efficiency domestic lighting and electronic components), in dentistry (as amalgam fillings), in instruments (e.g., thermometers and barometers), and pharmaceutical industries (mostly as a preservative and antimicrobial).

Mercury in many of its different forms occurs ubiquitously across the planet throughout the environment from fugitive natural and anthropogenic sources. Of most concern internationally is the fact that Hg is widely distributed throughout our food chain, air, water, and land at levels that were found toxic to flora and fauna and, in some instances, to humans (Amin-Zaki et al. 1978; Ratcliffe, Swanson, and Fischer 1996; Sweet and Zelikoff 2001; Counter et al. 2002). Mercury is a Category A Prescribed Industrial Waste, an ecotoxic waste, a poisonous (acute) waste, a toxic (chronic) reproductive waste, a neurotoxin, and is classified under the Australian Dangerous Goods Code as a *Class 6.1 (Toxic) and Class 8 (Corrosive) Substance*.

Elemental Hg in liquid and gaseous forms is a highly toxic substance affecting various human organs including brain, central nervous system (CNS), liver, and kidneys. It has also been shown to be corrosive to the renal proximal tubuli, and other human cells and organs (Nordberg et al. 2007). When combined with short chain alkyl groups (methyl- and ethyl-), it forms compounds that are both highly neurotoxic and readily absorbed in biological systems (Amin-Zaki et al. 1978; Ratcliffe, Swanson, and Fischer 1996; Counter et al. 2002; Nordberg et al. 2007; Lippmann 2009). When elemental or inorganic Hg is present in the environment it is converted to methylmercury (MeHg) and ethylmercury (EtHg) by various aerobic and anaerobic processes and bioaccumulates up the food chain (Nordberg et al. 2007; Mao et al. 2010).

### *Mercury, human toxicity, and the prenatal period*

At a human cellular level, mercury in all its forms (ionic $Hg^{2+}$ and $Hg^{+}$ ($Hg_2^{2+}$); vapor $Hg^0$ and organic alkyl forms $MeHg^+$, $Me_2Hg$, $EtHg^+$, and $Et_2Hg$) exerts demonstrable potent neurotoxicity for the human brain and particularly for the human fetal brain (Nordberg et al. 2007; Lippmann 2009). Mercury's strong affinity for sulfur and sulfydryl functional groups in biological molecules in membranes and enzymes interferes with their structure, function, and activity, and it is these complex binding interactions which give rise to toxicological properties of Hg compounds in mammalian organisms (Nordberg et al. 2007).

On the basis of Hg exposure and prenatal human (embryo/fetus), one of the particular concerns resulting from total dietary Hg intake is that small children, on a body weight basis, may receive a higher Hg exposure compared to adults (Lippmann 2009). Data documenting the impact of Hg exposure on humans are well-established (Ratcliffe, Swanson, and Fischer 1996; Sweet and Zelikoff 2001) and provides comprehensive evidence for detrimental effects that all forms of Hg exert on human fetal development. The direct causal link between cerebral palsy and Hg exposure to the developing human fetus was first established in 1971 by the Swedish Expert Group (Swedish Expert Group 1971) and more recently by others (Nordberg et al. 2007; Lippmann 2009; Tomljenovic, Dorea, and Shaw 2012).

Further, the United Nations Environment Program (UNEP DTIE 2008) Inter-Organization Program for the Sound Management of Chemicals (IOMC) and World Health Organization (WHO) document, "Guidance for Identifying Populations at Risk from Mercury Exposure," 2008 states that the CNS, kidneys, and cardiovascular system are the primary targets for Hg compounds-induced toxicity and that other affected systems may include respiratory, gastrointestinal, hematologic, immune, and reproductive systems. For a fetus, Hg brain levels may be significantly higher than in maternal blood; and the developing fetus CNS demonstrates the greatest sensitivity to Hg toxicity and is considered to be the main system of concern. Verstraeten et al. (2003) identified Hg exposure during early development to be associated with tic disorders (and possibly conditions falling within the definition of autism spectrum disorder (Bernard et al. 2001; Austin 2008; Leslie and Koger 2012; Tomljenovic, Dorea, and Shaw 2012) with genetic variables perhaps acting as mediators in this latter association (Leslie and Koger 2011; Shandley and Austin 2011).

### *Mercury in freshwater fish and crustacea*

At the *10th International Conference on Mercury as an Environmental Pollutant* (2011) held in Halifax, Nova Scotia, Canada, the overwhelming consensus of scientists presenting papers at this conference was that the maximum level of Hg in fish and crustacea should not exceed $0.3\,mg\,kg^{-1}$ (w/w) (Burger et al. 2003). This level is less than a third of the current maximum "mercury in fish" advisories adopted by many western regulators, including Australia where it is currently stated to be either $0.5\,mg\,kg^{-1}$ Hg (w/w) or $1\,mg\,kg^{-1}$ Hg (w/w) (Food Standards ASNZ 1.4.1 2011). It should be noted that while Food Standards Australia New Zealand (2011) Standard 1.4.1 states the maximum Hg in fish should not exceed $0.5\,mg\,kg^{-1}$ Hg, this lower limit, however, applies only to appropriately sampled commercial marine fishing lots. The higher limit of $1\,mg\,kg^{-1}$ Hg (w/w) applies to specific named marine species, to fish lots where there are insufficient numbers of fish to follow the required sampling regime, and to all freshwater fish not subject to commercial practices (e.g., those freshwater species caught and eaten by recreational anglers). Further, despite the latest recommendations for reduction of maximum "safe" Hg limits, there is growing support for the view "there is no safe level of mercury for the most vulnerable groups in the community" which includes pregnant women, their unborn babies, infants, and children less than 10 years (USEPA 2009).

In Australia, almost 33% of the entire continental land surface has been subjected to all forms of gold mining since early 1850s (GeoScience Australia 2011). Since elemental (liquid) Hg was extensively used for fine gold recovery on every goldfield in Australia up to the time Hg was eventually banned (*ca* early 1960s), there exists the likelihood that extensive Hg contamination occurs throughout all historical gold mining areas. Due to the

extent of Hg contamination across Australia the greatest likelihood of exposure to a population comes from contaminated freshwater sources derived from historical mining sites and from eating freshwater fish and crustacea living in these lakes, rivers and streams, or by drinking these waters during peak flows when sediment levels are highest, rather than from absorption of atmospherically distributed Hg or consumption of marine species. Freshwater fish, crustacea, and water in specific catchments were found to be contaminated by Hg to levels that frequently exceed National Regulatory Water and Food Standards and, in cases, up to several 10-fold greater than the maximum Hg limit (Ealey et al. 1978; McCredie 1982; Sutherland, Bell, and Bacher 1984; Jackson 1997; CSIRO 1998).

Burger and Gochfield (2012) stated another important determination that affects risk of a contaminant allowed by the US Clean Water Act is "Total Maximum Daily Load," which represents the maximum amount of a pollutant that a body of water can receive and still meet water-quality standards. In the US, most states have adopted a "mercury freshwater quality standard of 0.3 ppm (0.3 $mg\,kg^{-1}$ (w/w)) methylmercury in fish." Some states (Minnesota and Maine) adopted a more stringent standard of 0.2 $mg\,kg^{-1}$ (0.2 $\mu g\,g^{-1}$). Burger and Gochfield (2012) also indicated that these lower levels were set as a consequence of the important fact that levels of Hg in water do not relate directly to the levels of Hg in fish, and it is the levels of Hg in fish that are the crucial end point in terms of Hg risk. In addition, when fish Hg levels exceed the water-quality standard, the (US) Clean Water Act requires the calculation of how much Hg loading must be reduced.

Clearly, Food Standards ASNZ Standard 1.4.1 (2011) is not fully cognizant with "International Best Practice" and current scientific understanding of maximum levels of Hg in freshwater fish. The total Hg present in a single, "standard" 150 g portion of fish containing Hg at the maximum advisory of 1 $mg\,kg^{-1}$ Hg (w/w) is 150 µg. Mercury in fish and crustacea is present essentially wholly (>95%) as MeHg (Parsons and Percival 2005; FAO/WHO 2011). Mercury present in Australian drinking water is usually low (0.001–0.005 $\mu g\,L^{-1}$) but under Australian Drinking Water Guidelines, ADWG, Hg content is permitted to be as high as 1 $\mu g\,L^{-1}$ (NHMRC ADWG 2011). It is appropriate, therefore, to examine total Hg intake of three female family members to determine what a "typical daily intake" would be for each female by comparing their individual Hg intake after consumption of a "single normal portion" or a "single standard portion" (as defined by Australian Hg in fish advisories) of, for example, freshwater trout containing Hg at the maximum limit of 1 $mg\,kg^{-1}$ Hg (w/w).

Our typical scenario is based on a popular Victorian summer camping location where several thousand campers (mostly families) stay each summer. These camping locations are spread out along the Upper Goulburn River (UGR) between Jamison and Woods Point, in designated Parks Victoria Camping Areas, or in specific camping locations around the shoreline of Lake Eildon (LE). Normal camping activities in this area include drinking the UGR water when camped along the river (the only locally available drinking water in these parks along the river) and fresh-water fishing in this river, its nearby tributaries (Gaffney's & Raspberry Creeks), and in LE; the main reason campers usually visit these holiday locations. It is acknowledged that a small number of campers may bring drinking water from their homes for camping purposes but this is the exception, in our experience, of campers in this area, as most choose to drink the water direct from the river or lake. The UGR area holds the status of being one of Victoria's most "pristine" camping localities.

The main fish species likely to be angled in these waters are brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), redfin (*Perca fluviatilis*), carp (*Cyprinus carpio*), and

Table 1. Hg intake and consequential USEPA MeHg RfD exceedances/female (kg b.wt.)/portion/day.

| Family member | Weight (kg) | Age (year) | Fish portion (g) | | Hg intake/ portion (μg) | | USEPA RfD MeHg.[d] Exceedences[c] | |
|---|---|---|---|---|---|---|---|---|
| | | | Nor.[a] | Std.[a] | Nor. | Std. | Nor. | Std. |
| Mother | 65 | 39 | 200 | 150 | 200 | 150 | X 31 | X 23 |
| Daughter | 22 | 5 | 100 | 150[b] | 100 | 150[b] | X 45 | X 68[b] |
| Sister | 45 | 12 | 200 | 150 | 200 | 150 | X 44 | X 33 |

Notes: [a]Nor. – normal or typical; Std. – standard fish portions (as defined in advisories).
[b]This exceedance corresponds to the added daily risk a child would experience following consuming a single "standard 150 g fish portion."
[c]Exceedances are the total daily MeHg intake (per kg b.wt per person per day) expressed as a multiple of the USEPA RfD MeHg maximum Daily Limit (Lippmann 2009).
[d]USEPA daily RfD is 0.1 μg MeHg per kg b.wt per day.



spiny freshwater crayfish (*Euastacus armatus*), (LE and UGR) plus Murray cod (*Maccullochella peelii*) and silver perch (*Bidyanus bidyanus*) (LE). It is important to note that each of the named species taken from these waters, and in instances including the water itself, was analyzed for Hg and exceeded maximum Hg health limits on various occasions (Ealey et al. 1978; McCredie 1982; Sutherland, Bell, and Bacher 1984), and in more recent investigations conducted since then (Hg Recoveries PL, Victorian Stream Surveys 2010–2012 unpublished; Jackson 1997). The details and MeHg intake for each family member following consuming a single fish portion containing Hg at $1\,mg\,kg^{-1}$ Hg (w/w) and individual USEPA Daily MeHg reference dose (RfD) exceedances resulting from that consumption are shown in Table 1.

It must also be remembered that most, if not all, official fish advisories (e.g., USA, Europe, and Australia) state it is "safe" for a "standard fish portion" to be consumed once or twice per week for MeHg levels up to maximum advisory levels. Often a fish advisory is much less precise, wherein it is simply stated "up to a few standard portions per week" can be safely consumed. Of much greater concern for the "most at risk groups in a community" (i.e., pregnant women, their unborn babies, infants, and young children less than 10), it is implied that it is "safe" for all people to consume such Hg contaminated fish.

Our above analysis and those that follow clearly demonstrate that present fish advisories relating to consumption of MeHg contaminated fish are inadequate and do not offer the protection that is implied by following such advisories, nor does that of following the USEPA RfD limit of 0.1 μg MeHg per kg b.wt per person per day, when this maximum daily limit is applied to the recommended fish advisories. Of even greater concern is the absolute quantity of Hg transferred from the mother through the placenta to her developing embryo/fetus and the attendant impact this may exert for each additional occasion the mother consumes a portion of Hg contaminated fish during the same pregnancy.

Methylmercury's biogeochemistry is well-understood; however, there is now increasing evidence that EtHg too, follows similar biogeochemical processes and that this compound also accumulates up the food chain. Monoethylmercury was identified in Florida Everglades soils in 2010 (Mao et al. 2010), and this fact adds substantially to the understanding of EtHg biogeochemistry and how it gets into the environment when different forms of Hg are present. It also provides for a direct comparison with the process

of MeHg bioaccumulation in the food chain and this is a likely consequence for EtHg compounds. It is thus pertinent to modern studies dealing with the elucidation of the biogeochemistry of EtHg to comment on an observation made by the authors of a 1978 paper investigating the extent of Hg pollution from historic gold mining activities existing in the UGR Catchment, Victoria, Australia during the analysis of stream sediments, water, fish, invertebrates, and aquatic plants for MeHg and total Hg. Following acid digestion of freshwater fish and invertebrates with gas chromatographic identification of the components present (extracted as their chlorides) Ealey et al. (1978) stated without further qualification that "the ethylmercury peak elutes 1–3 mm after methylmercury peak."

The interesting conclusions drawn from this experimental observation are as follows:

(1) There is the possibility that EtHg not only forms under the same environmental conditions as MeHg, but that it also bioaccumulates up the food chain, since its presence and detection resulted from analyses of freshwater fish collected from this Hg-contaminated catchment, at that time.

    It is possible that either no one had previously looked for this compound, had not identified it, or was not interested in it to the same degree as MeHg. Alternatively, the analytical procedures used prior to this investigation may not have been sensitive enough to have detected the presence of monoethylmercury.

(2) Ethylmercury compounds have always been present in the environment as a direct consequence of microbiological interactions with inorganic or elemental Hg, as has MeHg. This is highly likely but requires confirmatory study.

### *Mercury in vaccines*

According to the Immunise Australia Website and the Australian Government Health Information telephone enquiry line (Personal Inquiry June 2011), a phase-out of Hg preservative in vaccines for babies and children began in Australia sometime between 2000 and 2008. There was no immediate cessation of supply of Hg-preserved vaccines during this period, and no government requirement for suppliers to do so, as pharmaceutical companies manufacturing (or supplying) vaccines into the Australian market were allowed to progressively run down their existing stocks. A similar process was followed in the US.

Thimerosal (Thiomersal) or sodium ethylmercuric thiosalicylate (SEMT) was the preservative of choice in childhood vaccines prior to the commencement of progressive depletion of existing stocks. SEMT was in use as a vaccine preservative since the mid-1930s and remains a common preservative in vaccines today, in particular, those designed for administration to adults including influenza vaccines (Table 2). It is also the preservative employed in a multi-dose influenza vaccine to be used as a first response during an influenza pandemic. Although the United Nations Environment Program is considering a draft global treaty mandating the elimination of Hg preservatives from all vaccines (UNEP 2011), and especially those that are employed in pregnant women, infants, and young children less than 10 years of age, Hg preservatives remain in use in various vaccines in 2012.

Influenza vaccine is generally administered to greater numbers of people at much greater frequencies (at least annually) compared to other Hg-preserved vaccines, due to the cyclical recurrence of influenza in the community each year. It is also important to note that this vaccine is particularly recommended for, and promoted to, pregnant women. In addition, current Australian Government Health Department Vaccination Schedules (as in May 2012) prescribes a course of four influenza injections for an infant not

Table 2. Vaccines containing mercury preservative in Australia.[a]

| Disease | Vaccine | Manufacturer/Distributor | Thiomersal/Vaccine dose |
|---|---|---|---|
| Hepatitis B: | *Engerix-B*[b] | GlaxoSmithKline | $<2\,\mu g\,mL^{-1}$ Thiomersal |
| | | Equiv. Hg conc.[c] | $<1\,\mu g/0.5\,mL$ |
| Influenza: | *Fluad* | Delpharm Consultants | 0.05 mg/0.5 mL Thiomersal |
| | | Equiv. Hg conc. | 24.8 μg/0.5 mL |
| | *Fluarix* | (GalxoSmithKline) | Traces of Thiomersal[d] |
| | | Equiv. Hg conc. | Traces of Thiomersal[d] |
| | *Panvax*[e,f] | CSL Biotherapies | 0.05 mg/0.5 mL Thiomersal |
| | | Equiv. Hg conc. | 24.8 μg/0.5 mL |
| Japanese encephalitis: | *Je-Rix* | Sanofi Pasteur | 0.007% Thiomersal ($m\,v^{-1}$)[g] |
| | | Equivalent to | 0.07 mg/1.0 mL Thiomersal |
| | | Equiv. Hg conc. | 17.35 μg/0.5 mL |
| Q-Fever | *Q-Max* | CSL Biotherapies | 0.01% ($m\,v^{-1}$) Thiomersal |
| | | Equivalent to | 0.05 mg/0.5 mL Thiomersal |
| | | Equiv. Hg conc. | 24.8 μg/0.5 mL |
| Q-Fever | *Q-Max:* | CSL Biotherapies | 0.05 mg/0.5 mL Thiomersal |
| | Skin test | Equiv. Hg conc. | 24.8 μg/0.5 mL |

Notes: [a]The Australian Government Department of Health and Ageing (2008), as in May 2012.
[b]A mercury-free vaccine is available.
[c]Equivalent weight of mercury per 0.5 mL dose of preserved vaccines.
[d]"Trace" is not quantified by the manufacturer.
[e]A single-dose mercury-free Panvax vaccine is available.
[f]CSL Ltd PANVAX Product pdf, December 2009; a multi-dose pandemic flu vaccine.
[g]Units of Thiomersal concentration are not stated; presumed to be ($m\,v^{-1}$).



previously inoculated against influenza, commencing at age two months and spanning the first year of life. It should be noted that preserved influenza vaccines containing a smaller level of SEMT are available for administration to infants but these do not appear to be listed on the Immunise Australia Website.

SEMT (trade names Merthiolate, Thimerosal, Thiomersal) is 49.57% Hg (by weight) and, therefore, 0.05 mg SEMT in a 0.5 mL vaccine dose (as in a standard preserved multi-dose influenza vaccine) contains 24.8 μg Hg. The absolute quantity of Hg preservative in vaccines prior to, and including 2000, in Australia varied between 25 and 50 μg SEMT per 0.5 mL dose, and this was the dose that babies, young children (<10 years), and adults (including pregnant women) routinely received with each vaccination. This was equivalent to 12.5–25 μg Hg per 0.5 mL dose, respectively. Of the current vaccines listed in Table 2, (and rounded to the nearest μg), four of seven vaccines contain 25 μg of Hg per 0.5 mL dose; one contains 17 μg, and the remaining two are not sufficiently quantified by the manufacturers to make a definitive statement.

### *On the question of the toxicity of ethylmercury compounds*

The toxicity of MeHg compounds to mammalian life is well-established; however, the toxicity of EtHg is less well-understood. Nevertheless, there is compelling evidence to support the conclusion that EtHg compounds in human fetuses, children, and adults even though the half-life is less than that of MeHg, produce adverse effects (Fagan et al. 1977; Nordberg et al. 2007; Mutter and Yeter 2008; Lippmann 2009; Sulkowski et al. 2011; Sharpe, Livingston, and Baskin 2012; Tomljenovic, Dorea, and Shaw 2012). Despite the shorter half-life in biological systems the metabolites from EtHg may be MeHg, Hg vapor

($Hg^0_{Vap}$), and inorganic mercuric ion ($Hg^{2+}$), which are then available via methylation and demethylation reactions to recommence the various modes and routes of Hg toxicity that these compounds undergo in the human body (Nordberg et al. 2007). The phenomenon of Hg ability to transform backwards and forwards into all of its multiple chemical states within biological systems (e.g., human fetus, children, and adults alike), no matter what its initial chemical form, is what both characterizes and underscores Hg-induced toxicity to all mammalian life.

While there are limited data available which suggest a safe level of EtHg exposure, both MeHg- and EtHg compounds are structurally similar and have similar modes of adversely impacting human critical organs and disrupting essential biological transport functions in cells, which then result in similar toxicities to the target organs or cells (Nordberg et al. 2007; Lippmann 2009; Sharpe, Livingston, and Baskin 2012). There is also increasing experimental evidence which identifies EtHg compounds as displaying neurotoxic properties in biological systems and behaving in an analogous manner to MeHg compounds (Nordberg et al. 2007; Lippmann 2009). Although the uptake of EtHg in the brain does suggest active transport, not all transporters have so far been positively identified, or Hg transferred by them quantified (Nordberg et al. 2007). Magos (2001) reported that clinical signs and symptoms of EtHg poisoning are similar to those for MeHg poisoning, including dysarthria, ataxia, constricted visual fields and paresthesias, and that brain pathology is also similar. He also reported that results from animal studies were consistent with clinical observations.

Ethylmercury characteristics of having a high solubility in lipids (Nordberg et al. 2007) and propensity to accumulate in biological systems (Lippmann 2009) means it is at least as mobile through this medium as MeHg despite having a shorter half-life and despite MeHg's aqueous transport capability, and may cross cell membranes and the blood-brain barrier just as readily, if not more so, than MeHg thus reducing the capacity of the body's natural defense mechanisms to mercury-induced damage. Ethylmercury resonance time in the body and particularly in a fetus, while shorter than MeHg may, nevertheless, be comparable to that of MeHg and therefore be significant. If this situation is experimentally confirmed it would, accordingly, warrant a great deal of concern for the continued use of SEMT as a preservative in human vaccines. Three recent clinical investigations established that Thimerosal produced irreversible disruption to mitochondrial function by inhibiting mitochondrial respiration in human astrocytes and irreversible damage to mitochondrial DNA by generation of specific oxidants; superoxide, hydrogen peroxide, and Fenton/Haber-Weiss generated hydroxyl radicals (Sharpe, Livingston, and Baskin 2012), lasting impairment of brain monoaminergic system in rat pups resulting in development disorders (Ida-Eto et al. 2012), and impacts to thyroid hormone-dependent gene expression in the cerebellum (Khan et al. 2012). Thus, a clinical basis for the cause of irreversible damage from exposure to Thimerosal (SEMT) in a developing fetus leading to postnatal developmental problems was demonstrated and has most concerning effect on human astrocytes. Accordingly, SEMT or Thimerosal is no longer considered as "safe" for use in vaccines for the "most at risk group in a community." When all of the observations pertaining to EtHg reactions and pathways and their undeniable similarities to their MeHg analogs are considered, there appears to be no scientific basis for continued claims that EtHg compounds are "*safe*" (Pichichero et al. 2002, 2008). Taking this into account, and the uncertainties surrounding EtHg placental transfer rates and toxicity to a developing fetus, it is possible that EtHg is at least as toxic as MeHg. Accordingly, our analyses did not distinguish between EtHg and MeHg and assumed equivalent human toxicities.

**Method**

To examine Hg exposures and their relative dose/weight ratios across the gestational period of the human infant, a group of pregnant women carrying individual fetuses of 1, 2, 4, 8, 12 16, 20, 24, 28, 32, 36, and 38 weeks old was considered, where each mother was exposed to Hg via either (1) consumption of a single "standard 150 g portion of fish" containing Hg at the maximum Australian advisory level of 1 mg $kg^{-1}$ (w/w), or, (2) a single Hg-preserved influenza vaccination during their pregnancy.

Although it is possible an adult and an infant 2 months – < 3 years, receiving their first inoculation, could receive two or more influenza vaccine doses in a year, our hypothetical scenario only considers the typical case where a single dose is administered to each pregnant woman. It is important to note that our primary exposure estimates are likely to be underestimates as one does not attempt to quantify other concurrent primary maternal Hg exposures through sources such as whole diet, dental amalgam, the atmosphere, and other vaccines. Equally, for simplicity in our discussion, one does not consider the likely situation that a pregnant woman could eat more than one single standard fish portion contaminated at the maximum permitted limit (i.e., 1 mg $kg^{-1}$ Hg (w/w)), throughout the gestation period. Obviously, doing so would increase the total Hg intake to the pregnant mother and, therefore, elevate Hg exposure to a developing fetus during the gestation period, in proportion to the number of additional portions consumed.

Similarly, it also needs to be realized that consumption of contaminated fish portions containing Hg levels below the maximum limit more frequently than our hypothetical "once only scenario" during gestation may exert an additive effect on total Hg body-burden of the pregnant mother, and, as a consequence, of total Hg actually crossing the placenta, which then impacts a developing fetus. This is a real likelihood for a pregnant female and her developing fetus given the elimination half-lives reported for Hg since they are quite variable for different organs; typically of the order of 3 – > 60 days for liver, kidneys, and CNS, and of the order of 2–5+ years for the fetal brain (Nordberg et al. 2007).

Given such long elimination half-lives there is the distinct possibility that Hg accumulation occurs after repeated exposures over a consumption frequency that is significantly less than the stated Hg elimination rates (Nordberg et al. 2007). Further, that Hg (as vapor, $Hg^{2+}$, and MeHg) administered to a pregnant female crosses through the placenta in all forms administered, but to varying extents, which then impacts the developing fetus, was confirmed by numerous animal studies (involving primates, rats, mice, rabbits, and hamsters) and in a number of limited cases human female/fetus pair exposures where this study was possible (Ask et al. 2002; Stern and Smith 2003).

Less research data investigating, and actually quantifying, the amount of Hg crossing the placenta compared to exposures the pregnant female receives are available. Dock, Rissanen, and Vahter (1994) quantified this aspect of maternal/placental transfer of Hg using Syrian golden hamsters. Data supported the validity of our chosen scenarios presented in the following sections. In our analyses a statistical average female weight of 65 kg, was assumed however, this is not a critical variable as it does not impact predictably on fetal Hg exposure. Ultimately, it is not the case that heavier women retain more, and lighter women retain less Hg as the primary variable impacting Hg metabolism is an individual's own genetics, metabolism, exposure histories, and susceptibilities, which vary markedly from person to person regardless of recipient body weight (Nordberg et al. 2007).

To simplify our discussion (and to be conservative) one needs to consider only three Hg exposure scenarios: (1) 100% Hg present in a vaccine or consumed fish crosses the placenta; (2) 10% Hg present in a vaccine or consumed fish crosses the placenta; and

(3) where only 1% Hg crosses the placenta. These three scenarios were selected to cover the complete range of likelihoods for passage of Hg directly from mother through the placenta to the developing embryo/fetus because investigators have not yet positively identified all of the transporting mechanisms carrying Hg across the placenta nor has the maximum possible amount of Hg associated with these processes been satisfactorily quantified. Thus, there still remains much uncertainty surrounding placental Hg transport, and its quantification, following each Hg exposure to the pregnant mother. Despite the above uncertainty, there is, nevertheless, sufficient experimental evidence confirming that Hg transfer across the placenta does occur and that fetal organs are adversely affected by this transfer, as established in animal and limited human fetal studies referred to in this article.



## Results

Table 3 shows the effective concentration of Hg in $\mu g\,kg^{-1}$ available to impact a fetus, corrected for fetal body weight, following a single fish consumption event or single vaccination throughout the gestation period. Graphical representation of the data is

Table 3. Fetal mercury exposure ($\mu g\,kg^{-1}$) throughout gestation as a function of fish consumption and maternal influenza vaccine administration.

| Fetal | | | Single 150 g standard fish portion consumption[h] | | | Single flu vaccination | | |
|---|---|---|---|---|---|---|---|---|
| | | | Fetal Hg exposure ($\mu g\,kg^{-1}$) | | | Fetal Hg exposure ($\mu g\,kg^{-1}$) | | |
| | | | Hypothetical placental elimination | | | Hypothetical placental elimination | | |
| Age (week) | Length (mm) | Weight (g) | @0%[b] | @90%[c] | @99%[d] | @0%[e] | @90%[f] | @99%[g] |
| 1 | 1[a] | 0.025[a] | 6,000,000 | 600,000 | 60,000 | 1,000,000 | 100,000 | 10,000 |
| 2 | 3[a] | 0.25[a] | 600,000 | 60,000 | 6000 | 100,000 | 10,000 | 1000 |
| 4 | 7 | 0.5 | 300,000 | 30,000 | 3000 | 50,000 | 5000 | 500 |
| 8 | 14 | 1.0 | 150,000 | 15,000 | 1500 | 25,000 | 2500 | 250 |
| 12 | 75 | 15 | 10,000 | 1000 | 100 | 1667 | 167 | 16.7 |
| 16 | 160 | 100 | 1500 | 150 | 15.0 | 250 | 25 | 2.5 |
| 20 | 240 | 300 | 500 | 50 | 5.0 | 83 | 8.3 | 0.83 |
| 24 | 300 | 650 | 231 | 23.1 | 2.31 | 39 | 3.8 | 0.38 |
| 28 | 350 | 1100 | 136 | 13.6 | 1.36 | 23 | 2.3 | 0.23 |
| 32 | 410 | 1700 | 88 | 8.8 | 0.88 | 15 | 1.5 | 0.15 |
| 36 | 460 | 2400 | 63 | 6.3 | 0.63 | 10 | 1.0 | 0.10 |
| 38 | 500 | 3300 | 45 | 4.5 | 0.45 | 7.6 | 0.76 | 0.076 |

Notes: [a]Interpolated from measured data (Fetal Development, Medline Plus 2009).
[b]Weight of Hg per 150 g fish portion at Maximum Fish Advisory Limit of $1\,mg\,kg^{-1}$ Hg (w/w): 150 µg Hg.
[c]Weight of Hg per 150 g fish portion @90% Hypothetical Placental Elimination: 15 µg Hg.
[d]Weight of Hg per 150 g fish portion @99% Hypothetical Placental Elimination: 1.5 µg Hg.
[e]Total weight of Hg per 0.5 mL single Influenza vaccine dose: 25 µg Hg.
[f]Weight of Hg per 0.5 mL single Influenza dose @90% Hypothetical Placental Elimination: 2.5 µg Hg.
[g]Weight of Hg per 0.5 mL single Influenza dose @99% Hypothetical Placental Elimination: 0.25 µg Hg.
[h]Data assume only one 150 g standard fish portion is eaten by a pregnant female at any specific week confinement with mercury content at the maximum limit of $1\,mg\,kg^{-1}$.

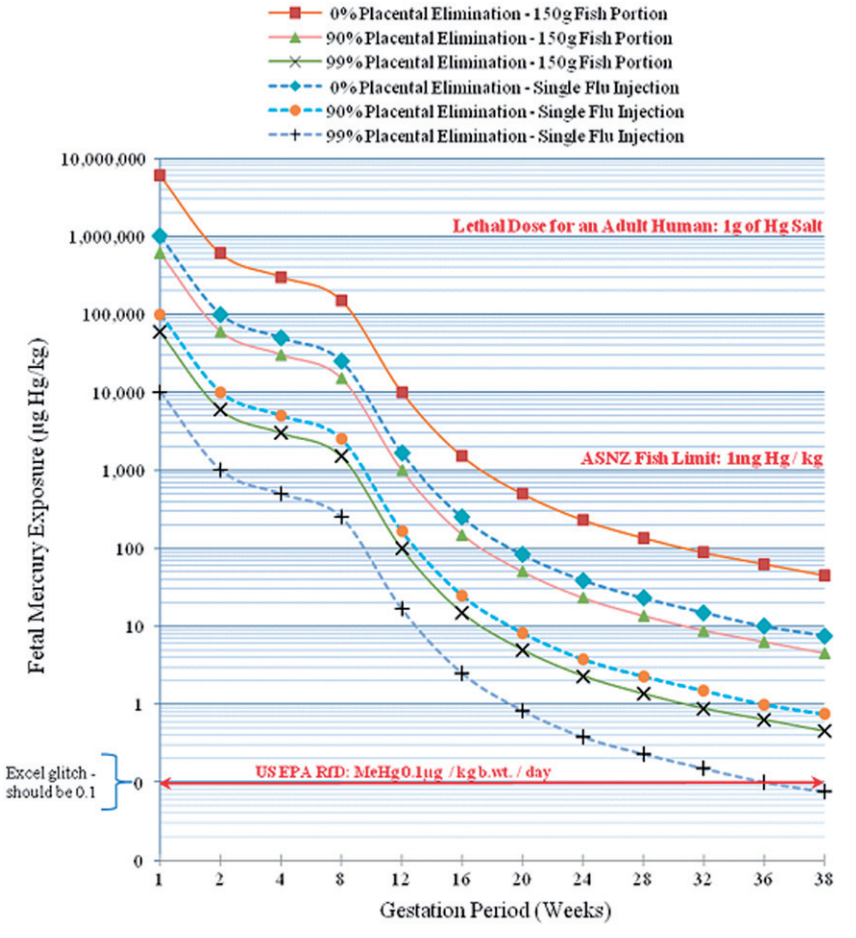


Figure 1. Fetal mercury exposure ($\mu g\,kg^{-1}$) as a function of maternal exposure and placental clearance following a single fish consumption event or single influenza vaccine administration.

presented in Figure 1, and reveals the extent of Hg exposure to a developing fetus resulting from consumption of a single standard fish portion or a single maternal vaccination throughout the gestation period. It is of particular interest to note that all exposure curves (based on all placental transfer scenarios save for the last two weeks gestation at 99% placental elimination – flu injection) remain in excess of the USEPA Daily RfD for MeHg.

## Discussion

When considering our graphical data it is immediately apparent that fetal Hg exposure in all of the scenarios exceeds the USEPA RfD of 0.1 µg MeHg per kg b.wt per day. What is also apparent is that the greatest danger to a developing fetus from Hg exposure to the mother occurs during the first few weeks following conception (during the differentiation phase of embryonic development), when body-weight of the embryo/fetus is smallest, Hg concentration is highest, fetal brain development is fastest, and the embryo/fetus is most susceptible to damage (Fetal Development, Medline Plus 2009). Further, it should also be

noted that human fetal brain development is a dynamic process wherein significant rates of growth occur in the different brain structures, throughout the whole gestation period (Huang et al. 2009). Thus, there is no one single period of fetal brain development that could be considered to be less sensitive to Hg.

It is important to acknowledge limitations in our analyses and a critical one relates to the notion that most Hg a pregnant mother receives may be distributed throughout her body organs in preference to the developing fetus, thus severely reducing the likelihood for Hg to be available for transfer across the placenta to impact a developing embryo/fetus. Although one acknowledges 100% placental Hg transfer scenario is an extreme value and, therefore, unlikely, there is compelling evidence to suggest our remaining scenarios (1% and 10% placental Hg transfer) are more likely possibilities, if not overly conservative.

Dock, Rissanen, and Vahter (1994) determined in Syrian golden hamsters after a single oral dose of $^{203}$Hg-labeled MeHgCl and a single intravenous injection of $^{203}HgCl_2$ that fetal content of $^{203}$Hg in hamsters administered radio-labeled MeHg on gestational day 2 and 9 corresponded to 1.3% and 4.6% of the administered dose, respectively. The distribution of $^{203}$Hg in the fetus was more even than in the dam and the concentration of $^{203}$Hg in the fetal brain, liver, and kidneys was similar to that of the placenta. Hg was found in maternal liver (18% of total Hg), kidneys (31%), placenta (21%), and fetal liver (3%).

These findings establish the following factors:

(1) Maternal Hg events impacted fetal organs corresponding to overall transfer rates of 1% and 5% of the maternal dose (gestational days 2 and 9, respectively).
(2) MeHg toxicokinetic studies using a Syrian golden hamster model provide better comparisons for human MeHg impacts than do rat models because rat fetuses are unable to demethylate MeHg and because of preferential binding of MeHg to rat hemoglobin: ''The brain/blood ratio of Hg in the rat is two orders of magnitude lower (0.06–0.08) than man (3–30) and other animals, such as the Syrian golden hamster (2–4).''
(3) Comparison of metabolically formed inorganic Hg in the fetal liver following intravenous injection of $^{203}HgCl_2$ compared to that found in fetal liver following oral administration of MeHg to the dam (both on gestation day 9) yielded twice the level of fetal liver inorganic $^{203}$Hg suggestive of there being a doubling of impact to the fetal liver for the intravenous route over that of the oral route.
(4) The higher content of $^{203}$Hg in the fetal brain compared to the corresponding level for the dam yielded 5.8% and 0.2%, respectively, of the total body burden of $^{203}$Hg; a 29-fold increase for the fetal brain over that of the dam. This confirms that much higher transfer rates do, in fact, occur for the CNS, and particularly for the fetal brain. The fetal brain, therefore, has the ability to preferentially accumulate Hg compared to the maternal brain following Hg impact to the maternal female.

If these are valid conclusions to draw from this study then our hypothesized fetal transfer rate of 10% of the initial maternal dose gains much realistic support for our scenario at this transfer level. Similarly, consideration of already established transfer characteristics for a pregnant female and embryo/fetus pair from animal and human fetus studies have determined the following factors:

(1) Developing embryo/fetus or placenta accumulates Hg from the mother's blood at a higher concentration than the corresponding maternal blood, at the time of exposure (Nordberg et al. 2007; Lippmann 2009).

(2) Hg crossing the placenta accumulates in various developing fetal organs to differing extents but specifically targets the fetal brain and kidney accumulating at levels 6–10 times higher than that found in other organs of the same fetus (Nordberg et al. 2007; Lippmann 2009).
(3) The human fetus is more at risk of MeHg impacts transferred through the placenta than current animal models (rats, primates, or hamsters) indicate since the human placenta appears to facilitate preferential distribution of Hg to a developing embryo or fetus to a greater extent compared to placental transfer levels determined from animal studies (Nordberg et al. 2007).

*USEPA RfD MeHg daily limit*

It is instructive to draw comparisons between our data and conclusions made in a USEPA study examining the distribution of concentrations of Hg in blood of women of child-bearing age (USEPA 2009), wherein it is concluded that

(1) USEPA RfD for MeHg is 0.1 μg per kg b.wt per day, which is approximately equivalent to a blood Hg concentration of 5.8 parts per billion (ppb).
(2) Current research indicates that there is no safe level of MeHg in blood within the range of exposures measured in the human studies of the health effects of Hg.
(3) The EPA determined that children born to women with blood Hg concentrations above 5.8 $\mu g\,L^{-1}$ are at some increased risk of adverse health effects.

Despite this final assertion of risk only presenting beyond blood-Hg concentrations greater than 5.8 $\mu g\,L^{-1}$, there is evidence that at, or some level below, this level, neurological damage to the unborn child may still occur (Greim and Snyder 2009). Further, caution needs to be exercised in relying on the "5.8 $\mu g\,L^{-1}$ blood-mercury level" as being a marker for determining the possibility of experiencing adverse impacts above this blood-Hg level due to the following estimates:

(1) The "5.8 $\mu g\,L^{-1}$ blood-mercury level" was determined from the original study which identified this value was actually 58 $\mu g\,L^{-1}$ blood-Hg, and following application of a statistical "10% safety margin," it was reduced to the present blood-Hg level, as reported in the USEPA study. Significant caution should, therefore, be exercised by setting a "bench mark" for a "mercury in blood limit above which abnormalities may likely be expected to occur," when based entirely on a statistical consideration which has no basis on whether or not Hg impacts will or will not actually take place at this reduced blood-Hg level.
(2) Organic Hg in cord blood was correlated with postnatal neurobehavioral effects; blood levels of 5.8 $\mu g\,L^{-1}$ in children were associated with losses in IQ (Greim and Snyder 2009). Therefore, neurological damage was shown to have already occurred at (or some level below) the USEPA Hg in blood level of 5.8 $\mu g\,L^{-1}$.

Further, while blood concentrations are highly useful, they do not reflect Hg retained in the brain, where Hg from vapor inhalation has a half-life of several years (Lippmann 2009), it, therefore, follows that investigators should not use blood-Hg levels as a measure of total Hg body burden, as has been the case in some recent scientific investigations.

*Graphical fetal Hg exposures*

Considering that experimental support for the likelihood of 1% and 10% placental transfer scenarios were established, it is further proposed these are highly likely to be

conservative estimates because of the present lack of understanding of the complete placental transfer mechanisms, or knowledge of all of the transporting molecules responsible, and greater sensitivity and susceptibility of the human fetus to exposure from MeHg transferred maternally, compared to data determined from experiments on lab animals and primates. Should this subsequently prove to be the case then the implications for a developing fetus after exposure to Hg (on a weight adjusted basis) during the first week of gestation from either a single Hg-preserved influenza vaccine or from the pregnant woman eating a single *standard* portion of fish, resulting in a Hg impact that is somewhere between our 100% and 10% limits, are so grave they warrant stating,

- At 100% placental transfer (0% placental reduction) scenario:
  - Though unlikely to occur at this level, fish consumption case is >seven orders of magnitude higher than USEPA RfD daily limit.
  - Vaccination case is seven orders of magnitude higher than USEPA RfD daily limit.
  - The fetal Hg exposure from a single influenza injection given to the mother at this stage potentially equates to a lethal adult dose of an equivalent Hg salt (Nordberg et al. 2007). Fetal Hg exposure from consumption of a single fish portion is potentially six-fold higher than the lethal adult dose for this same Hg salt.
- At 10% placental transfer (90% placental reduction) scenario:
  - This transfer rate is highly likely and has experimental support that it does occur at this level. Fish consumption case is greater than six orders of magnitude higher than USEPA RfD daily limit. Vaccination case is six orders of magnitude higher than USEPA RfD daily limit.
- At 1% placental transfer (99% placental reduction) scenario:
  - Transfer rates have been experimentally confirmed to occur above this level. Fish consumption case is still greater than five orders of magnitude above USEPA RfD daily limit.
  - Vaccination case is still five orders of magnitude above USEPA RfD daily limit.

Additional risk to a fetus may occur because this time coincides with a period when a pregnant woman may be unaware she is pregnant and, therefore, would be unable to practice any Hg avoidance as advised by various agencies including the World Health Organization. Given the extremely high concentration of Hg from fish or vaccines available to impact a fetus within the first few weeks following conception, there is likely a proportion of pregnancies that do not survive this event which may go unnoticed by the mother. While such Hg induced terminations are tragic, they are unlikely to represent the entire population of victims of this exposure as, of the children who do survive the initial Hg exposure, some proportion will likely be found to have developmental problems, delays, or even devastating conditions such as cerebral palsy or autism spectrum disorder. Given the magnitude of these potential Hg impacts on a fetus and the high degree of lack of knowledge presently surrounding maternal transfer of Hg across the placenta, one should not continue to ignore such horrendous potential Hg impacts to a developing human embryo/fetus.

*Recommendation to authorities*

Our data suggest that health and medical regulators would be wise to undertake a comprehensive re-evaluation of the potential consequences of Hg exposure to a developing fetus from the perspective of fetal body weight rather than from that of the maternal adult recipient. Authorities should also re-evaluate all fish/crustacea advisories for pregnant women given the above calculations for our scenarios. Even under the most conservative of scenarios presented (just 1% placental transfer of Hg to the developing child), our analyses suggest that fetuses right up to the gestational age of week 38 may be exposed to doses of Hg far greater than any generally accepted safe levels, and at a developmental period of high vulnerability.

Health officials and regulators therefore need to be concerned by recommending that weekly consumption of standard portions of contaminated fish (or crustacea), for all levels of Hg up to the maximum limit, is "safe" for the "most at risk group in a community." Clearly, such simplistic advisories are not supportive of health protection for the most at risk groups in a community when Hg elimination rates for the human fetal brain, CNS, and major organs are considered together with what are, supposedly, "*acceptable*" consumption frequencies.



## Conclusion

Current Hg exposure advisories do not appear to account for (or offer protection of) developing embryos and fetuses which are exposed to Hg via maternal (placental) transfer. Data presented suggest that fetal Hg exposure is intolerably high even under conservative scenarios of maternal exposure and placental transfer. Critical perusal of the data (Table 3 and Figure 1) demonstrates that available Hg crossing the placenta throughout the entire gestation period following a single fish consumption event ranges between 6 and 6,000,000 times higher than the USEPA RfD maximum recommended daily exposure for MeHg, even under conservative exposure and placental transfer scenarios. The equavalent fetal Hg exposure following a single SEMT-preserved maternal influenza vaccination ranges between 1 and 1,000,000 times higher than the USEPA RfD maximum. There is, therefore, no safe level of maternal transfer of Hg to a developing human embryo/fetus.

## Competing interests

IAB is Technical Director of Hg Recoveries Pty Ltd, an Australian company involved in remediation of environmental Hg contamination.

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