



# A Review of Thimerosal in Vaccines

**WHO Informal Consultation to develop further guidance  
on vaccines for the UNEP-convened Intergovernmental  
Negotiating Committee Meeting 4 (INC4)**

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# Outline

- Provide an update on the safety profile of thimerosal
  - In vitro, in vivo animal, human, and epidemiological data
  - USFDA quantitative risk assessment
- Address the biological plausibility of thimerosal contributing to cumulative mercury toxicity



# Safety Profile for Thimerosal (based on data until 2008)

# Background

- United States Code of Federal Regulations (CFR) requires, in general, the addition of a preservative to multi-dose vials of vaccines, and the preservative used “[s]hall be sufficiently non-toxic so that the amount present in the recommended dose of the product will not be toxic to the recipient” [21 CFR 610.15(a)]
- The FDA Modernization Act (1997) required the Agency “...to compile a list of drugs and foods that contain intentionally introduced mercury compounds and...[to] provide a quantitative analysis...”
- In July 1999, the US Public Health Service and American Academy of Physicians coauthored a statement to remove thimerosal from vaccines as soon as possible, as a precautionary measure

# In vitro data

- Thimerosal has been tested in numerous in vitro assays in nanomolar to micromolar range
- In vitro effects have been observed on cellular/intracellular endpoints (e.g., glutathione levels, signaling pathways, cytotoxicity) across dose range and study
- While useful for hypothesis generation, the utility for making quantitative predictions of in vivo effects is limited

# Animal data

- Effects at very low doses overlapping with human infant dose range were mixed
  - 1 or 6  $\mu\text{g}/\text{kg}$  bw/day in adult squirrel monkeys (intranasal x 6 months) – no histopathology despite distribution to brain and kidney (Blair 1975)
  - 5.6-14.2  $\mu\text{g}/\text{kg}$  bw in neonatal mice (x 4 doses im) – no effects in normal strains; genetically modified autoimmune disease-susceptible pups exhibited decreased body weight gain and several behavioral and neuropathological effects not observed in normal strains (Hornig et al 2004)
- 25-fold higher doses or more: changes in body weight gain, neurotoxicity (motor incoordination, neuropathology), renal toxicity, death
- Taken together, doses much higher than human exposure levels and/or or repeated exposures, were necessary to induce toxicity in animals

# Clinical data

- Case reports of clinical neurotoxicity following accidental poisonings with ethylmercury or suicide attempts using thimerosal
- However, doses in these cases ranged from 0.5-83 mg/kg bw ( $\geq 250$ -fold higher than in US influenza vaccines)
- Allergic reaction at site of injection has been only demonstrable adverse effect in humans at levels found in vaccines (doses  $\leq 1-4 \mu\text{g/kg}$  bw)

# Epidemiological data

- Studies “consistently provided evidence of no association between thimerosal-containing vaccines and autism” (US Institute of Medicine Report 2004)
- Well-designed epidemiological studies (2004-2008) have failed to find a causal relationship between prenatal, neonatal, or postnatal exposures to thimerosal in vaccines and a host of neuropsychological outcomes, including autism





# Key Studies Conducted Since 2008

# Pharmacokinetic (in humans)

- Pichichero et al (2008): blood mercury levels in normal-weight newborns and infants receiving multiple vaccinations (+thim: up to 57.5  $\mu\text{g}$  Hg per dose) in Argentina returned to pre-vaccination levels 30 days post dose ( $t_{1/2} = 3.7$  days)
- Pichichero et al (2009): blood mercury levels in premature and low birth weight infants vaccinated with single-dose HBV (+thim: 12.5 or 32.5  $\mu\text{g}$  Hg) in Argentina returned to pre-vaccination levels 30 days post dose ( $t_{1/2} = 6.3$  days)
- Barregard et al (2011): adults vaccinated with staphylococcus toxoid (+thim: 25  $\mu\text{g}$  Hg) vaccine (once every 3-4 weeks) for at least a year did not accumulate mercury in blood ( $t_{1/2} = 5.6$  days)
- Conclusion: thimerosal mercury is cleared from blood relatively quickly in infants and adults and excreted mainly in feces

# Toxicology (animal)

- Berman et al (2008): up to 56-142  $\mu\text{g}/\text{kg}$  bw in neonatal mice (x 4 doses im) – no behavioral effects in genetically modified autoimmune disease-susceptible pups mentioned earlier
- Hewitson et al (2010): delays in attaining root, suck, and snout (survival) reflexes in neonatal macaques vaccinated with HBV (+thim) at 4  $\mu\text{g}$  Hg/kg bw (x 1 dose im) on day of birth
  - Magnitude of dose identical to single HBV dose in infants outside US
  - Results preliminary and follow-up study underway, according to authors
- Conclusion: preliminary evidence of behavioral neurotoxicity in infant macaques following a single dose of HBV containing a clinically relevant dose of thimerosal on day of birth

# Toxicology (contd)

- Olczak et al (2011): preliminary neuropathology in neonatal rat brain (PND 7,9,11,15) at doses  $\geq 12 \mu\text{g Hg/kg bw}$  (x 4 doses im)
  - Dosing regimen not ideal (multiple), but magnitude of doses approaching single dose range for LBW infants outside US (5-12.5  $\mu\text{g Hg/kg bw}$ )
  - Pathology slides should be peer-reviewed to confirm results
  - Neuronal, behavioral alterations in two other studies by same group at similar or higher doses (Olczak et al 2009, 2011)
  - No effect on social interaction except at much higher doses
- Conclusion: preliminary evidence of neuropathology in neonatal rats exposed to clinically relevant doses of thimerosal mercury; however, short dosing interval makes extrapolation of results to human situation with much longer dosing interval difficult

# Summary of data since 2008

- Preliminary evidence of neurotoxicity in neonatal rat and macaque has been published, but studies have limitations so replication is required before they could be used to characterize ethylmercury toxicity in animals
- Well-designed epidemiological studies have failed to find a causal relationship between prenatal, neonatal, or postnatal exposures to thimerosal in vaccines and a host of neuropsychological outcomes, including autism



# Quantitative Risk Assessment

## Approach taken in 1999 semi-quantitative risk assessment (USFDA)

- Comparison of cumulative exposures to thimerosal from vaccines with a regulatory standard for dietary MeHg
- However, the routes and lengths of exposure for each compound are very different
- In addition, each compound has different behavior in the body (pharmacokinetics) and, as a result, differential toxicities

# Comparison of thimerosal and MeHg pharmacokinetics

- Peak blood levels of mercury are similar following single doses of thimerosal or MeHg; however, mercury from thimerosal is cleared from the blood faster than mercury from MeHg leading to a lower body burden of mercury following exposure to thimerosal
- Mercury accumulates in the brain and is converted to inorganic mercury following repeated exposures to either thimerosal or MeHg; however, brain mercury levels from MeHg are higher than those from thimerosal, and mercury is retained in the organic form (the putative CNS toxicant) in brain longer following MeHg exposures
- Conclusion: more toxicity would be expected from equivalent exposures to MeHg relative to thimerosal



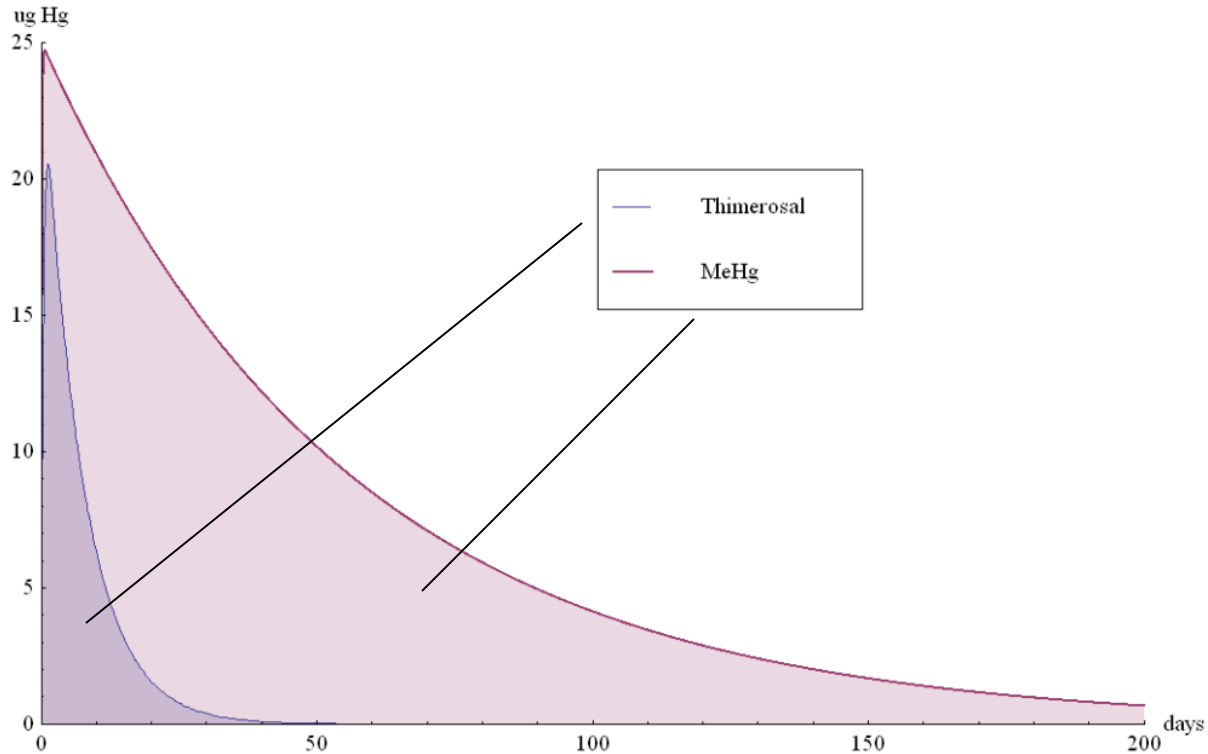
# Current USFDA approach to QRA for thimerosal

- Well-designed epidemiological studies have failed to find a causal relationship between prenatal, neonatal, or postnatal exposures to thimerosal in vaccines and a host of neuropsychological outcomes, including autism
- Half-life of ethylmercury in blood has been shown to be similar in human and macaque infants
- USFDA developed a kinetic model to facilitate interpretation of animal and human toxicokinetic studies

# FDA Pharmacokinetic Model

- Modeled original blood mercury data from Burbacher et al (2005) study, in which infant macaques were exposed to either thimerosal (im) or MeHg (po), using sophisticated statistical methods
- Applied derived pharmacokinetic parameters to estimate body burden of mercury in
  - US infants exposed (im) to thimerosal following a single dose from an influenza vaccine packaged in multidose vials
  - US infants exposed (po) to an identical single dose of MeHg
- Margin of safe exposure estimated by comparing resulting peaks and areas under each curve

# Comparison of a single 25 $\mu$ g dose



# Remaining uncertainties in QRA for thimerosal

- Data from human epidemiological studies of thimerosal in vaccines are reassuring
- However, acute exposures to thimerosal in infants outside US begin at birth, and no safe level for thimerosal exposure has been established as it has for dietary methyl mercury
- This would provide a standard against which to compare results from any future studies involving blood or tissue mercury levels, or assessment of contribution to cumulative mercury toxicity
- May be difficult to accomplish without extensive animal studies, but constructing a quantitative model of the differences between ethyl and methyl mercury might help bridge the gap

# Possible next steps for kinetic model development and QRA

- Model mercury plasma concentrations measured in human infants following single HBV, DTaP vaccinations containing thimerosal (Pichichero 2002, 2008, 2009 data) and compare to infant macaque (Burbacher et al 2005 data)
- Model levels of mercury in brain following exposures to thimerosal in infant macaques, since blood levels underestimate brain levels of mercury
  - Requires development of model of ethyl mercury transfer across the blood brain barrier
  - Relevant for cumulative mercury toxicity assessment
- Model toxicity of ethylmercury on brain
- Goal: link external dose, plasma/brain concentrations, and neurotoxicity to make quantitative predictions in humans

# Summary of quantitative risk assessment for thimerosal

- Ethyl and methyl mercury toxicity are not directly comparable and methyl mercury is likely more toxic than ethyl mercury
  - Further development of kinetic model might provide a tool for better interpretation of any future animal or human toxicokinetic studies and quantitative risk assessment
  - Without accounting for in vivo kinetic and toxicological differences, in vitro studies are not helpful
  
- Recent preliminary toxicity data in animals have identified a potential hazard at relevant WHO exposures to thimerosal that would require further study for confirmation



# Biological Plausibility of Thimerosal Contributing to Cumulative Mercury Toxicity

# Cumulative toxicity considerations

- Which toxicity? which organ? which species of mercury?
- There are several species of mercury to which humans may be exposed
- Exposure levels to these species varies based on country, medicinal practices, etc



# Species of mercury

- $\text{Hg}^0$  (elemental, inorganic)
- $\text{Hg}^{1+}$  (inorganic)
- $\text{Hg}^{2+}$  (inorganic)
- $\text{MeHg}^+$  (organic)
- $\text{EtHg}^+$  (organic)
- $\text{PhHg}^+$  (organic)

# Additional cumulative toxicity considerations

- Epidemiological studies that evaluated the safety of thimerosal in vaccines have included (unmeasured) environmental exposures to other sources of mercury in the studied populations, and no evidence of harm was detected
- Routes and realistic levels of exposure, as well as in vivo metabolic information for each mercury species would need to be ascertained and integrated to properly assess cumulative risk using a QRA framework
- Since each mercury species can cause distinct, and sometimes overlapping toxicities, differential hazard information would also need to be taken into account

# Overall summary

- A complete Quantitative Risk Assessment model accounting for the kinetic and toxicological differences between ethyl and methyl mercury might be a useful area of further research
  - Would incorporate any future rigorously conducted and confirmed animal studies demonstrating toxicity from thimerosal
  - Contribute to cumulative toxicity assessment from exposure to multiple mercury species
- Well-designed epidemiological studies have failed to find a causal relationship between prenatal, neonatal, or postnatal exposures to thimerosal in vaccines and a host of neuropsychological outcomes, including autism
- Benefits of vaccination with thimerosal containing vaccines outweigh any potential risk from thimerosal

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