Thimerosal in Vaccines: Some Recent Research

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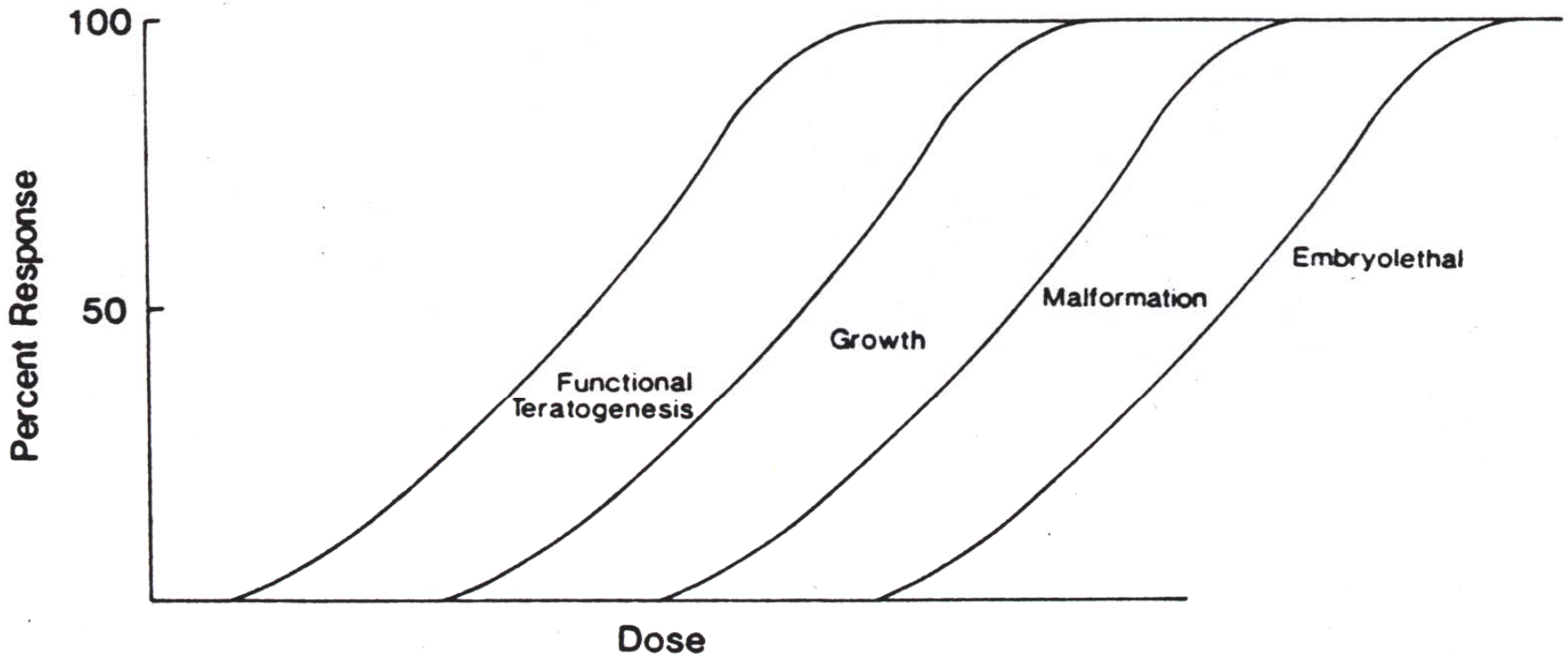
Behavioral Teratogens/Developmental Neurotoxicants (Functional Effects)



Idealized dose-response curves for the major manifestations of teratogenesis.

Behavioral Teratogens/Developmental Neurotoxicants (Functional Effects)

List of Human Behavioral Teratogens/Developmental Neurotoxicants

Environmental Chemicals

Lead

Mercury

PCBs

Manganese

Pesticides

Drugs of Abuse

Ethanol

Cocaine

Maternal Medications

Epilepsy

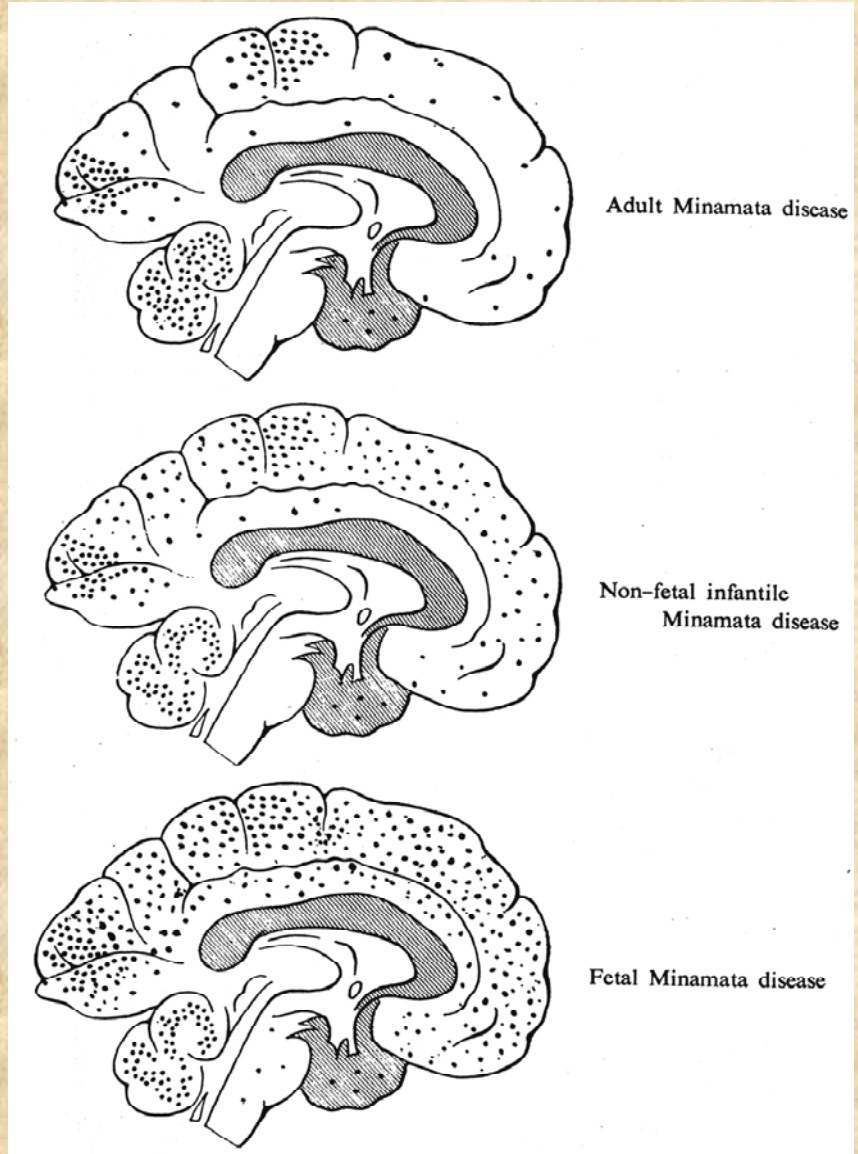
Depression

Methylmercury -Fetal Minamata Disease

http://www.geocities.com/minoltaphotographyw/williameugenesmith.html

Methylmercury -Pathology of Minamata Disease

Comparison of the distribution of lesions among the adult, non-fetal infantile and fetal infantile Minamata disease.



Minamata Disease, Study Group of Minamata Disease, Kumamoto University, Japan, 1968

Methylmercury -Functional Effects

Faroe Island Study

Cognitive Deficit in 7-Year-Old Children with Prenatal Exposure to Methylmercury

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Neurotoxicology and Teratology, Vol. 19, 417-428, 1997

Methylmercury -Functional Effects

Faroe Island Study

Cord Blood Mercury Levels Associated with Poor Performance on Neuropsychological Tests.

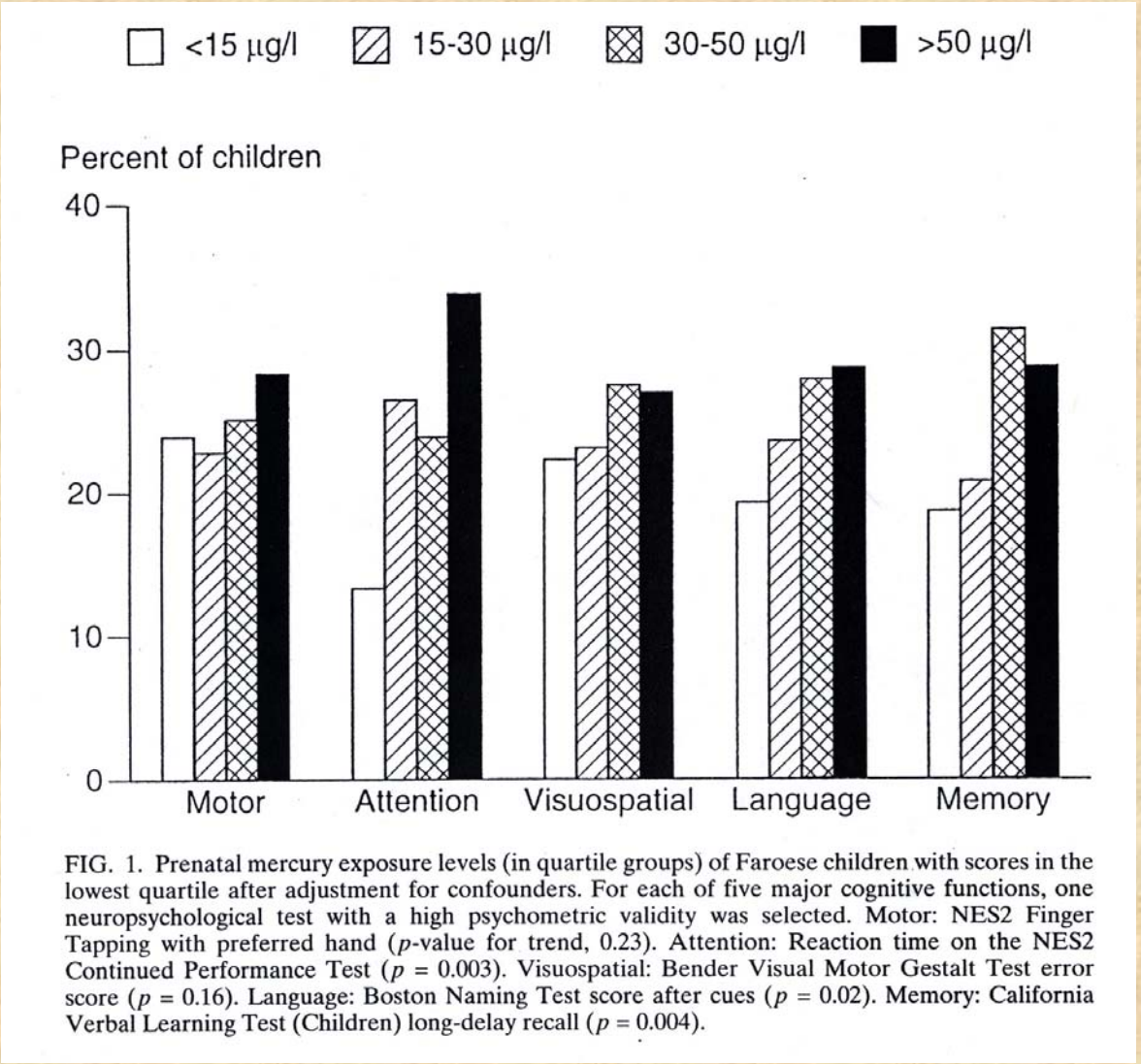
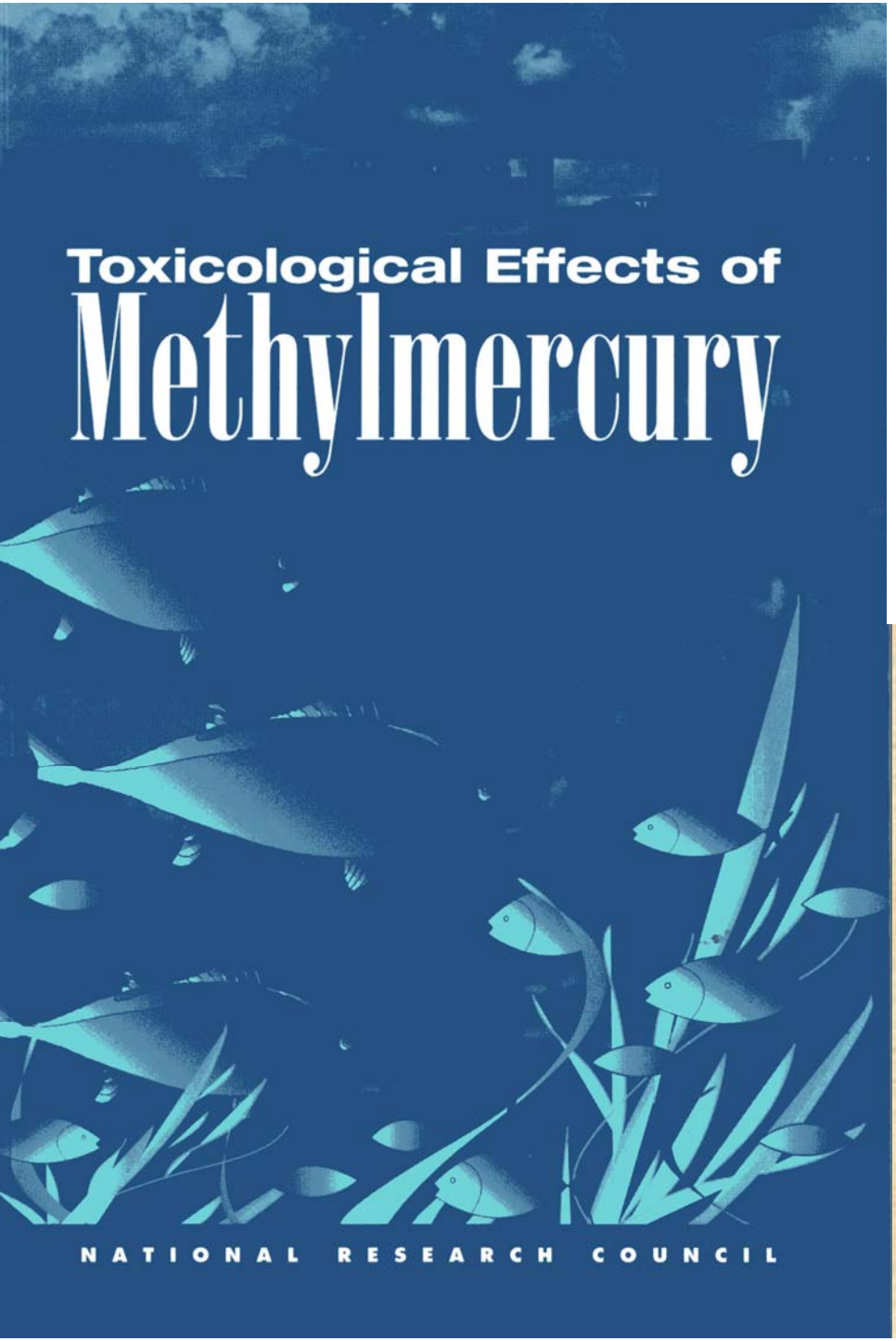


FIG. 1. Prenatal mercury exposure levels (in quartile groups) of Faroese children with scores in the lowest quartile after adjustment for confounders. For each of five major cognitive functions, one neuropsychological test with a high psychometric validity was selected. Motor: NES2 Finger Tapping with preferred hand (p-value for trend, 0.23). Attention: Reaction time on the NES2 Continued Performance Test ($p = 0.003$). Visuospatial: Bender Visual Motor Gestalt Test error score ($p = 0.16$). Language: Boston Naming Test score after cues ($p = 0.02$). Memory: California Verbal Learning Test (Children) long-delay recall ($p = 0.004$).

Neurotoxicology and Teratology, Vol. 19, 417-428, 1997



COMMITTEE ON THE TOXICOLOGICAL EFFECTS OF METHYLMERCURY

ROBERT A. GOYER (*Chair*), University of Western Ontario (Professor, Emeritus), Chapel Hill, North Carolina
H. VASKEN APOSHIAN, University of Arizona, Tucson, Arizona
LENORE ARAB, University of North Carolina, Chapel Hill, North Carolina
DAVID C. BELLINGER, Harvard Medical School, Boston, Massachusetts
THOMAS M. BURBACHER, University of Washington, Seattle, Washington
THOMAS A. BURKE, The Johns Hopkins University, Baltimore, Maryland
JOSEPH L. JACOBSON, Wayne State University, Detroit, Michigan
LYNDA M. KNOBELOCH, State of Wisconsin Bureau of Environmental Health, Madison, Wisconsin
LOUISE M. RYAN, Dana-Farber Cancer Institute, Boston, Massachusetts
ALAN H. STERN, New Jersey Department of Environmental Protection, Trenton, New Jersey

EPA Safety Standard for Methylmercury Intake (RfD) is based on Human Studies of Exposure through Fish Consumption During Pregnancy

In 2000, NAS panel supported EPA's action to lower Safety Standard (RfD) to 0.1ug/kg/day

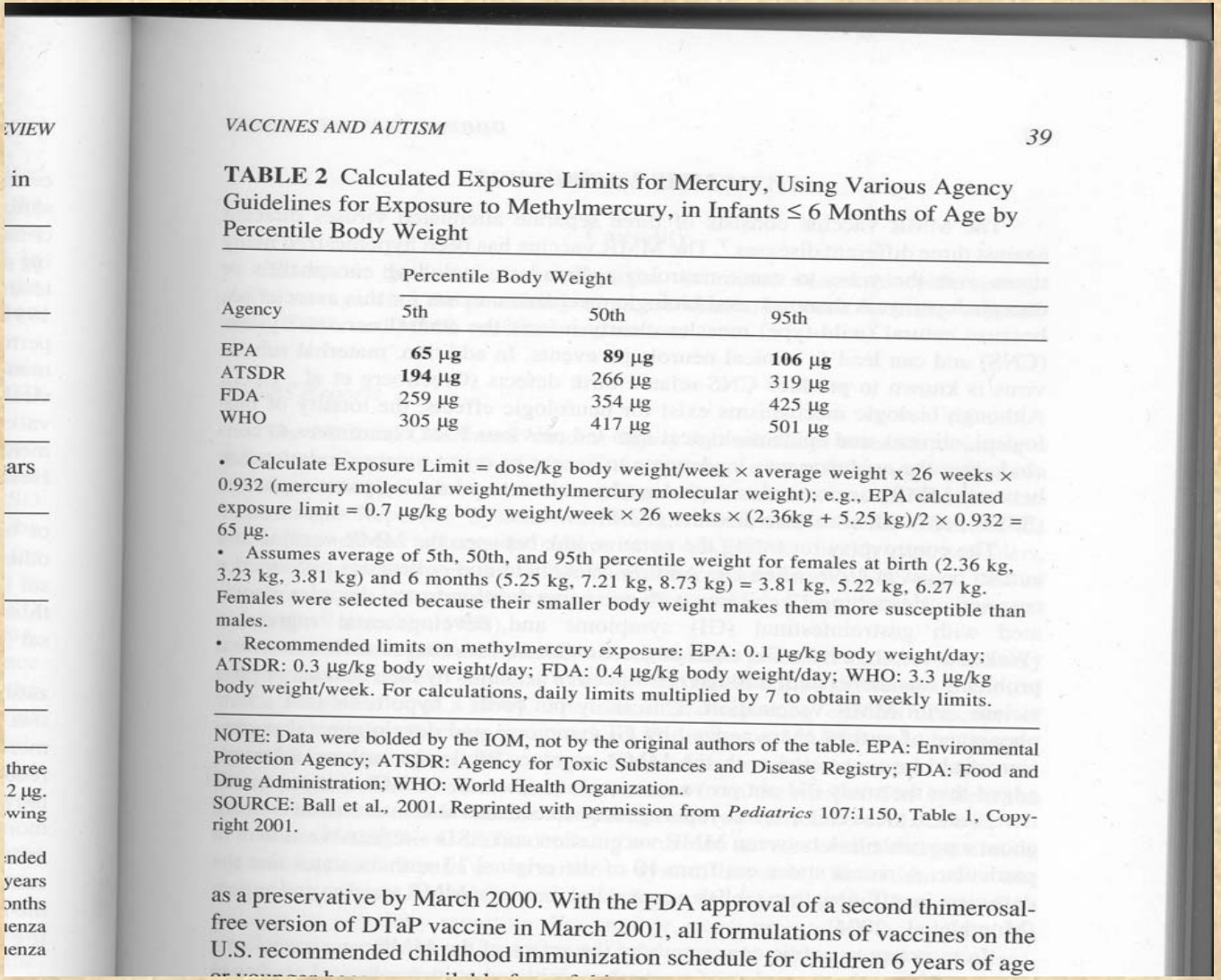
The Controversy: Thimerosal in Vaccines

TABLE 2 Estimated Exposure to Mercury from Vaccines in U.S. Infants in 1999 and in 2001. (≤ 6 Months)

Vaccines	1999 Maximum Mercury Dose (µg)	2001 Maximum Mercury Dose (µg)
3 doses of DTaP	75.0	0
3 doses of Hep B	37.5	0
3 doses of HIB	75.0	0
3 doses of IPV	0	0
[1 dose of influenza] *(selected populations)	[12.5]	[12.5]
TOTAL	**187.5 [200]**	**[12.5]**

The Controversy: Thimerosal in Vaccines

Mercury exposure from vaccines during first 6 months of life exceeds EPA safety standard for methylmercury



VACCINES AND AUTISM 39

TABLE 2 Calculated Exposure Limits for Mercury, Using Various Agency Guidelines for Exposure to Methylmercury, in Infants ≤ 6 Months of Age by Percentile Body Weight

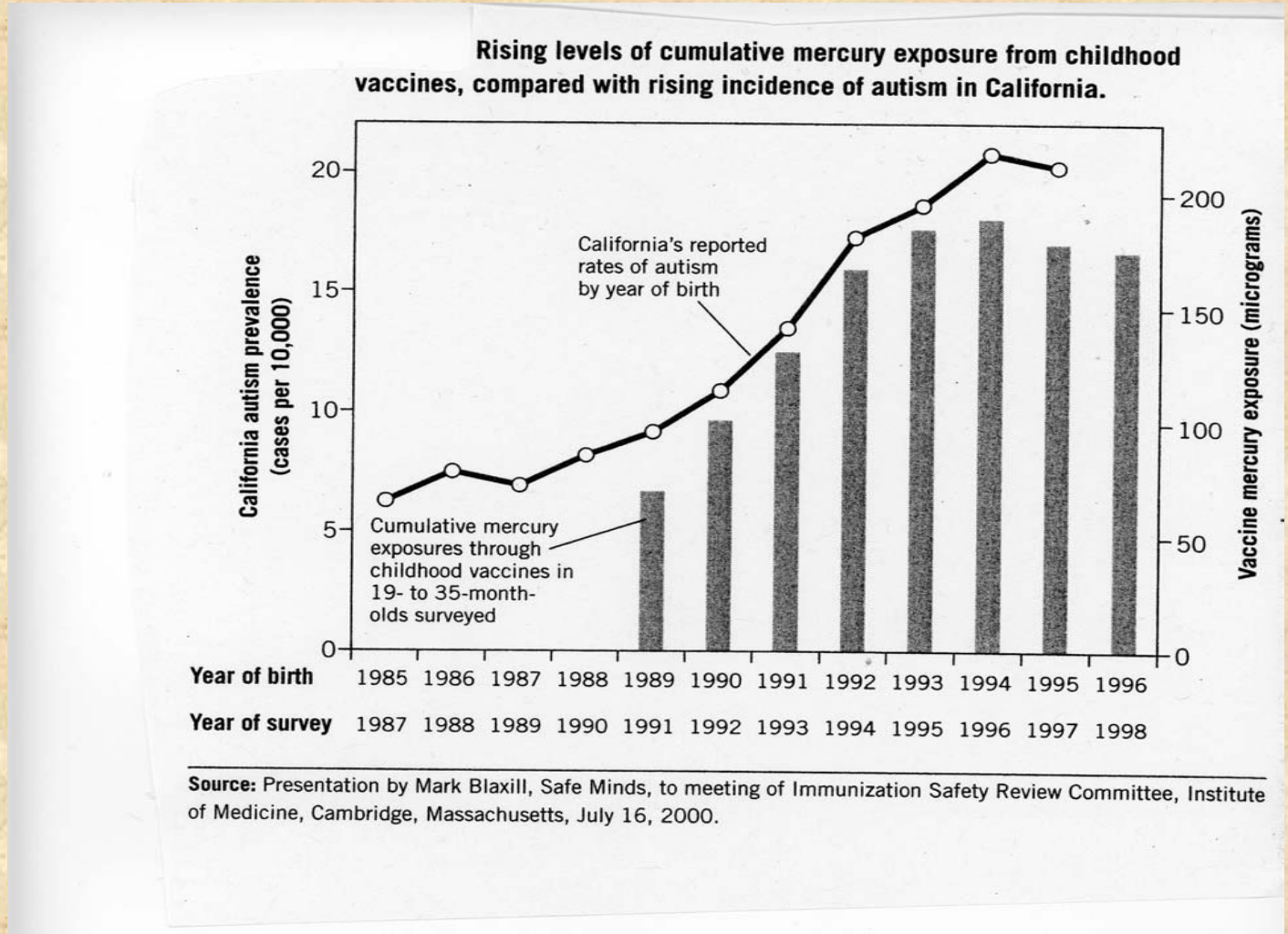
	Percentile Body Weight		
Agency	5th	50th	95th
EPA	**65 µg**	**89 µg**	**106 µg**
ATSDR	**194 µg**	266 µg	319 µg
FDA	259 µg	354 µg	425 µg
WHO	305 µg	417 µg	501 µg

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

NOTE: Data were bolded by the IOM, not by the original authors of the table. EPA: Environmental Protection Agency; ATSDR: Agency for Toxic Substances and Disease Registry; FDA: Food and Drug Administration; WHO: World Health Organization.
SOURCE: Ball et al., 2001. Reprinted with permission from *Pediatrics* 107:1150, Table 1, Copyright 2001.

as a preservative by March 2000. With the FDA approval of a second thimerosal-free version of DTaP vaccine in March 2001, all formulations of vaccines on the U.S. recommended childhood immunization schedule for children 6 years of age

The Controversy: Thimerosal in Vaccines

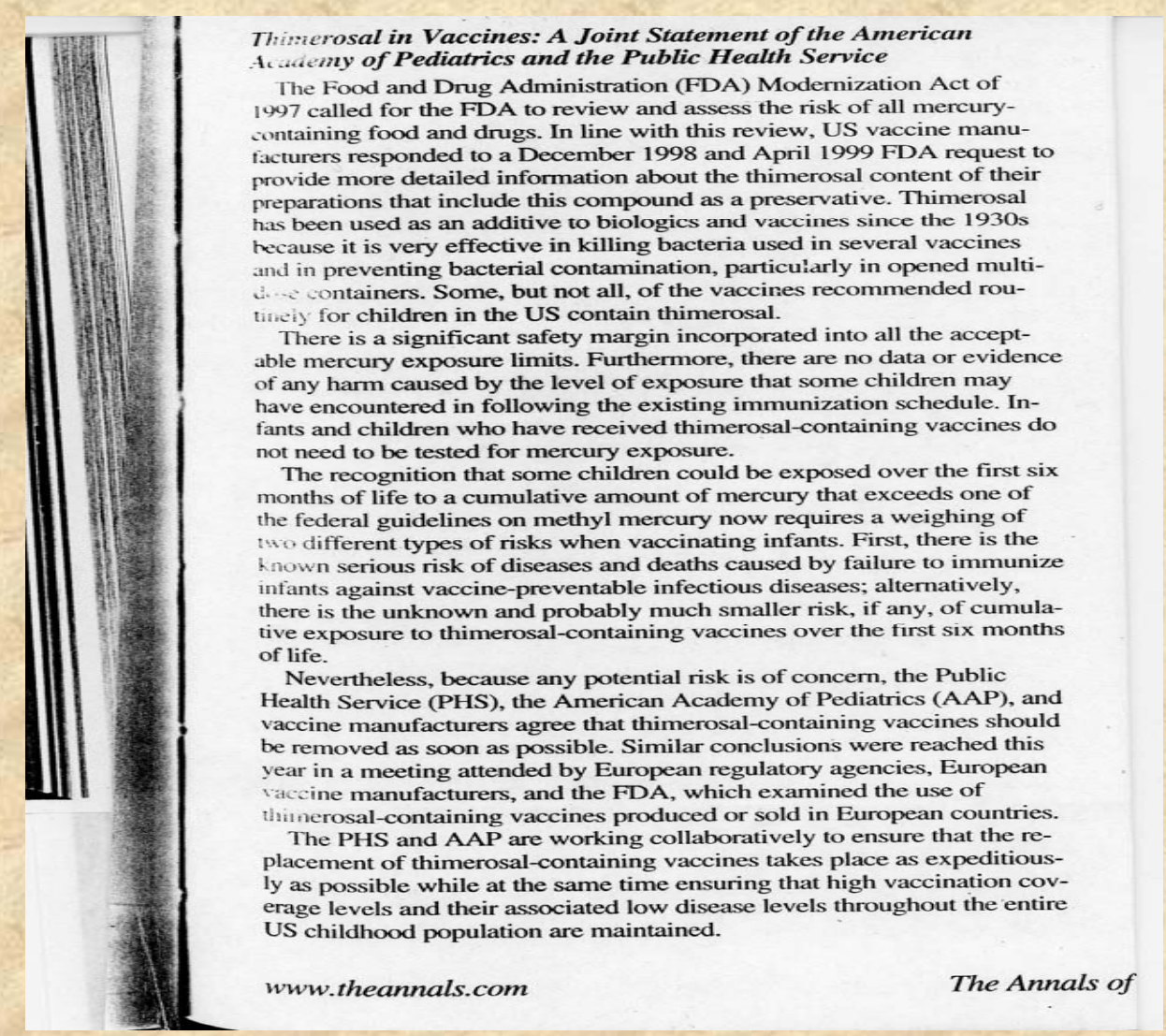


Source: Presentation by Mark Blaxill, Safe Minds, to meeting of Immunization Safety Review Committee, Institute of Medicine, Cambridge, Massachusetts, July 16, 2000.

The Controversy: Thimerosal in Vaccines

1999 Statement from American Academy of Pediatrics and US Public Health Service

Conclusion: Thimerosal containing vaccines should be removed as soon as possible to reduce exposure to mercury.



Thimerosal in Vaccines: A Joint Statement of the American Academy of Pediatrics and the Public Health Service

The Food and Drug Administration (FDA) Modernization Act of 1997 called for the FDA to review and assess the risk of all mercury-containing food and drugs. In line with this review, US vaccine manufacturers responded to a December 1998 and April 1999 FDA request to provide more detailed information about the thimerosal content of their preparations that include this compound as a preservative. Thimerosal has been used as an additive to biologics and vaccines since the 1930s because it is very effective in killing bacteria used in several vaccines and in preventing bacterial contamination, particularly in opened multi-dose containers. Some, but not all, of the vaccines recommended routinely for children in the US contain thimerosal.

There is a significant safety margin incorporated into all the acceptable mercury exposure limits. Furthermore, there are no data or evidence of any harm caused by the level of exposure that some children may have encountered in following the existing immunization schedule. Infants and children who have received thimerosal-containing vaccines do not need to be tested for mercury exposure.

The recognition that some children could be exposed over the first six months of life to a cumulative amount of mercury that exceeds one of the federal guidelines on methyl mercury now requires a weighing of two different types of risks when vaccinating infants. First, there is the known serious risk of diseases and deaths caused by failure to immunize infants against vaccine-preventable infectious diseases; alternatively, there is the unknown and probably much smaller risk, if any, of cumulative exposure to thimerosal-containing vaccines over the first six months of life.

Nevertheless, because any potential risk is of concern, the Public Health Service (PHS), the American Academy of Pediatrics (AAP), and vaccine manufacturers agree that thimerosal-containing vaccines should be removed as soon as possible. Similar conclusions were reached this year in a meeting attended by European regulatory agencies, European vaccine manufacturers, and the FDA, which examined the use of thimerosal-containing vaccines produced or sold in European countries.

The PHS and AAP are working collaboratively to ensure that the replacement of thimerosal-containing vaccines takes place as expeditiously as possible while at the same time ensuring that high vaccination coverage levels and their associated low disease levels throughout the entire US childhood population are maintained.

NIAID Meeting on Thimerosal, November 28, 2000

Basic Question

"What do we know about what happens to the mercury in Thimerosal (or ethylmercury) when it is injected in infants (human or animal)."

Answer: Very Little

- **Delayed-type hypersensitivity reported in humans**
- **Acute poisonings reported in humans (coma, death)**
- **Acute toxicity studies reported in adult animals (several species)**
- **Only one study could be found that actually measured mercury levels in infants following a vaccine**

Levels of Mercury in Blood of Preterm and Term Newborns after administration of Hepatitis B Vaccine

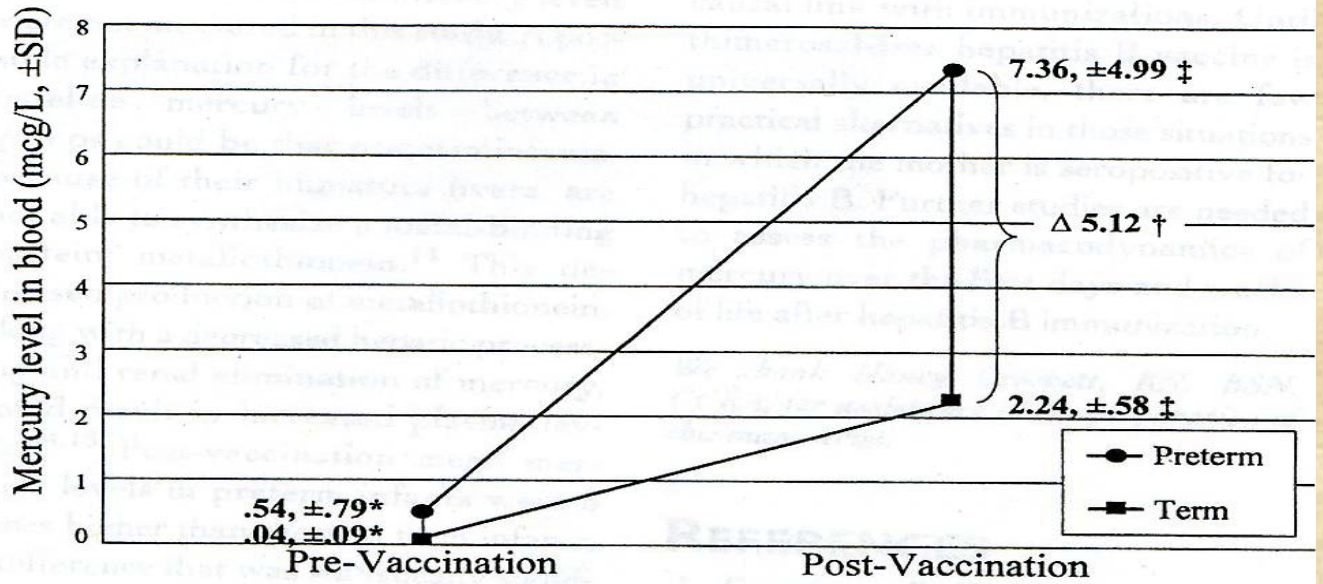


Figure. Difference in mean mercury levels (in micrograms per liter) between preterm and term infants. ‡$P < .01$; †$P < .01$; *$P = .2$.

From: Journal of Pediatrics, Vol 136, Number 5, May 2000

Estimates of Mercury Doses from Vaccines

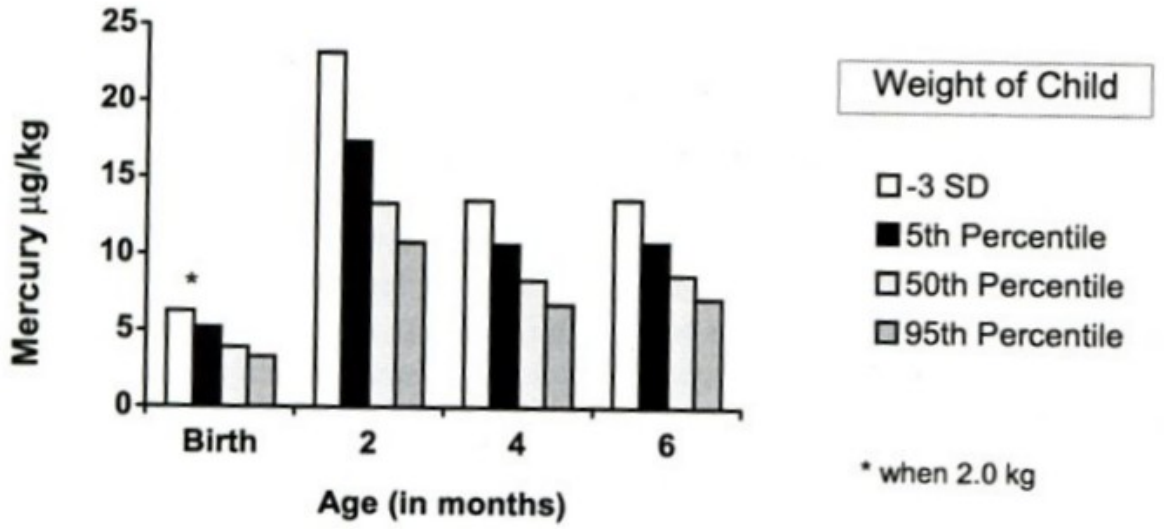


FIGURE 2 Mercury (µg/kg) administered by age and weight if thimerosal-containing vaccines are given for Hepatitis B, Hib, and DTaP. *Amount of Hg received (in micrograms) = 12.5 at birth, 62.5 at 2 and 6 months, 50 at 4 months.*

SOURCE: Halsey, 1999a. Reprinted with permission from the author.

Thimerosal Monkey Study

(Comparison of Thimerosal and Methylmercury Toxicokinetics)

	Age (days)																		
	Birth (0)	2	4	7	9	11	14	16	18	21	23	25	28	31	35	38	42	45	49
Mercury Dose[1] (Oral MeHg)	20			20			20			20									
Mercury Dose[2] (I.M. Thimerosal in Vaccine)	OPV-0 HB-20			OPV-0 HB-4 DTP-8 Hib-8			OPV-0 DTP-10 Hib-10			OPV-0 HB-4 DTP-8 Hib-8									
Blood Draws[3]	0	2	4	7	2	4	7	2	4	7	2	4	7	10	14	17	21	24	28
Sacrifice Day[4]											2	4	7						28

[1]Dose of MeHg in µg/kg

[2]Dose of ethylmercury in µg/kg

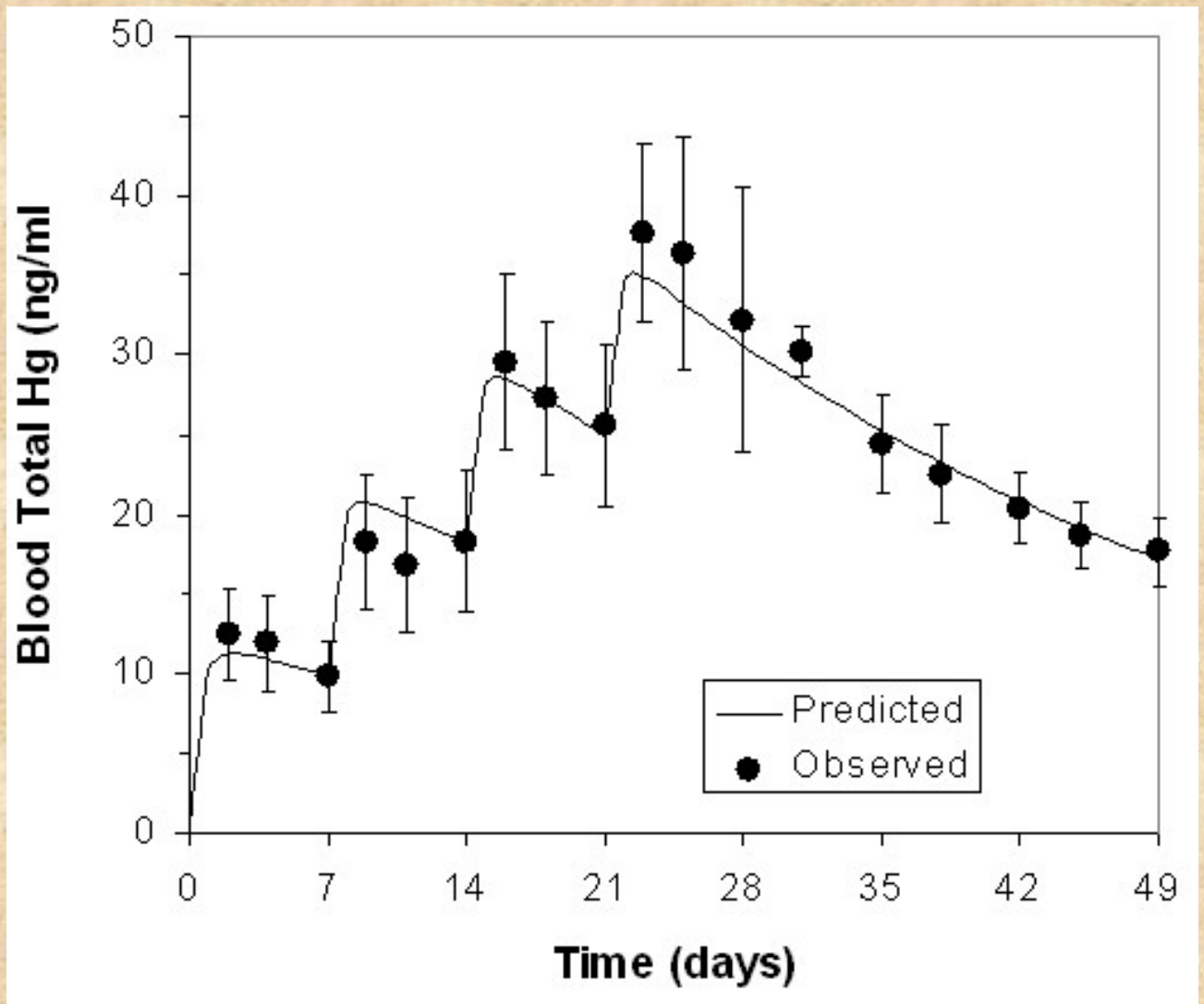
[3]Days after most recent dose

[4]Days after last (4th) dose

From: Burbacher, et al. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosa

Mean Blood Total Hg Concentration During and After Four Weekly Oral Doses (20 μg/kg) of Methylmercury

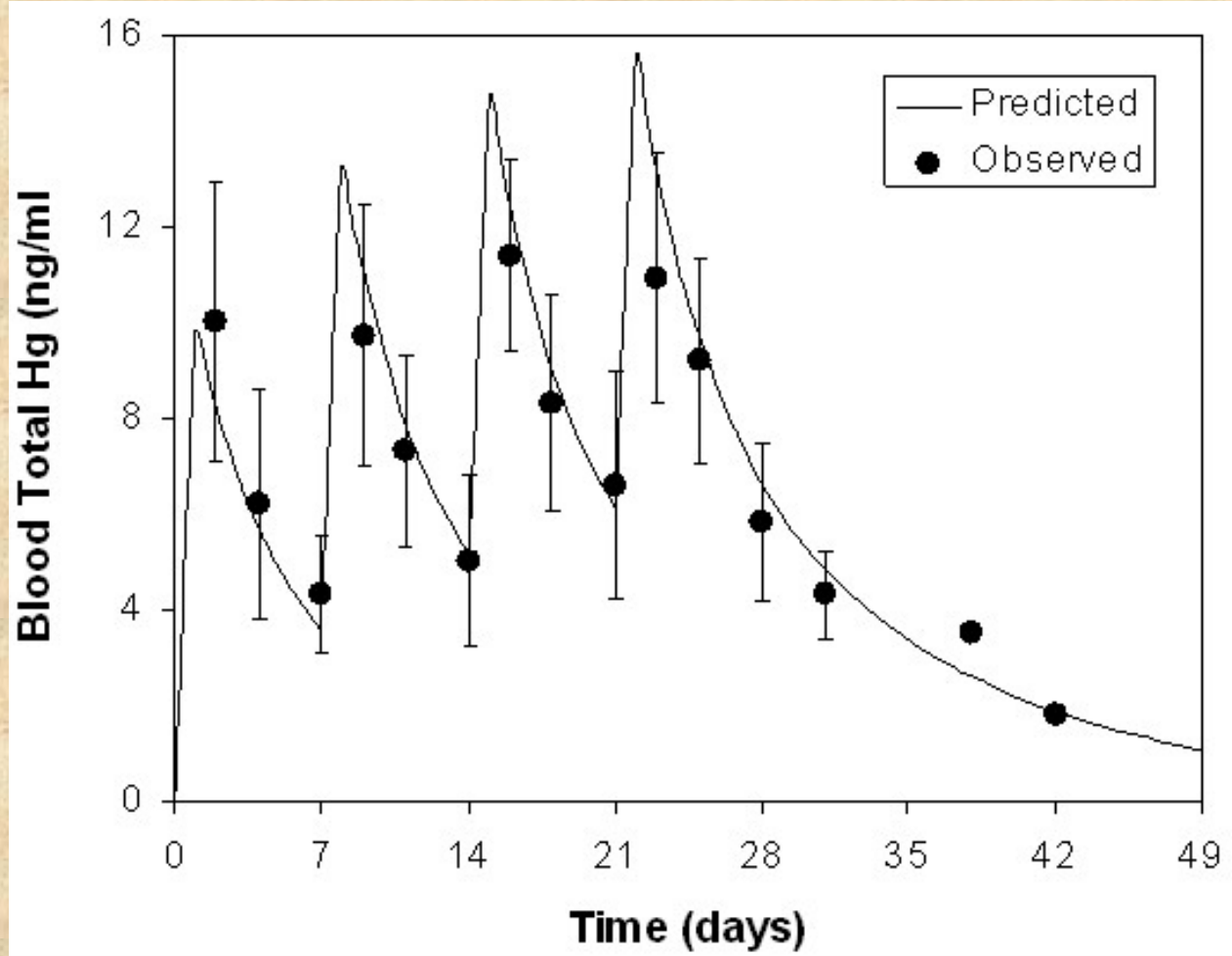
Methylmercury
Total Mercury in Blood
Half-Life = 21.5 days



From: Burbacher, et al. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosa

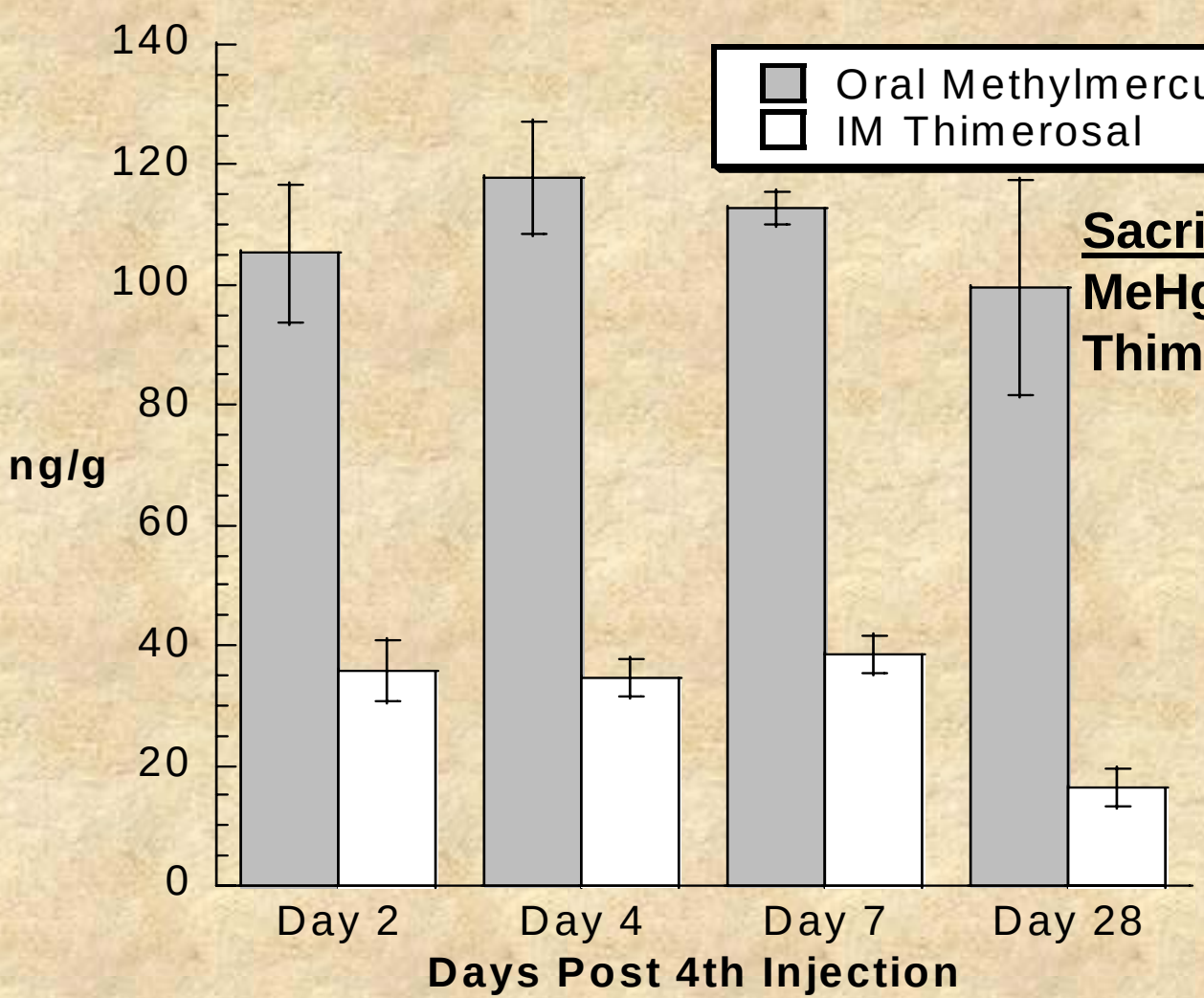
Mean Blood Total Hg Concentration During and After Four Weekly I.M. Injections of Vaccine Containing Thimerosal at 20 μg/Kg Hg

Thimerosal
Total Mercury in Blood
Half-Life = 6.9 days



From: Burbacher, et al. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosa

Mean (SE) Total Mercury Concentrations in Brain of Oral Methylmercury and I.M. Thimerosal Groups



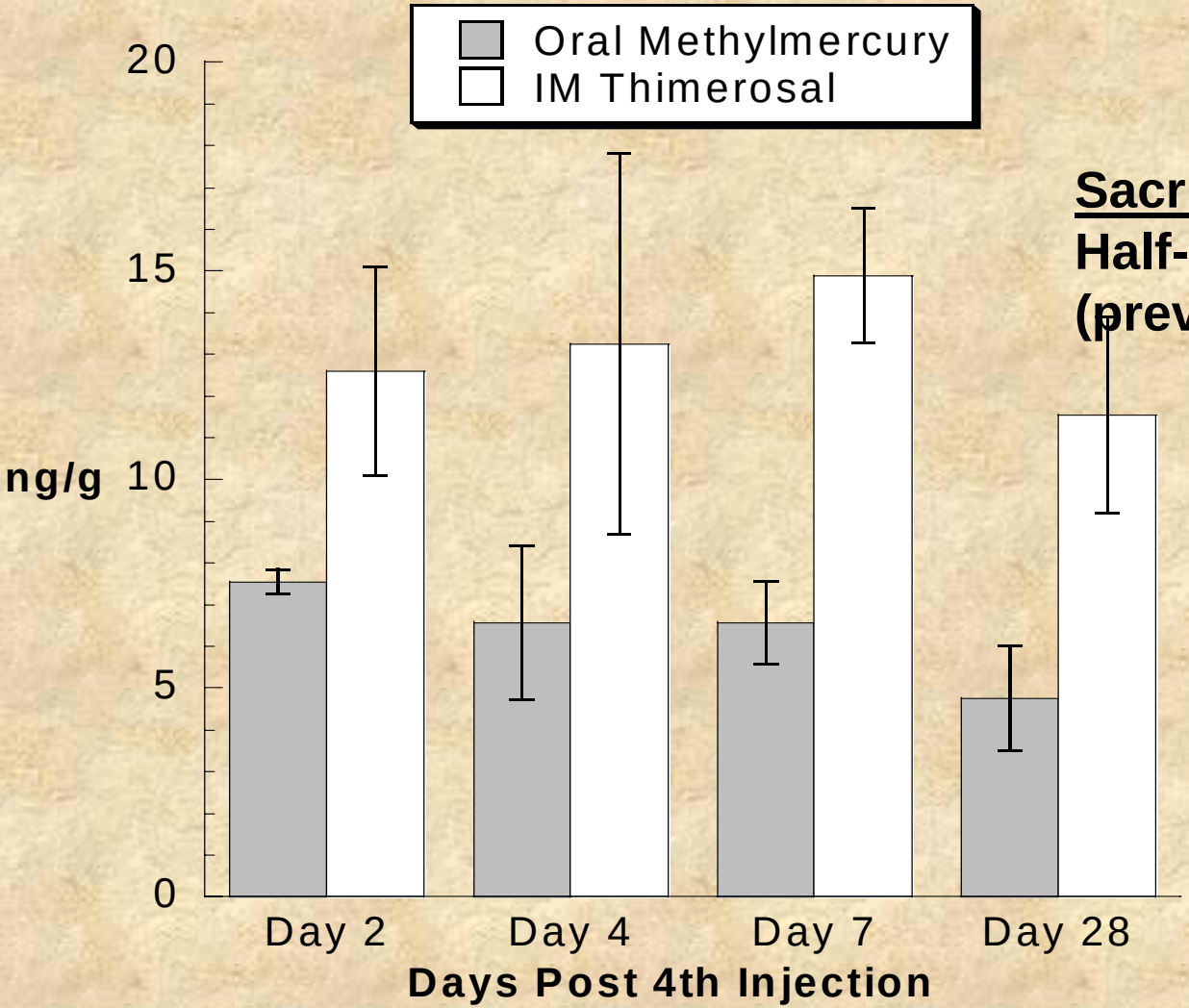
Sacrifice or Washout Period (Brain)

MeHg Half-Life = 59.5(24.1) days

Thimerosal Half-Life = 24.2(7.4) days

From: Burbacher, et al. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosa

Mean (SE) Inorganic Mercury Concentrations in Brain of Oral Methylmercury and I.M. Thimerosal Groups



Sacrifice or Washout Period (Brain)

Half-Life could not be estimated (previous estimates over 1 year)

From: Burbacher, et al. Comparison of Blood and Brain Mercury Levels in Infant Monkeys Exposed to Methylmercury or Vaccines Containing Thimerosa

Review of Study Results and Recommendations

- **Methylmercury is not a good reference for Thimerosal Risk**
  - Absorption rate and initial distribution are similar
  - Blood clearance rates are different (thimerosal cleared more quickly)
  - Brain levels of total mercury are different (levels are lower for thimerosal)
  - Brain clearance of total mercury are different (thimerosal cleared more quickly)
  - Ratio of total mercury in brain to blood is different (thimerosal is higher)
  - Brain levels of inorganic mercury are different, with no clearance (levels are higher for thimerosal)

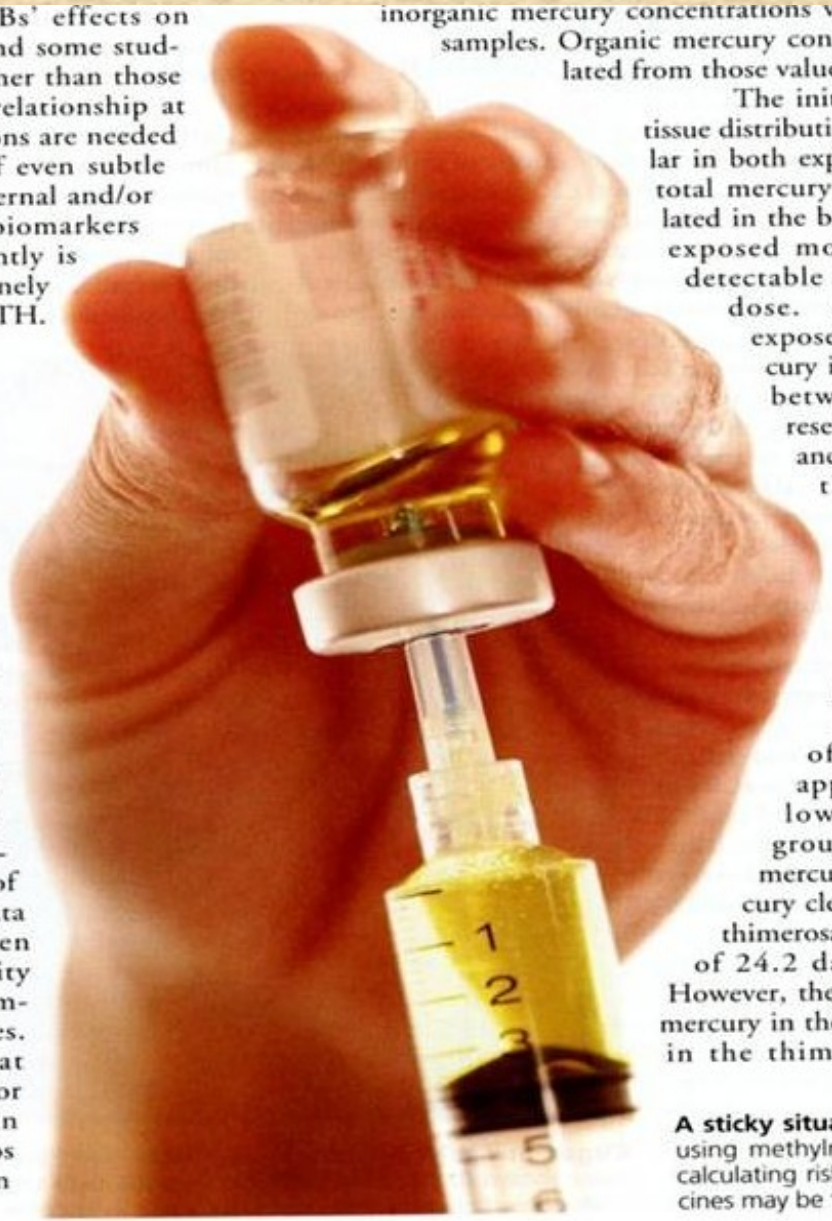
- **Future studies of Thimerosal should focus on the neurotoxic effects of organic and inorganic mercury following exposure.**
  - Inorganic mercury has been associated with activation of microglia in the brain of adult non human primates (neuroinflammation)
  - Inorganic mercury has a very long half-life in the brain (years)
  - A recent report suggests that neuroinflammation is present in brains of autistic children
  - Information on the developmental neurotoxicity of thimerosal is critical if we are to respond to public concerns regarding the safety of childhood immunizations.

Recent epidemiologic research into PCBs' effects on human TH function has been inconsistent, and some studies have found no effect at exposure levels higher than those in this study. But the current finding of a relationship at such low levels indicates that more investigations are needed in pregnant women, including monitoring of even subtle environmental exposures that can disturb maternal and/or fetal thyroid status. For this purpose, the biomarkers should include not only TSH—which currently is the only element of the thyroid system routinely monitored in pregnant women—but all forms of TH.
–Valerie J. Brown

Thimerosal and Animal Brains

New Data for Assessing Human Ethylmercury Risk

Since the 1930s, vaccines have contained thimerosal, a mercury-based preservative that breaks down to ethylmercury and thiosalicylate in the body. By some calculations, children given the usual schedule of vaccines containing thimerosal receive ethylmercury in doses exceeding the U.S. Environmental Protection Agency's guidelines for methylmercury, a known neurotoxicant. Because of the lack of pharmacokinetic and toxicity data for ethylmercury, methylmercury has been used as a reference for ethylmercury toxicity based on the assumption that the two compounds share similar toxicokinetic profiles. However, a new animal study shows that methylmercury is an inadequate reference for ethylmercury due to significant differences in tissue distribution, clearance rates, and ratios of organic to inorganic mercury in the brain [*EHP* 113:1015–1021].



inorganic mercury concentrations were measured in brain samples. Organic mercury concentrations were calculated from those values.

The initial absorption rate and tissue distribution of mercury was similar in both exposed groups. However, total mercury progressively accumulated in the blood of methylmercury-exposed monkeys and remained detectable 28 days after the last dose. Among thimerosal-exposed monkeys, total mercury in blood declined rapidly between doses, and the researchers estimated clearance to be 5.4-fold higher than in the methylmercury group. In the thimerosal group, the half-life of total mercury in blood was 6.9 days, compared to 19.1 days for the methylmercury group.

Brain concentrations of total mercury were approximately 3–4 times lower in the thimerosal group than in the methylmercury group, and total mercury cleared more rapidly in the thimerosal group (with a half-life of 24.2 days versus 59.5 days). However, the proportion of inorganic mercury in the brain was much higher in the thimerosal group (21–86%

A sticky situation. New data show that using methylmercury as a reference for calculating risk from ethylmercury in vaccines may be fraught with problems.

Projected Levels of Mercury in Blood of Infants after administration of Vaccines

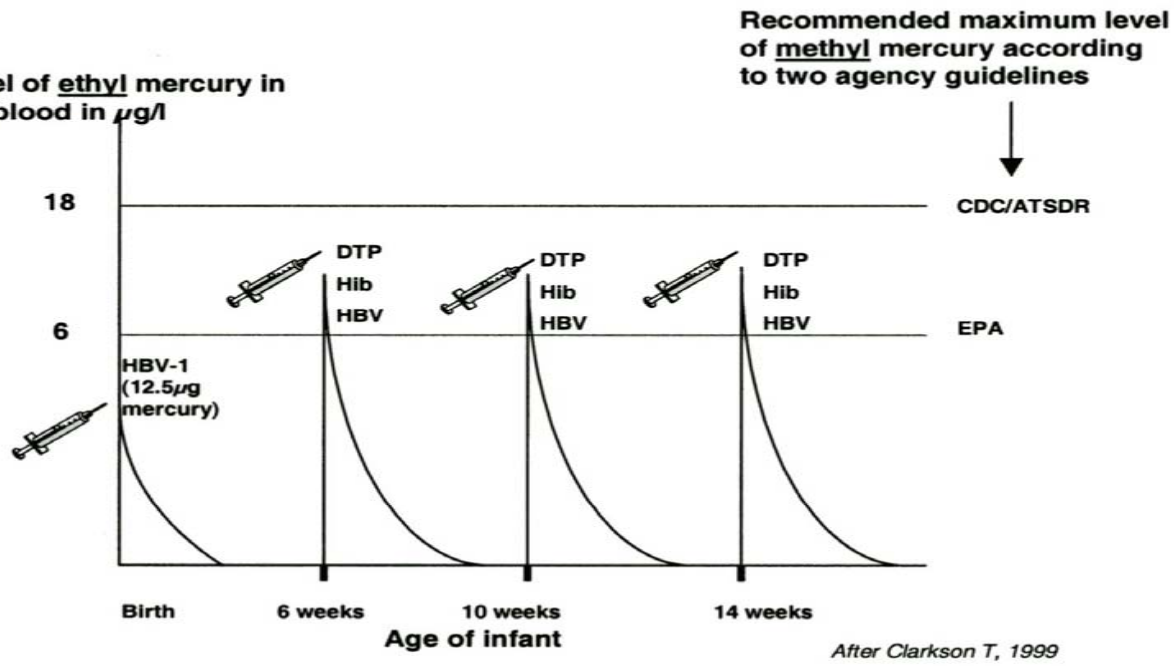


Fig. 1. Projected levels of ethyl mercury in blood after administration of a birth dose of HBV and other infant vaccines. The shape of the peaks drawn above are modeled from the paper by Pichichero et al. [43], but the paper was not able to ascertain the entire curve. Thus, there remains speculation about the peak values that may well be rounded peaks and lower maximum values than are shown above. The maximum value could well be below the 5.8 level EPA cut-off. A healthy 3 kg infant can be expected to increase his body mass to around 5.5 kg (80%) by 3 months. Thus, the blood concentration of ethyl mercury will be proportionately less (not shown graphically). This is being tested empirically in up-coming studies. In any case, the immediate post-immunization level is not directly relevant as the blood compartment does not equilibrate with the brain compartment for several days after vaccination, at a time when the blood level has reached a steady state at a yet-to-be determined (but lower) value.

Projections based on data from 40 full-term infants aged 6 months and younger.
From: The Lancet, Vol 360, November 2002

Disorder in Mercury Excretion in Children with Autism?

Reduced Levels of Mercury in First Baby Haircuts of Autistic Children

Amy S. Holmes,[1] Mark F. Blaxill,[2] and Boyd E. Haley[3]

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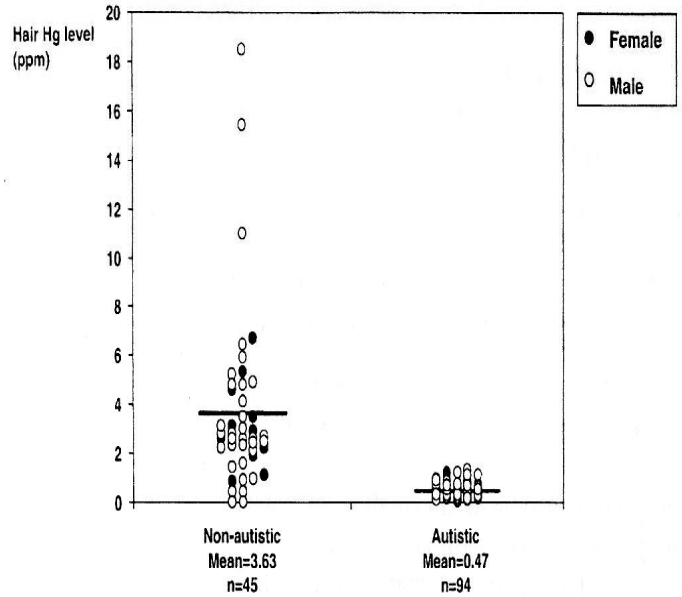

FIGURE 1

A plot of the birth hair mercury levels of nonautistic versus autistic children. Solid circles represent individual female subjects and open circles represent individual male subjects.

TABLE 2

Exposure differences in autistic group as compared to controls

	Autistic group ($N = 94$)	Control group ($N = 45$)
Mercury levels in first baby haircut (ppm, mean ± *SD*)	0.47 (±0.28)[a]	3.63 (±3.56)
Rho D immunoglobulin shots during pregnancy (number per mother, mean ± *SD*)	0.53 (±0.67)[b]	0.09 (±0.29)
Amalgam fillings during pregnancy (number per mother, mean ± *SD*)	8.35 (±3.43)[c]	6.60 (±3.55)

[a]Statistically different from control group ($p < .0000004$).
[b]Statistically different from control group ($p < .0000004$).
[c]Statistically different from control group ($p < .01$).

From: International Journal of Toxicology, Vol 22, 277-285, 2003

Disorder in Mercury Excretion in Children with Autism?

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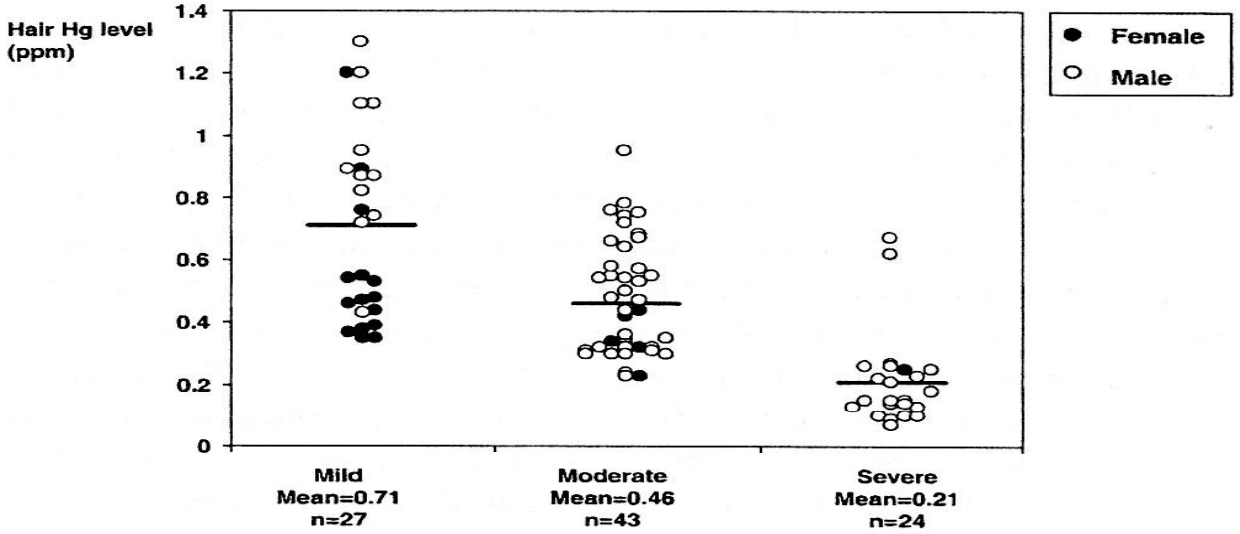


FIGURE 2

A plot of the birth hair mercury levels in autistic children based on the clinical severity of the disease. Solid circles represent individual female subjects and open circles represent individual male subjects.

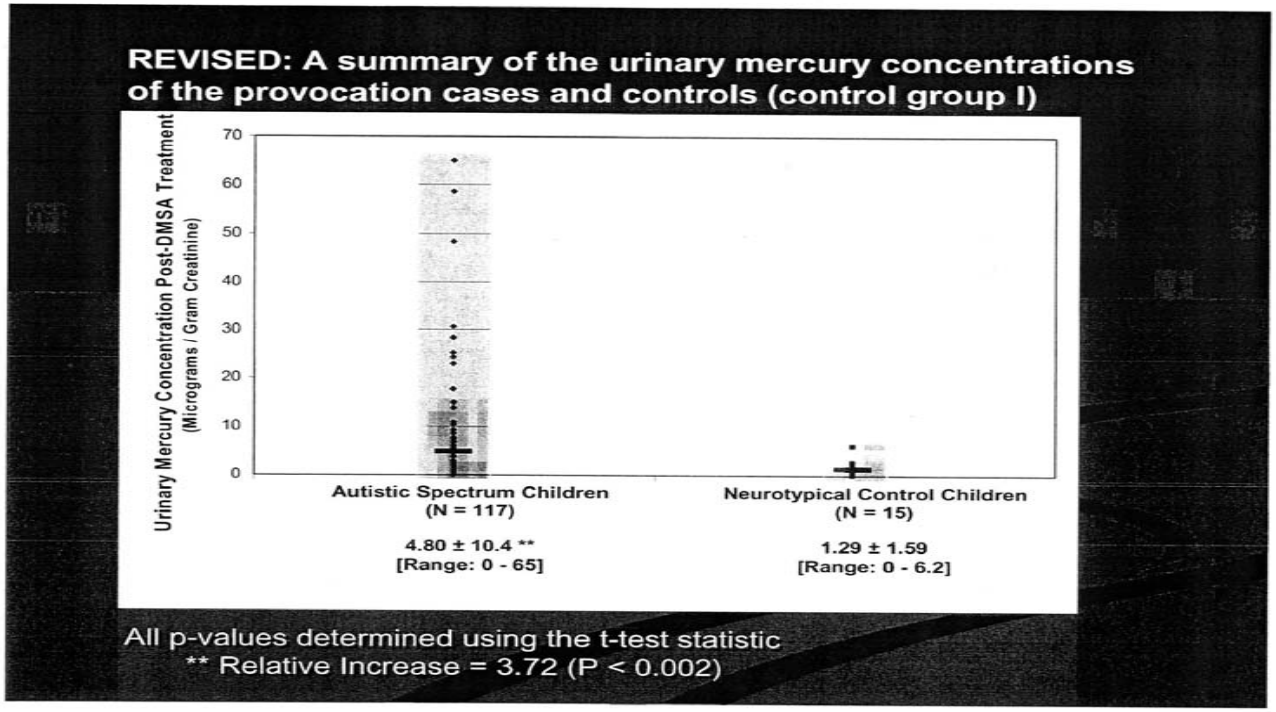
From: International Journal of Toxicology, Vol 22, 277-285, 2003

Disorder in Mercury Excretion in Children with Autism?

A Case-Control Study of Mercury Burden in Children with Autistic Spectrum Disorders

Jeff Bradstreet, M.D.
David A. Geier, B.A.
Jerold J. Kartzinel, M.D.
James B. Adams, Ph.D.
Mark R. Geier, M.D., Ph.D.

Journal of American Physicians and Surgeons Volume 8 Number 3 Summer 2003



From: Journal of American Physicians & Surgeons, Vol 8, 2003

Biochemical Basis of Thimerosal Neurotoxicity?

ELSEVIER

NeuroToxicology 26 (2005) 1–8

NeuroToxicology

Thimerosal Neurotoxicity is Associated with Glutathione Depletion: Protection with Glutathione Precursors

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Abstract

Thimerosol is an antiseptic containing 49.5% ethyl mercury that has been used for years as a preservative in many infant vaccines and in flu vaccines. Environmental methyl mercury has been shown to be highly neurotoxic, especially to the developing brain. Because mercury has a high affinity for thiol (sulfhydryl (–SH)) groups, the thiol-containing antioxidant, glutathione (GSH), provides the major intracellular defense against mercury-induced neurotoxicity. Cultured neuroblastoma cells were found to have lower levels of GSH and increased sensitivity to thimerosol toxicity compared to glioblastoma cells that have higher basal levels of intracellular GSH. Thimerosal-induced cytotoxicity was associated with depletion of intracellular GSH in both cell lines. Pretreatment with 100 μM glutathione ethyl ester or N-acetylcysteine (NAC), but not methionine, resulted in a significant increase in intracellular GSH in both cell types. Further, pretreatment of the cells with glutathione ethyl ester or NAC prevented cytotoxicity with exposure to 15 μM Thimerosal. Although Thimerosal has been recently removed from most children's vaccines, it is still present in flu vaccines given to pregnant women, the elderly, and to children in developing countries. The potential protective effect of GSH or NAC against mercury toxicity warrants further research as possible adjunct therapy to individuals still receiving Thimerosal-containing vaccinations.

Keywords: Thimerosal; Neurotoxicity; Glutathione; *N*-acetylcysteine

INTRODUCTION

Thimerosal (sodium ethylmercurithiosalicylate) was developed by Eli Lilly in the 1930s as a effective bacteriostatic and fungistatic preservative and has been widely used in multidose vials of vaccines and in ophthalmic, otic, nasal, and topical products. Until the removal of Thimerosal from most pediatric vaccines in 2001, the largest human exposure in the US (μg/kg body weight) was in children under 18 months of age undergoing routine childhood immunization schedules. Prior to 2001, a child may have received a cumulative dose of over 200 μg/kg in the first 18 months of life (Ball et al., 2001). Although the neurotoxicity of methyl mercury has been relatively well studied, limited information is available on the relative neurodevelopmental toxicity of ethylmercury, the mercury metabolite of Thimerosal. Based on the known toxicity of methylmercury, the cumulative ethylmercury exposure to US pediatric populations in Thimerosal-containing vaccinations was re-examined in 1999 and found to exceed EPA recommended guidelines

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IMMEDIATE COMMUNICATION

Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal

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Methylation events play a critical role in the ability of growth factors to promote normal development. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might exert adverse effects on methylation. We found that insulin-like growth factor-1 (IGF-1)- and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells, via a PI3-kinase- and MAP-kinase-dependent mechanism. The stimulation of this pathway increased DNA methylation, while its inhibition increased methylation-sensitive gene expression. Ethanol potently interfered with IGF-1 activation of MS and blocked its effect on DNA methylation, whereas it did not inhibit the effects of dopamine. Metal ions potently affected IGF-1 and dopamine-stimulated MS activity, as well as folate-dependent phospholipid methylation: Cu^{2+} promoted enzyme activity and methylation, while Cu^{+}, Pb^{2+}, Hg^{2+} and Al^{3+} were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC_{50} of 1 nM and eliminated MS activity. Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. The potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins.
Molecular Psychiatry advance online publication, 27 January 2004; doi:10.1038/sj.mp.4001476

Keywords: autism; attention deficit hyperactivity disorder; P13-kinase; D4 dopamine receptor; DNA methylation; phospholipid methylation; lead; mercury

Introduction

Developmental disorders include a spectrum of neurological conditions characterized by deficits in attention, cognition and learning, frequently accompanied by abnormal behaviors. Severe deficits may be recognized at birth, but a failure to achieve standard milestones during initial years of life remains the primary basis of diagnosis in most cases. While the underlying cause(s) remains obscure for many developmental disorders, metabolic abnormalities involving purine synthesis (eg Lesch–Nyhan Syndrome and adenylsuccinate lyase deficiency)[1,2] or impaired methylation-dependent gene silencing and/or imprinting (Rett and Fragile-X syndromes)[3,4] suggest biochemical mechanisms that may be involved. The development disorders can also be caused by exposure to toxins (eg ethanol, in fetal alcohol syndrome; heavy metals, in lead poisoning),[5,6] although the precise mechanisms underlying their toxicity are not known. The recent increase in the incidence of autism has led to the speculation that environmental exposures including vaccine additives (ie aluminum and the ethylmercury-containing preservative thimerosal) might contribute to the triggering of this developmental disorder.[7]

Normal development is closely related to cellular differentiation, and growth factor-initiated signaling promotes differentiation of pluripotent cells.[8] Furthermore, altered patterns of DNA methylation and associated gene silencing underlie phenotypic differences between undifferentiated and differentiated cells.[9] Together, these observations suggest that growth factors promote cellular differentiation by producing effects on DNA methylation. This suggestion is reinforced by the observation that

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Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism[1,2]

S Jill James, Paul Cutler, Stepan Melnyk, Stefanie Jernigan, Laurette Janak, David W Gaylor, and James A Neubrander

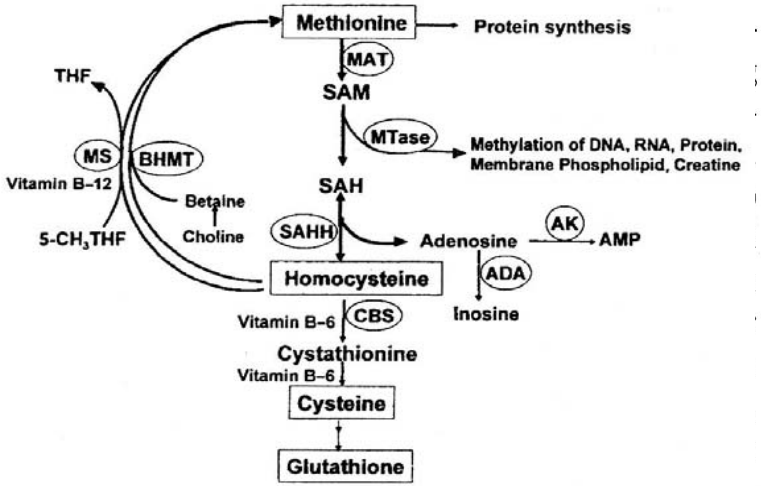

FIGURE 1. The methionine cycle involves the remethylation of homocysteine to methionine by either the folate–vitamin B-12–dependent methionine synthase (MS) reaction or the folate–vitamin B-12–independent betaine homocysteine methyltransferase (BHMT) reaction. Methionine is then activated by methionine adenosyltransferase (MAT) to *S*-adenosylmethionine (SAM), the major methyl donor for cellular methyltransferase (MTase) reactions. After methyl group transfer, SAM is converted to *S*-adenosylhomocysteine (SAH), which is further metabolized in a reversible reaction to homocysteine and adenosine. Adenosine may be phosphorylated to adenosine nucleotides by adenosine kinase (AK) or catabolized to inosine by adenosine deaminase (ADA). Homocysteine may be permanently removed from the methionine cycle by irreversible conversion to cystathionine by vitamin B-6–dependent cystathionine β-synthase (CBS). Cystathionine is converted to cysteine, which is the rate-limiting amino acid for the synthesis of the tripeptide glutathione (Glu-Cys-Gly). THF, tetrohydrofolate; 5-CH_3 THF, 5-methyltetrahydrofolate; SAHH, SAH hydrolase.

TABLE 1

Comparison of methionine cycle and transsulfuration metabolites between autistic children and control children[1]

	Control children (n = 33)	Autistic children (n = 20)
Methionine (μmol/L)	31.5 ± 5.7 (23–48)	19.3 ± 9.7 (15–25)[2]
SAM (nmol/L)	96.9 ± 12 (77–127)	75.8 ± 16.2 (68–100)[3]
SAH (nmol/L)	19.4 ± 3.4 (16–27)	28.9 ± 7.2 (14–41)[2]
SAM:SAH	5.2 ± 1.3 (4–8)	2.9 ± 0.8 (2–4)[2]
Adenosine (μmol/L)	0.27 ± 0.1 (0.1–0.4)	0.39 ± 0.2 (0.17–0.83)[4]
Homocysteine (μmol/L)	6.4 ± 1.3 (4.3–9.0)	5.8 ± 1.0 (4.0–5.8)[3]
Cystathionine (μmol/L)	0.17 ± 0.05 (0.1–0.27)	0.14 ± 0.06 (0.04–0.2)[5]
Cysteine (μmol/L)	202 ± 17 (172–252)	163 ± 15 (133–189)[2]
tGSH (μmol/L)	7.6 ± 1.4 (3.8–9.2)	4.1 ± 0.5 (3.3–5.2)[2]
Oxidized glutathione (nmol/L)	0.32 ± 0.1 (0.11–0.43)	0.55 ± 0.2 (0.29–0.97)[2]
tGSH:GSSG	25.5 ± 8.9 (13–49)	8.6 ± 3.5 (4–11)[2]

[1] All values are $\bar{x}$ ± SD; range in parentheses. SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; tGSH, total glutathione; GSSG, oxidized glutathione.

[2–5]Significantly different from control children: [2] $P < 0.001$, [3] $P < 0.01$, [4] $P < 0.05$, [5] $P < 0.002$.

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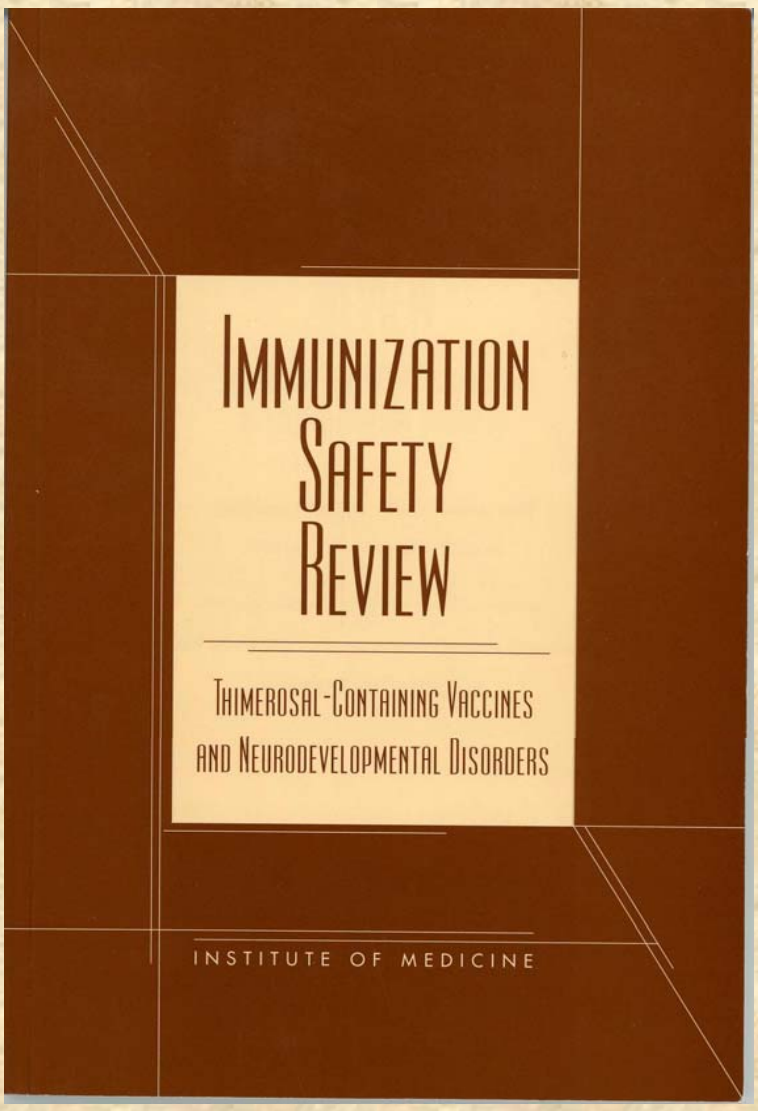
The Controversy: Thimerosal in Vaccines

2002 IOM Report on Thimerosal and Neurodevelopmental Disorders

Conclusions
Hypothesis of a link is biologically plausable.

Current evidence is inadequate to accept or reject a causal relationship.

Additional studies are needed at all levels, epidemiology, clinical, basic science.



The Controversy: Thimerosal in Vaccines

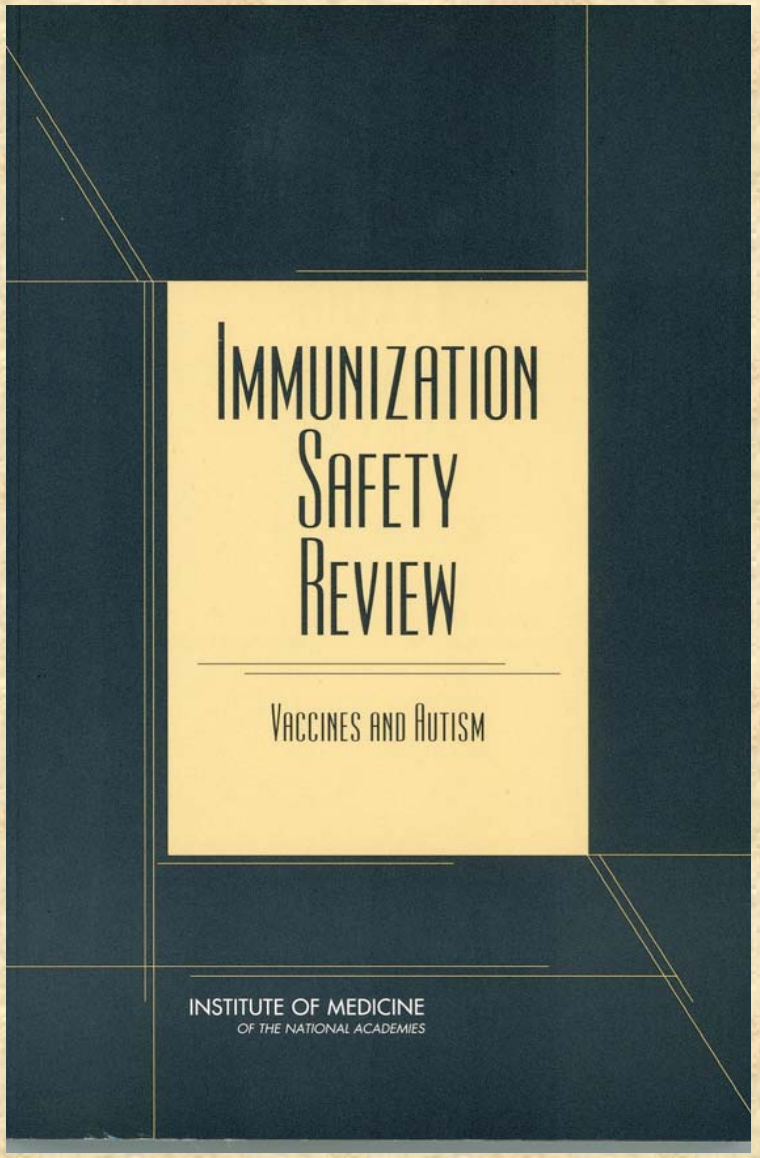
2004 IOM Report on Thimerosal and Autism

Conclusions
Evidence favors rejection of a causal relationship.

Continue surveillance for autism as exposure to thimerosal declines.

Increase efforts to quantify the level of prenatal and postnatal exposure to thimerosal and other forms of mercury in infants, children, and pregnant women.

Available funding for autism research be channeled to the most promising areas.



Early Downward Trends in Neurodevelopmental Disorders Following Removal of Thimerosal-Containing Vaccines

David A. Geier, B.A.
Mark R. Geier, M.D., Ph.D.

ABSTRACT

Contemporaneously with the epidemic rise in neurodevelopmental disorders (NDs), first observed in the United States during the 1990s, the childhood immunization schedule was expanded by the U.S. Centers for Disease Control and Prevention (CDC) to include several additional thimerosal-containing vaccines (TCVs). On July 7, 1999, a joint recommendation was made by the American Academy of Pediatrics (AAP) and the U.S. Public Health Service (PHS) to remove thimerosal from vaccines. A two-phase study was undertaken to evaluate trends in diagnosis of new NDs entered into the Vaccine Adverse Event Reporting System (VAERS) and the California Department of Developmental Services (CDDS) databases on a reporting quarter basis, from 1994 through 2005. Significant increasing trends in newly diagnosed NDs were observed in both databases 1994 through mid-2002. Significant decreasing trends in newly diagnosed NDs were observed in both databases from mid-2002 through 2005. The results indicate that the trends in newly diagnosed NDs correspond directly with the expansion and subsequent contraction of the cumulative mercury dose to which children were exposed from TCVs through the U.S. immunization schedule.

Thimerosal is an ethylmercury-containing compound (49.6% mercury by weight) that was historically added to many vaccines at the preservative level (0.005% to 0.01%). The U.S. Centers for Disease Control and Prevention (CDC), from the late 1980s through the 1990s, expanded the number of doses of TCVs to be administered to U.S. infants. To five doses of diptheria-tetanus-whole-cell-pertussis (DTP) vaccine were added three doses of hepatitis B (Hep b) vaccine and four of *Haemophilus influenzae* type b (Hib) vaccine. Additionally, the CDC began recommending three doses of influenza vaccine for certain infant populations. An infant who received all of these vaccines on schedule could have received as much as 200 micrograms (µg) of mercury during the first 6 months of life.[1,4]

In response to theoretical concerns about the cumulative doses of mercury from TCVs, the AAP and the U.S. Public Health Service (PHS) issued a joint statement on July 7, 1999, calling for the removal of thimerosal from all vaccines.[1,4] It has been estimated that the last thimerosal-containing Hep b, diphtheria-tetanus-acellular-pertussis (DTaP) and Hib vaccines were manufactured in 2000-2001 and expired at the end of 2002 (or early 2003).[1,4] Table 1 summarizes significant historical dates in the use of pediatric TCVs in the United States.

Considering all significant environmental exposures to mercury, such as through breast milk, TCVs represent almost 50% of the total mercury dose some infants received.[1,5] The 187.5 µg of

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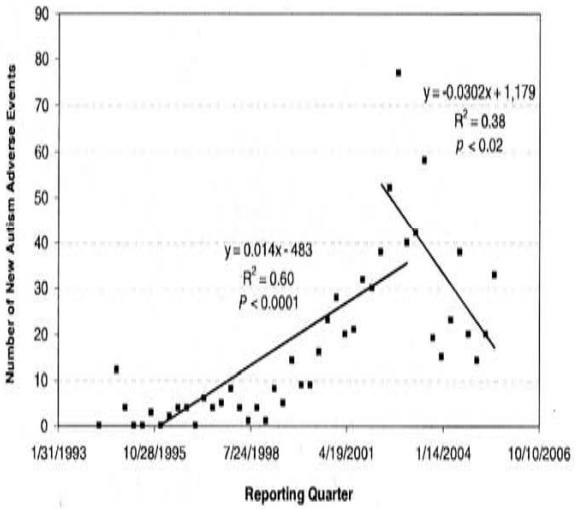

Figure 1. Trends in New Autism Adverse Events Reported to VAERS. The trend from Jan 1, 1994, through Dec 31, 2002, is significantly increasing, with $P < 0.0001$. The trend from Jan 1, 2002, through June 30, 2005, is significantly decreasing, with $P < 0.02$. The difference in the slope of the regression lines for the number of new autism adverse events in the earlier compared with the later periods is significant, with $P < 0.0005$.

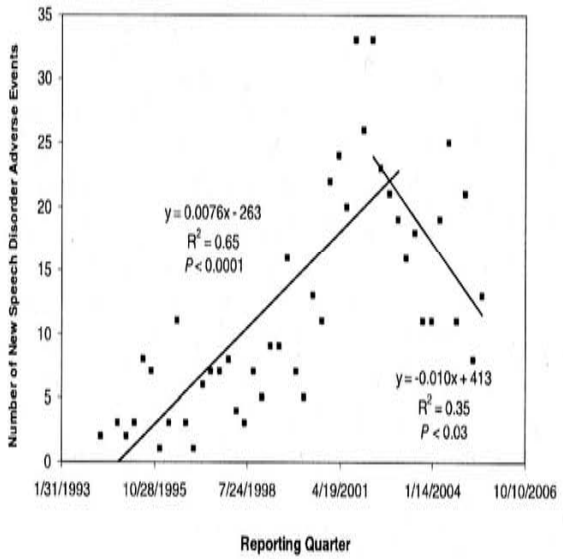

Figure 2. Trends in New Cases of Speech Disorders Reported to VAERS. The trend from Jan 1, 1994, through Dec 31, 2002, is significantly increasing, with $P < 0.0001$. The trend from Jan 1, 2002, through June 30, 2005, is significantly decreasing, with $P < 0.03$. The difference in the slope of the regression lines for the number of new speech disorder adverse events in the earlier compared with the later periods is significant, with $P < 0.005$.

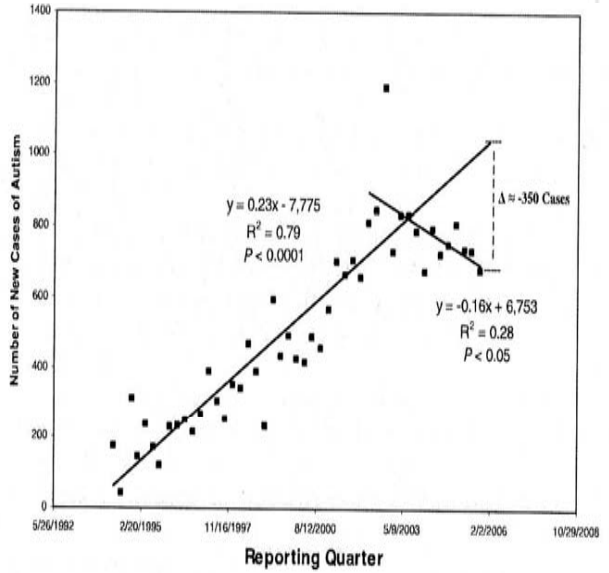

Figure 3. Trends in New Cases of Autism Entered into the CDDS. The trend from Jan 24, 1994, through Jan 6, 2003, is significantly increasing, with $P < 0.0001$. The trend from Jan 6, 2002, through Oct 4, 2005, is significantly decreasing, with $P < 0.05$. The difference in the slope of the regression lines for the number of new autism cases in the earlier compared with the later periods is significant, with $P < 0.0001$.

Note: The NIEHS is working with CDC to convene an expert panel in May 2006 to review the use of the CDC-supported Vaccine Safety Data Link (VSDL) to address questions about changes in autism rates and their potential association with thimerosal exposure through childhood vaccination.