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Supplemental Report in lieu of rebuttal testimony:

Mercuric mercury in the developing brains of young children exposed to thimerosal-containing vaccines.

US Federal Court of Claims, Vaccine Trial

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by

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I. Introduction

The major purpose of this report is to rebut the claim of Dr. Jeff Brent that there would be insufficient amounts of Hg^{++} in the brains of infants exposed to TCV's to trigger a neuroinflammatory process, and that the amount of Hg^{++} from breast milk would result in greater amounts of Hg^{++} than that derived from TCV's. In so doing, I will present to the court the estimated concentration of mercuric mercury in brains of human infants who received thimerosal-containing vaccines. In addition, other relevant facts will be presented.

It is well known to biochemists and toxicologists that mercuric mercury (also designated as Hg^{2+}, or sometimes described as inorganic mercury) is chemically very reactive in the brain of human infants that have received thimerosal-containing vaccines. In addition, mercuric mercury has a strong affinity for and combines with thiol (sometimes called sulfhydryl or -SH) groups of brain proteins resulting in the inhibition of crucial enzymes or of proteins that are part of the brain's structure and cytoarchitecture (Nordberg et al., 2007, PMRL0213).

When thimerosal-containing vaccines are administered to humans the thimerosal is quickly biotransformed in the tissues to ethylmercury which is then converted by oxidation to mercuric mercury (Clarkson and Magos, 2006, PMRL0035).

In the case of the brain, once the ethylmercury enters the brain it is either released from the brain or converted to mercuric mercury in the brain (Burbacher et al., 2005, PMRL0026). Ethyl mercury is released from the brain at a rate faster than is methyl mercury but the conversion in the brain of ethyl mercury to mercuric mercury occurs more rapidly than the conversion of methyl mercury to mercuric mercury.

When the amount of Hg in blood and brain of primates and humans is determined in controlled experiments,, wide variations among individuals, often by an order of magnitude, have been found in every study done (Burbacher et al., 2005, PMRL0026).

II. Brain Mercury Levels

Burbacher et al., compared the blood and brain mercury levels in infant monkeys exposed to methyl mercury and vaccines containing thimerosal. The $t_{1/2}$ values for ethyl mercury blood levels in infant monkeys (Burbacher et al., 2005, PMRL0026) and humans (Pichichero et al., 2002, PMRL0223, and 2008, PMRL0497) are quite similar.

Stereologic and autometallographic studies of the brains of adult monkeys chronically exposed to methyl mercury (Vahter et al., 1994, 1995, PMRL0060, PMRL0064) demonstrated that the persistence of inorganic mercury in the brain was associated with an increase in the microglia in the brain but the number of astrocytes decreased. Mercuric mercury can cause gliosis (Davis 1994, PMRL0183). The effects in the adult monkeys were associated with brain inorganic mercury levels on average five times higher (Charleston et al., 1994, 1995, 1996, PMRL0033, PMRL0032, PMRL0116) than those associated with the infant monkeys (Burbacher et al., 2005, PMRL0026).

The total Hg in brain of the MeHg fed infant monkeys was about 105 ng/g at peak, and dropped only to about 90 at the end of the experiment. At that point in time the average Hg^{++} concentration was about 6 or 7 ng/g, but was below detection limits in 8 of 17 monkeys. The text calculates that only about 6-10% of total Hg was converted to Hg^{++} in these animals.

Dr. Brent argues that because there was still almost 90 ng/g of organic MeHg in the infant monkey brains at end of experiment, much would still be converted to Hg^{++}. However, if the conversion rate is only 6-10%, then the total converted would be at most 5-9 more ng/g of additional Hg^{++} once the conversion of MeHg to Hg^{++} was fully made in the brain.

Using the same analysis on the TCV infant monkeys, they had 16ng/g of Hg^{++} on average, with about 8 ng/g of organic Hg left at the end of experiment; if 34% were converted (the conversion % for ethyl mercury to Hg^{++} as found by Burbacher et al, 2005), that would add another 3 ng to the 16, leaving around 19 ng/g on average.

Now, since the human brain/blood ratio is 2.3 times higher than for monkeys (per Magos 1987, PMRL0666 as cited in Burbacher 1990, PMRL0224): the formula is 6.0/2.6=2.3), then the 19 is multiplied by 2.3 = 44 ng/g.

We are now already to a level about 73% of the amount of Hg necessary to set off neuroinflammation in the adult monkeys, which was as low as 60 ng/g—see table Table 2 in Vahter 1994 "Speciation of Hg in Primate blood and brain." (PMRL0060)

See also the text on p. 203 of Charleston 1994 (PMRL0033), "Increases in the number of reactive glia . . .", where it says that even the I-Hg-fed monkeys, which had the lowest levels of Hg^{++} in brain, still had lots of reactive microglia (left hand column); note that in animal 82177, one of those fed HgCl, the level of Hg^{++} was only .06 micrograms/g, or 60 ng/g, very, very close to the projected human value for TCV's. (To convert mcg/g to ng/g, one multiplies the number of mcg's by 1000; hence, .06 x 1000 = 60.)

It is likely that the rapidly developing human brain from birth to 1.5 years of age is more sensitive to neuroinflammation than a mature adult brain, just because the microglia and astrocytes are so involved in the orchestration of the complex and rapid growth of connections. In other words, just because the adult monkeys showed no symptoms despite confirmed chronic, active neuroinflammation there should not be an assumption the same kind of neuroinflammation in an infant's developing brain is harmless. One would expect that the developing brain of an infant would have more developmental processes and events occurring than the adult brain. It needs to be remembered that in the Minamata methylmercury spill disaster, severely damaged central nervous systems occurred in children born to mothers without symptoms.

III. Brain cumulative inorganic mercury levels based on USA children from Pichichero et al, 2002

I have used the only human infant ethylmercury blood data we have after TCV's, from the two Pichichero studies (PMRL0223, PMRL0497) and the Stajich study (PMRL0249), to calculate

what the likely concentration of Hg^{++} in the brain of human infants would be. Those calculations are set out in Table 1 of this supplemental report.

The estimated uncorrected cumulative brain inorganic Hg content is 5.2 X 7 = 36.4 ng inorganic Hg/g brain tissue plus an injection-collection correction factor ***of 20% or giving a corrected estimated cumulative value of 43.7ng inorganic Hg per g brain tissue. 7.3 ng

It needs to be clearly understood that the basis for the conclusions of the below data in the following table is:
a)- thimerosal is metabolized to ethyl mercury.
b)- ethyl mercury is metabolized to mercuric mercury
c)- mercuric mercury is tenaciously held in the brain for years.
The above statements can be verified in the following references: Clarkson TW and Magos L. 2006 (PMRL0035); Burbacher et al., 2005 (PMRL0026); *Handbook Of The Toxicology Of Metals* edited by Nordberg, Fowler, Nordberg and Friberg, Academic Press 2007 (PMRL0213).

Table 1- BRAIN INORGANIC AND TOTAL MERCURY LEVELS AT IMMUNIZATON TIMES 2 MONTHS AND 6 MONTHS
(This TCV schedule does not include flu vaccine)

TIME	VACCINE	Hg Dose mcg	Cum. Dose mcg	Blood Hg nmol/L	Blood Hg ng/ml	Brain Hg ng/ml - *Total	Brain Hg ng/ml - *Inorganic
Birth	HepB #1	12.5	12.5				
1 mo.	HepB #2	12.5	25				
2 mos.	DTP #1	25					
	HIB #1	25	75	**20.55	4.12	24.7	8.4
4 mos.	DTP #2	25					
	HIB #2	25	125				
6 mos.	DTP #3	25					
	HIB #3	25					
	HepB #3	12.5	187.5	**6.9	1.4	8.4	2.0
12-18	DTP #4	25					
	HIB #4	25	237.5				
4-5 yrs	DTP #5	25					
	HIB #5	25	287.5				
Sum of brain mercury based on only 2 month value and 6 month value above						33.1	10.4
The average, ng /ml, of the above 2 and 6 months values for **inorg** Hg							5.2
***Corrected estimated cumulative value of 43.7ng inorganic Hg per g brain tissue							43.7

**For these calculations the infant blood mercury levels after vaccination were taken from Pichichero et al., 2002 (PMRL0223). A brain/blood ratio for mercury in the human of 6.0 was used (Magos, 1987, PMRL0666); and from Burbacher et al., 2005 (PMRL0026) 34% was used as the percentage that inorganic Hg was of the total Hg in the brain of infant monkeys receiving TCVs.*

***Highest value from taken from Pichichero et al., 2002 (PMRL0223).. However, the collection of blood in this infant took place five days after vaccination, so the blood levels had to be higher on days 1 –2 than when measured; this is also true of all the blood measurements in the study—they were taken on average several days after*

vaccination. Therefore, to use the data in the Pichichero study will lead to a significant underestimate of the total Hg in blood, and thus in the proportion going into the brain, in the day or two after vaccination when blood levels are highest. Thus an injection-collection correction factor is necessary, and I think a reasonable estimate would be to project that blood levels at their peak post vaccination would be about 20% higher..

****Vaccines were given at 7 different times. Therefore estimated cumulative brain inorganic Hg content is 5.2 X 7 = 36.4 ng inorganic Hg/g brain tissue plus an injection-collection correction factor of 20% or 7.3 ng giving a corrected estimated cumulative value of 43.7ng inorganic Hg per g brain tissue.*
I have assumed throughout that 1 ml brain tissue = 1 gram brain tissue. Such an assumption was obviously made in the Burbacher et al., 2005 (PMRL0026).

IV. Brain cumulative inorganic mercury levels based on USA children from Pichichero et al, 2008

If the same sort of calculations are done using the highest blood concentrations of the Pichechero 2008 paper (PMRL0497) the uncorrected, incremental brain **inorganic mercury** concentrations of the highest outliers are:
Newborns.....17.1 ng/ml brain tissue
2 month olds.....10.2
6 month olds.....10.0

The final corrected values are shown in Table 2.

Table 2 High end of the doses of Hg^{++} in human infant brains

TIME	*Blood – total Hg	Brain Total Hg	Brain inorganic Hg^{++}	**Corrected Brain inorganic Hg^{++}
New born	7.9 ng/ml	47.4 ng/ml	17.1 ng/g	20.5 ng/g
2 mos.	5.0 ng/ml	30.0 ng/ml	10.2 ng/g	12.2 ng/g
6 mos.	4.9 ng/ml	29.4 ng/ml	10.0 ng/g	12 ng/g
Cumulative corrected brain inorganic Hg				44.7 ng/g

**For these calculations the infant blood mercury levels after vaccination were taken from Pichichero et al., 2008 (PMRL0497). A brain/blood ratio for mercury in the human of 6.0 was used (Magos, 1987, PMRL0666); and from Burbacher et al., 2005 (PMRL0026), 34% was used as the percentage that inorganic Hg was of the total Hg in the brain of infant monkeys. However, these values are from Argentinian children who received TCVs according to a different vaccination protocol than USA children (Pichichero et al., 2008).*

*** see legend of table 1 for definition of corrected value.*

The paper by Vahter 1994 "Speciation of Hg in Primate blood and brain." (see table 2)states that 60ng of Hg^{++}/g (or inorganic Hg/g) will cause neuroinflammation in the brain. This is remarkably close to the 43.7 and 44.7 ng inorganic Hg that we have determined independently.

V. Additional Rebuttals of Dr. Brent's Testimony

Since my testimony in May, there have been some published studies that back up my opinions and which tend to refute those of Dr. Brent.

First, with respect to evidence for a mercury efflux disorder in autistic children, the Holmes et al (PMRL0237) studies indicating that autistic children have less mercury in their hair indicating that they have an efflux disorder has been confirmed recently by Adams et al., 2008 (PMRL0667).

Citations for evidence that autistic children have less mercury in their hair and bodies and support the concept that a mercury efflux disorder is involved in some or all autism cases.

1. **Holmes et al., 2003. (PMRL0237)**
2. **Hu et al., 2003 (PMRL0016)**
3. **Bradstreet et al., 2003 (PMRL0244)**
4. **Adams et al., 2007 (PMRL0138)**
5. **Adams et al., 2008 (PMRL0667)**

Adams et al., 2008 demonstrated that at hair mercury concentrations of below 0.55μg/g, children are 2.5 times more likely to manifest autism. This study was done in collaboration with people at the National Inst of Health. This supports the initial paper of Holmes et al., 2003 (PMRL0237) and Hu et al., 2003 (PMRL0016). All three studies plus the Bradstreet et al., report together show that autistic children tend to be slow excretors of Hg.

Dr. Brent and other DOJ witnesses criticized the Hornig mouse study (PMRL0015) model of thimerosal leading to autistic symptoms in animals, but since then there has been publication of another good animal model of EtHg toxicity from TCV's: the Peruvian hamster study by Laurente et al. (PMRL0668).

Dr. Brent also criticized the Bradstreet 2003 chelation challenge study (PMRL0224) for having no pre-challenge results and for not having any standard reference for post-chelation results. However, traditionally there have been two ways of doing challenge tests: (1) a control group also is given the challenge, or (2) a pre-challenge urine collection is begun on the subjects 6 hrs before the challenge. Experienced investigators know that when working with autistic children it is difficult to get a pre-challenge collection for 6 hrs and then give them the challenge and again collect urine, so they used the control protocol. This was a reasonable and valid way to do the study.

It is pertinent to note that Windham et al., 2006 (PMRL0018) in their conclusions "suggest a potential association between autism and estimated metal concentrations, and possibly solvents, in ambient air around the birth residence..." and that since my testimony in May, Windham et al. have refined their data to show a statistically significant correlation between distance from the Hg release point source and the rate of autism four years later. Windham et al., 2008 (PMRL0670).

Dr. Brent cited a study from Brazil for his claim that the average exposure to MeHg ingested from breast milk in the first six months was about 280 micrograms. About 95% of the MeHg is absorbed by the gut (266 micrograms). A certain percentage of this will be delivered to the brain. We are not certain as to how much because during this period the infant blood brain barrier is not in its mature barrier form. Note, however, that the Hg++ delivered to an infant's brain from TCV's would only add to the total Hg++ in the brain, thus making TCV's even more dangerous to infants whose mothers have MeHg in breast milk.

In summary, it is my opinion to a reasonable degree of scientific certainty, that in some infants receiving the normal schedule of TCV's in the mid 1990's in the USA, there would be sufficient concentrations of Hg++ deposited in their brains to trigger the same kind of neuroinflammation and other brain cell changes seen in the adult monkeys exposed to MeHg. Given the fact that many infants will already be exposed to some Hg++ in their brains from breast milk, and ambient air sources, it is even more likely that the additional amount of Hg++ from TCV's would push some kids over the toxic threshold.

Signed: H. Vasken Aposhian Date: 7/8/2008

REFERENCES

PMRL #	AUTHOR	TITLE	CITATION	DATE
0015	Hornig, M, et al	Neurotoxic Effects of Postnatal Thimerosal Are Mouse Strain Dependant	Molecular Psychiatry 2004:1-13	5/4/2004
0016	Hu, L et al.	Neutron activation analysis of hair samples for the identification of autism.	Poster presentation:Trans Am Nucl Soc 2003;89	1/1/2003
0018	Windham GC, Zhang L, Gunier R, Croen LA, Grether JK	Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco Bay Area.	Environ Health Perspect. 2006 Sep;114(9):1438-44.	9/1/2006
0026	Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T	Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thimerosal.	Environ Health Perspect. 2005 Aug;113(8):1015-21	08/00/2005
0032	Charleston JS, Body RL, Mottet NK, Vahter ME, Burbacher TM;	Autometallographic determination of inorganic mercury distribution in the cortex of the calcarine sulcus of the monkey Macaca fascicularis following long-term subclinical exposure to methylmercury and mercuric chloride.	Toxicol Appl Pharmacol. 1995 Jun;132(2):325-33	6/1/1995
0033	Charleston JS, Bolender RP, Mottet NK, Body RL, Vahter ME, Burbacher TM	Increases in the number of reactive glia in the visual cortex of Macaca fascicularis following subclinical long-term methyl mercury exposure.	Toxicol Appl Pharmacol. 1994 Dec;129(2):196-206.;	12/1/1994
0035	Clarkson TW, Magos L	The toxicology of mercury and its chemical compounds.	Crit Rev Toxicol. 2006 Sep;36(8):609-62	1/1/2006
0060	Vahter M, Mottet NK, Friberg L, Lind B, Shen DD, Burbacher T	Speciation of mercury in the primate blood and brain following long-term exposure to methyl mercury	Toxicol Appl Pharmacol. 1994 Feb;124(2):221-9	2/1/1994
0064	Vahter ME, Mottet NK, Friberg LT, Lind SB, Charleston JS, Burbacher TM	Demethylation of methyl mercury in different brain sites of Macaca fascicularis monkeys during long-term subclinical methyl mercury exposure	Toxicol Appl Pharmacol. 1995 Oct;134(2):273-84	10/1/1995
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0116	Charleston JS, Body RL, Bolender RP, Mottet NK, Vahter ME, Burbacher TM. ;	Changes in the number of astrocytes and microglia in the thalamus of the monkey Macaca fascicularis following long-term subclinical methylmercury exposure.; ;	Neurotoxicology. 1996 Spring;17(1):127-38. ; ;	4/1/1996
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0183	Davis LE, Kornfeld M, Mooney H, Fielder KJ, Haaland KY, Orrison WW, Cernichiari E, Clarkson TW	Methylmercury Poisoning: Long-Term Clinical, Radiological, Toxicological, and Pathological Studies of an Affected Family	Annals of Neurology;35(6): 680-6	6/1/1994
0213	Nordberg, GF, Fowler BA, Nordberg M, Friberg LT.	Handbook on the toxicology of metals,	3d edition, Academic Press 2007	
0223	Pichichero ME, Cernichiari E, Lopreiato J, Treanor J	Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study	Lancet;360(9347): 1737-40	11/30/2002
0237	Holmes AS, Blaxill MF, Haley BE	Reduced Levels of Mercury in First Baby Haircuts of Autistic Children	International Journal of Toxicology;22(4): 277-85	7/1/2003
0244	Bradstreet J, Geier DA, Kartzinel JJ, Adams JB, Geier MR	A Case-Control Study of Mercury Burden in Children with Autistic Spectrum Disorders	Journal of American Physicians and Surgeons;8(3): 76-9	6/1/2003
0497	Pichichero ME, Gentile A, Giglio N, Umido V, Clarkson T, Cernichiari E, Zareba G, Gotelli C, Gotelli M, Yan L, Treanor J.	Mercury levels in newborns and infants after receipt of thimerosal-containing vaccines.	Pediatrics. 2008 Feb;121(2):e208-14.	2/1/2008
0666	Magos L, Clarkson TW, Sparrow S, Hudson AR	Comparison of the protection given by selenite, selenomethionine and biological selenium against the renotoxicity of mercury	Archives of Toxicology;60(6): 422-26	8/1/1987
0667	Adams JB, Romdalvik J, Levine KE, Hu LW	Mercury in first-cut baby hair of children with autism versus typically-developing children.;	Toxicol Environ Chem 2008, May 2008 (Abstract)	5/1/2008
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(On April 3, 2009, the Petitioners' Steering Committee filed a compact disc containing the PSC's Updated Master Reference List. This disc has been placed into the record of the Omnibus Autism Proceeding, but its contents, except for the list of titles that follows this page, are not being placed on the website at this time, due to the fact that copyrighted material is included.)

[List of scientific articles and other materials follows.]