

### **Thimerosal-Containing Vaccines and Neurodevelopment Outcomes**

**FORWARD:** Thimerosal or merthiolate is a derivative of thioisalicylate where ethyl-mercury is attached through the sulfur. It is defined as a preservative or anti-microbial in medical use. This anti-microbial action is dependent on thimerosal breaking down releasing ethyl-mercury that can penetrate cell membranes and bind to intracellular enzymes, inhibiting them, and causing cell death. Further, in certain biological environments the ethyl-mercury can further break down releasing mercury cation ( $\text{Hg}^{2+}$ ).  $\text{Hg}^{2+}$  is also very reactive with enzymes and proteins inhibiting their biological functions and causing cell injury or death. Both ethyl-mercury and  $\text{Hg}^{2+}$  are very neuro-toxic compounds.

However, ethyl-mercury is more rapidly partitioned into the hydrophobic (fatty) tissues of the central nervous system and is a more potent neuro-toxin than  $\text{Hg}^{2+}$  based on this "partitioning factor". It is this partitioning factor that makes organic-mercurials such as dimethyl-mercury so neuro-toxically lethal (this is the compound that caused the death of a Dartmouth University chemistry professor after she was exposed to a drop or two on her gloved hand). The concern with organic-mercurials, such as thimerosal, is that such compounds can be perceived as "pro-toxicants" just as certain pharmaceuticals can be classified as "pro-drugs". This means that the original compound, e.g. thimerosal, is less reactive giving the compound time to partition into certain areas of the body before it breaks down releasing the ethyl-mercury and then further releasing  $\text{Hg}^{2+}$ . However, while attaching ethyl-mercury to thioisalicylate makes the ethyl-mercury less reactive it most likely allows increased partitioning into the central nervous system before the ethyl-mercury is released and thereby, increases the neuro-toxicity per unit ethyl-mercury involved.

Considerable caution must be taken when stating what is the "toxic level" of mercury and any mercury containing compound. Humans are not rats in a pristine cage where their environment can be controlled to ensure that other toxicities and infections are not occurring. The level of mercury that would cause toxicity in a healthy individual is much higher than what would be needed to cause a toxic effect in an individual that is ill, aged or under oxidative stress. This is because additional stresses and increased age lower the amount of protective compounds that bind mercury and render it less harmful. If an individual is low on these protective compounds, then less mercury or thimerosal would be needed to cause a clinical effect. Below I will present my interpretation of our research and that from other laboratories that focus on the potential toxicity of injected thimerosal in the vaccine mixture. The conclusion I have reached after both personal laboratory research and a thorough evaluation of the research literature is that the injection of thimerosal into expectant mothers and newborn infants represents without a doubt a severe, major toxic exposure and is most likely causal in autism spectrum disorders.

**BIOCHEMICAL TOXICITY STUDIES:** In my laboratory we have recently done an evaluation of the potential *in vitro* toxicity of vaccines containing thimerosal as a

“preservative” versus those vaccines not containing thimerosal. In these preliminary studies, vaccines with thimerosal added consistently demonstrated in vitro toxicity that was markedly greater than the non-thimerosal or low thimerosal containing vaccines. We also compared the toxicity of the vaccine solutions with solutions of pure thimerosal and with solutions of mercury chloride. Mercury is a known neurotoxin and its mechanism of neurotoxicity has been studied in our laboratory for the past 10 years. To determine the relative toxicity we used two different biological testing systems: (i) brain homogenates and (ii) a mixture of four purified mammalian enzymes. In human brain homogenates we had earlier observed that mercuric ion rapidly inhibited tubulin viability at low micromolar levels, mimicking the situation in Alzheimer’s diseased brain, but was less toxic to actin (see **Figures 1& 2**). Both tubulin and actin are polymerizing proteins that are actively involved in neurite growth cone activity. In contrast to mercuric ion, vaccines containing thimerosal inhibited both tubulin and actin viability (see **Figure 3**). This would indicate that thimerosal has the potential to be much more damaging to neurite development than equivalent levels of mercuric ion. It is my hypothesis that thimerosal releases ethyl-mercury which most certainly interferes with neurite growth and neuronal development in infants through rapid inhibition of several thiol-sensitive enzymes/proteins including actin, tubulin and creatine kinase. This supports the concept that thimerosal in biological solutions injected into the human body could cause a number of systemic problems identified as disease states. Recently, in preliminary studies using rat hippocampal neurons in culture we observed that toxicity started in the low nanomolar level.

**CELL CULTURE WORK ON THIMEROSAL TOXICITY:** The toxicity results obtained in our biochemical toxicity studies were not at all unexpected since thimerosal and other compounds containing a similar thiol-organic mercury group are widely known to be especially potent neurotoxic agents. Our biochemical toxicity results are very consistent with the reported toxicity of thimerosal containing vaccines versus non-thimerosal containing vaccines as observed in cell culture studies (*Kravchenko et al., Evaluation of the Toxic Action of Prophylactic and Therapeutic Preparations on Cell Cultures III. The Detection of Toxic Properties in Medical Biological Preparations by the Degree of Cell Damage in the L132 Continuous Cell Line. Zh. Mikrobiological Epidemiol. Immunobiol. (3):87-92, 1983*). The results of this research demonstrated the toxicity of thimerosal (merthiolate) by showing cell damage of the 1:10,000 concentration found in vaccines after dilution of this mixture to 1 part per 128. The conclusion was that thimerosal use for medical and biological preparation (i.e. vaccines) manufacturing is inadmissible, especially in pediatrics. Other studies on cytotoxicity of thimerosal compared it to another mercury containing preservative (phenylmercuric acetate) and thimerosal was 5 times more toxic with only a two minute exposure to the cells. The LD50 for thimerosal was 2.2 micrograms/ml for a 24 hour exposure to human conjunctival cells and the comment was made that “the longer the contact time of these preservatives, the severer the damage to the ocular tissue”.

In collaboration with another professor in our department we have now included toxicity studies using human brain neurons in culture. Our initial studies have shown that thimerosal is quite toxic to these neurons in culture with neuron death being observed at

as low as 10 nanomolar concentration of thimerosal with 50 nanomolar thimerosal causing 43% neuron death in 24 hours. 100 nanomolar thimerosal caused about 83% death occur in 24 hours.

Studies using vaccines with and without thimerosal present demonstrated that the presence of thimerosal greatly enhanced the neurotoxicity of the vaccine. Further, the vaccines seemed more toxic than the level of thimerosal they contained should have caused. This would be expected as the vaccines also contain aluminum that is in itself neurotoxic (see section on synergistic toxicity below). However, the neuron toxicity studies on the pure thimerosal and vaccines mirror the results we observed in the enzyme toxicity studies mentioned above with the thimerosal being equally or more toxic than inorganic mercury to many critical enzymes. Further studies are underway at the present time.

**SYNERGISTIC TOXICITIES WITH THIMEROSAL:** Since about 1989 my laboratory has been actively involved in research regarding the toxic effects of elemental mercury and the relationship of this toxicity to neurological diseases, primarily Alzheimer's disease. One fact that has become extremely obvious to me during this past 11 years is that it is impossible to determine the exact toxic level of mercury or mercury containing compounds that is safe for all humans. There are several reasons why mercury should not be considered safe for humans at the measurable levels currently reported as "safe" by current government monitoring agencies. One of these is the obvious effects of other metals on increasing the toxicity of identical levels of mercury. An example is that of zinc ion, an essential metal for normal cell function. Yet, in the presence of mercuric ion, the addition of zinc enhances the toxicity level significantly (see **Figure 4**). Cadmium and lead are even more potent at enhancing the toxicity of mercuric ion. This concept of synergistic toxicity of mercury with other metals is supported by prior research that demonstrated that a mixture of mercury at LD-1 level with lead at 1/20<sup>th</sup> the LD-1 level produced a mixture with an LD-100 effect, at least 50 to 100 times the additive effect minimally expected (*Schubert, J., Riley, E.J. and Tyler, S.A., Combined Effects in Toxicology—A Rapid Systematic Testing Procedure: Cadmium, Mercury and Lead. J. of Toxicology and Environmental Health, 4:763-776, 1978*).

Using pure thimerosal and aluminum solutions the possible synergistic effects of combining these two toxicants was studied. Levels of thimerosal that caused about 50% neuron death gave nearly 90% death in the presence of non-toxic levels of aluminum hydroxide. This represents a significant synergy of the toxicity as shown in **Figure 5**. Therefore, mixing thimerosal with aluminum does enhance toxicity and likely follows the basic chemistry described where aluminum with thimerosal effected severe skin burns in patients (*H.T. Jones, Danger of Skin Burns from Thiomersal, British Medical J. #2, p504-505, 1972*).

The synergistic effects of different compounds with thimerosal are not all known but some do exist. For example, the commonly used antibiotic, tetracycline, is known to enhance thimerosal toxicity. *Crook and Freeman, Reactions Induced by the Concurrent Use of Thimerosal and Tetracycline, American J. of Optometry & Physiological Optics v60,#9, pp759-761 1983*, reported that the use of tetracycline in humans induced and increased the irritation and inflammation of the ocular tissues caused by thimerosal. These results were confirmed in studies using rabbits. Therefore, it is obvious that

concurrent treatment of infants with other drugs and/or antibiotics has the possibility to enhance the toxic effects of thimerosal exposures. Further, it was postulated that the synergistic effects of tetracycline was due to the metal binding properties of this antibiotic that may have delivered the toxic metal more effectively to the site(s) inducing enhanced toxicity.

Studies were done to see if various antibiotics enhanced the toxicity of thimerosal against the neurons in culture system. Tetracycline, ampicillin and neomycin all enhanced the toxicity to somewhat different extents. However, using this system it would be difficult to prove the effects in a whole body animal without the appropriate studies. This data clearly demonstrates that there is no know level of safety for the use of thimerosal, especially in infants being treated with other medicinals that would enhance the toxicity of the ethyl-mercury released such as occurred with tetracycline (a commonly used antibiotic).

One of the conundrums of autism is the explanation of the 4:1 ratio of boys to girls that get the disease. It has been reported that estrogen therapy reduces the risk of females to Alzheimer's disease, a clinical condition we hypothesize is exacerbated by mercury. We therefore decided to test the effects of both female and male hormones on the neurotoxicity of thimerosal. The results were eye-opening. For example, 50 nanomolar thimerosal causes less than 5% neuron death within the first three hours incubation and 1 micromolar testosterone causes no significant death within this time frame. However, mix these two together and 100% neuron death was observed at the earliest time point checked. This represents a severe enhancement of thimerosal toxicity (see **Figure 6**). Further, at 12 hours the neuron death effected by 50 nanomolar thimerosal alone could be reversed by 1 micromolar estrogen. Estrogen also significantly reduced the testosterone enhanced toxicity of thimerosal. While much research is yet to be done it is apparent that these results fit into the "thimerosal being causal" hypothesis as it may be used to explain the high rate of boys being affected. Interestingly, a Dr. Simon Baron Cohen of London has reported in a meeting that, in a study of the amniotic fluid of mothers who gave birth to autistic children, the major anomaly was an increased level of testosterone in their amniotic fluid when compared to that of mothers who gave birth to non-autistic children.

The data above on the effects of antibiotic, other heavy metals and hormones on thimerosal toxicity imply that it would be impossible to predict the exact level of mercury that would induce observable toxicity in each human. Many environmental toxicants could work synergistically with ethyl-mercury rendering the ethyl-mercury much more toxic than it would be in the absence of these other toxicants (e.g., elemental mercury from dental amalgams, cadmium from smoking, lead from paint and drinking water, aluminum, etc.). Humans are not rats in a pristine cage, eating rat chow carefully prepared to eliminate any toxicants. Humans smoke, drink alcohol, have numerous mercury emitting amalgam fillings, eat questionable food, and drink water known to contain other toxicants. Finally, it is impossible to state the toxic effect of any injection of thimerosal unless one knows the toxic exposure of the individual to other heavy metals or other environmental toxicants.

**CASE HISTORIES ON THE TOXICITY OF THIMEROSAL AND OTHER ETHYL-MERCURY RELEASING COMPOUNDS:** A recent review covers much of

the case history literature on the little that is known about ethylmercury toxicity (*L. Magos, Review on the Toxicity of Ethylmercury, Including its Presence as a Preservative in Biological and Pharmaceutical Products, J. Applied Toxicology 21, 1-5, 2001*). The conclusions reached by the author of this review is that “ethylmercury may present a risk when blood mercury concentrations approaches or exceeds 1.0 microgram per ml and severe intoxication occurs when blood mercury concentration approaches or exceeds 2 micrograms per ml.”

In the context of the literature reviewed the conclusions by Dr. Magos seems reasonable. However, this conclusion was based primarily on ethylmercury and methylmercury exposures from occupational exposures, dietary intake, externally applied tinctures along with vaccination data on adults. It should be noted that in considering deceased patients the one infant had a blood mercury (from an externally applied tincture) that was measured at 1.34 micrograms per ml, a young boy had a blood mercury of 5 micrograms per ml (from eating pork from a pig feed ethylmercury) and adults had 15 micrograms per ml (from eating bread made with seed treated with a compound that generated ethylmercury). Without the needed extensive data to make a conclusion, it appears as if the younger the patient the more deadly or toxic the ethylmercury is at a lower concentration. This is further supported by the other (*Kostial, K., et al. Influence of Age on Metal Metabolism and Toxicity, Environmental Health Perspectives, v25, 81-86, 1978*) who state “results obtained in sucklings show a very high intestinal absorption of all metals which is partly attributed to milk diet; a higher whole body retention, higher blood levels and a much higher accumulation in the brain”. Certainly, no conclusion of safe levels of exposure to ethylmercury on infants could be made from the data reviewed by Dr. Magos.

The exposures reviewed were from different delivery modalities and there is a considerable difference in the toxicity of many materials when oral intake is compared to injections via the vaccine route. Total mercury in the blood stream does not distinguish between bound mercury (e.g. that coupled with glutathione and being removed from the body) and unreacted mercury (that available to cause further damage). Ratios of bound and free ethylmercury are likely to be different if ethylmercury is eaten or inhaled versus injected, bypassing the protective systems available in the intestines. It was also pointed out in the review that the blood/urine ratios varied from 3.4 to 18 indicating that urine mercury levels are inferior for monitoring ethylmercury exposures. However, since ethylmercury should partition between blood and urine at a consistent ratio this data could also be interpreted to indicate that the mercury in some of these patients is coming from more than just ethylmercury (e.g. dental amalgams that are the major source of human mercury body burden). In a report on mercury levels in squirrel monkeys treated intranasally with thimerosal (*Blair, A., Clark, B., Clarke, A and Wood, P., Tissue Concentrations of Mercury After Chronic Dosing of Squirrel Monkeys with Thimerosal, Toxicology, v3, 171-176, 1975*) it was shown that exposure to 0.002% thimerosal daily for 6 months, with a total of 2,280 µg given, lead to a 174/29 or about 6.0 ratio of mercury in the brain/blood ratio indicating that thimerosal leads to a more rapid build up of brain versus blood mercury. However, it was pointed out that the highest brain total (250ng/g) was still below the 3-9 µg/g where neurological symptoms appear, but this later value would depend on the oxidative stress of the patient and could be much lower.

The review states that “ethylmercury in medicinal preparations declines with time” and gave examples of 38%, 64% and 85% decreases in ethylmercury in plasma and immunoglobulin G samples. This mercury did not disappear and the loss of ethylmercury has to be due to ethylmercury reacting covalently with the protein-thiols in the medicinal preparations. In aged medicinal preparations, increased ethylmercury reaction with protein-thiols in the preparations would likely change the neurotoxicity effects of the resulting mercury complexes compared to pure ethylmercury. How this pre-reacted ethylmercury would contribute to blood levels of mercury appears unknown, but it is likely to be quite different from pure ethylmercury. However, what is known is that ethylmercury retains its severe toxicity after prolonged exposure in living animals. This is supported by a case mentioned in the Magos review where ethylmercury obtained by “consumption of meat from a pig fed with ethyl-mercury” caused severe damage to adults and killed two young boys. It seems as if ethylmercury can retain its severe toxicity after a period of incubation time in a living pig, butchering and storage of meat, followed by cooking. Then the concept that the faster decomposition of ethylmercury, relative to methylmercury, decreases its toxicity compared to methylmercury seems to be such a small difference as to be insignificant. What is solidly observed is that ethylmercury (and other organic-mercurials) can withstand considerable exposure to a living system, storage in a biological environment, exposure to high heat in the presence of muscle tissue, and still produce a lethal toxicity when taken orally.

In a 1972 *(National Geographic , Quicksilver and Slow Death, v142, #4, 507-527, 1972)* a similar report was presented where the pig was fed seed coated with Panogen, a methylmercury pesticide. The family ate the pig as above and the four children suffered severe neurological damage. But, in contrast to the ethylmercury poisoning above, they all lived. One of the children was *in utero* during the consumption of the pork, suffered the most and was born blind and mentally retarded. Again, this supports the concept that the younger the human the more detrimental the toxic effect the organic mercury compounds will have.

It appears certain that much of the blood level mercury in these patients presented in the Magos review could be from sources other than pure ethylmercury. In my opinion, I do not believe that a safe level of ethylmercury can be arrived at by only comparing blood levels of mercury if we do not know the chemical nature of all of the contributing mercury sources, the initial source of the mercury or if the presence of other compounds were involved (e.g. antibiotics that bind heavy metals such as tetracycline and enhance thimerosal toxicity:see below in Synergistic Toxicity).

It is of major concern that ethylmercury from thimerosal in vaccines is a special situation. It is injected with millimolar levels of aluminum and it is probable that thimerosal, a negatively charged molecule, has formed a salt compound with the positively charged aluminum cation that would change its partitioning, breakdown rate, and may have a synergistic effect on the toxicity of any mercuric ion produced from the ethylmercury. Aluminum is a known neurotoxin and to be causally involved in macrophagic myofasciitis. The enhanced toxicity of ethylmercury in the presence of

other toxic agents is to be expected. Few of the clinical cases included in the Magos review were from vaccine but the one that was discussed problems which occurred in a 44 year old adult with a blood mercury of 0.104  $\mu\text{g}$  per ml, so low that Dr. Magos called the diagnosis “unconvincing”. Perhaps co-administration of thimerosal with aluminum in the Hepatitis-B vaccine represents the “other etiological factors than ethylmercury” that might have been responsible for his mercury like induced symptoms at such low concentrations. The authors of the report on this patient state “this patient had evidence of previous environmental exposure to mercury” and this data can imply that thimerosal is more toxic in patients previously exposed to materials that sensitize them.

**DR. MAGOS REPORT TO THE IOM, SUMMER 2001:** Dr. Magos makes several statements that reasonable individuals with scientific experience could disagree about. First, “The consequence of faster decomposition is that, compared with methylmercury, the neurotoxic potential of ethylmercury declines faster.” This requires the assumption that ethylmercury breaks down to  $\text{Hg}^{2+}$  as a toxic factor. What if the breakdown product was a conjugate of cysteine known to enhance the toxicity of mercuric ion? What if the breakdown was caused by reactive oxygen species generated in response to an infection? It is known that ethylmercury breaks down 10 times faster in the presence of reactive oxygen species (*Suda, I, and Takahashi, H., Degradation of methyl and ethyl mercury into inorganic mercury by other reactive oxygen species besides hydroxyl radical. Arch. Toxicol. 66, 34-39, 1992*) making the production of toxic  $\text{Hg}^{2+}$  occur more rapidly at sites of high level of reactive oxygen, and in the body this would be at sites of infection or inflammation or within mitochondria, the important energy producing organelle. In my opinion, the enhanced chemical ability to breakdown ethylmercury versus methyl mercury at sites of reactive oxygen production (usually sites of oxidative stress) makes ethylmercury a much more dangerous compound than methylmercury as it attacks chemically at a site of infectious damage.

In section 2.b.a Dr. Magos quotes his research as showing that methylmercury treated rats had 1.55 (males) and 2.4 (females) the mercury in their brains as did ethylmercury treated rats. In addition, the ethylmercury treated rats had 3.4 fold more inorganic mercury in their brains. He states that this “excludes the possibility that the cleavage itself of the formed inorganic mercury is responsible for the brain damage. If this were the case, the brain ethylmercury treated rats would be more affected than the brain of methylmercury treated rats (which didn’t occur by his analysis).” The problem with this conclusion is that Dr. Magos expects the mechanism of damage caused by methylmercury to be the same as that caused by a combination of ethylmercury and 3.4 fold extra  $\text{Hg}^{2+}$ . This is not likely as methyl and ethyl mercury would partition into the hydrophobic areas of the brain whereas  $\text{Hg}^{2+}$  would most likely react in the hydrophilic aspect of the brain. The inhibition of specific brain enzymes by thimerosal (ethylmercury) compared to  $\text{Hg}^{2+}$  are markedly different. Further, our data on the effects of thimerosal on pure enzymes shows rapid inhibition at times that would not allow the breakdown of the ethylmercury to inorganic mercury.

**THE EFFECTS OF AGE AND HEALTH ON THIMEROSAL TOXICITY:** The detrimental effect of any specific level of mercury or mercury containing compound

would have on any one individual's metabolic system would be directly proportional to both the level of "protective bio-compounds" (e.g., glutathione, metallothionein) that exist within that person on the time of exposure and, the ability to physiologically clear such toxicants from the body. The level of the protective compounds would certainly be directly dependent on two factors, age and health. Infants, with their immature physiology and metabolism would not be expected to handle mercury as efficiently as mature adults. The elderly have been shown to have decreased "protective" glutathione levels compared to middle aged and young adults. Melatonin, a hormone, is known to be decreased in the aged and melatonin is known to increase the neuron and cellular concentration of glutathione. Glutathione is the natural compound that binds mercuric ion and aids in its removal from the body. This explains partly why the aged are also more susceptible to oxidative toxicants such as mercury.

The elderly also have weakened immune systems and are more susceptible to microbial infections are known to lower their chemical energy levels and, further, to reduce their ability to synthesize the proteins that protect them from heavy metals. Infants have their own weaknesses regarding toxic exposures. Infants do not make much bile in their early months of life and are less able to remove mercury through biliary transport, the major route for mercury removal. They also do not have a fully developed renal system that would remove other heavy metals (e.g. aluminum) as effectively as adults. The age factor must always be considered for response to heavy metal exposure as well as spurious microbial infections.

#### **THE EFFECTS OF GENETIC SUSCEPTIBILITY ON MERCURY TOXICITY:**

Genetically susceptibility is of critical importance. For example, other researchers have shown that genetic carriers of the brain protein APO-E2 are protected against Alzheimer's disease (AD) whereas genetic carriers of the APO-E4 genotype are at enhanced risk factor for developing AD. APO-E proteins are synthesized in the brain with the assigned physiological task of carrying waste material from the brain to the cerebrospinal fluid, across the blood-brain barrier into the plasma where the material is cleared by the liver. The biochemical difference between APO-E2 and APO-E4 is that APO-E2 has two additional thiol groups, capable of binding and removing mercury (and ethyl-mercury) that APO-E4 does not have. The second highest concentration of APO-E proteins is in the cerebrospinal fluid. Therefore, it is my opinion that the protective effects of APO-E2 is due to its ability to protect the brain from exposure to oxidants like mercury and ethyl-mercury by binding these toxicants in the cerebrospinal fluid and keeping them from entering the brain. I strongly object to labeling those "genetically susceptible" as "having a genetic disease" because they are the first injured on exposure to modern toxicants. Humans did not evolve breathing mercury vapor or having organic-mercury compounds injected in them as infants.

**SIMILARITY TO ACRODYNIA:** The argument that the thimerosal containing vaccines could not deliver the amount of mercury to cause a systemic illness is somewhat refuted by the history of the disease classified as acrodynia. Perhaps autism will end up like acrodynia, where the removal of the causative material (i.e. the mercury containing teething powders) lead to cessation of the disease and the identification of the cause. Due

to the perceived low levels of mercury in the teething powders and the wide-spread use of mercury in medicine at that time it was 10 years after the removal of the mercury containing teething powders before medicine acknowledged that mercury exposure was the causal factor. It is significant to notice that many of the symptoms of acrodynia are similar to the clinical symptoms of children identified today as autistic, with attention deficit disorder, etc. that have no family history of such diseases or illness classifications.

**SUMMARY:** It is the inability to see the effects of chronic, low level toxicities on human health that has been, and remains, our greatest failing as intelligent beings. For example, within the past year two publications in refereed scientific journals have emerged from major foreign research universities demonstrating that mercury can induce the formation of three major pathological diagnostic hallmarks of Alzheimer's disease. The production of these diagnostic hallmarks occurred at non-lethal concentrations near or below the levels of mercury reportedly found in most human brains. First, mercury has been shown to induce an increase in amyloid protein secretion (the component of amyloid plaques) and to increase the phosphorylation of a protein called Tau {see Oliveri et al., *J. of Neurochemistry*, V 74, p231, 2000}, and to produce neurofibrillary tangles {Leong et al., *NeuroReports* V12(4), 733, 2001}. All of this was done with neurons in culture and represent observations found and considered diagnostic of Alzheimer's disease. Further, in a very recent article by Dr. Ashley Bush in the journal *Neuron* it is implied that Alzheimer's disease may be caused by heavy metal buildup. This article focused on removal of zinc and copper by chelation decreasing amyloid plaque formation in rats--mercury was not studied. However, these metals, along with silver, are the components of dental amalgams. This work is in agreement with data published earlier from my laboratory in refereed articles and summarized in one single article {Pendergrass and Haley, *Metal Ions in Biological Systems* V34, Chapter 16, *Mercury and Its Effects on Environment and Biology*, Siegel and Sigel EDS., Marcel Dekker, Inc. 1996}. This data basically demonstrated that addition of very low amounts of mercury to normal human brain homogenates inhibited critical thiol-sensitive enzymes (creatine kinase, glutamine synthetase and tubulin) that are also dramatically inhibited in Alzheimer's diseased brain. Research in our laboratory clearly demonstrates that thimerosal rapidly inhibits these enzymes as well as several other metabolically important enzymes.

Further, data presented in Aschner et al. in *Methylmercury Alters Glutamate Transport in Astrocytes, Neurochemistry International*, v37, #2-3, pp 199-206, 2000 indicate that organic-mercury compounds dysregulate excitatory amino acid homeostasis and may cause glutamate-mediated excitotoxic mechanisms to be involved on exposures that cause neuron death or injury. Glutamate toxicity is one hypothesis proposed to explain the slow deterioration of AD as it was reported that the enzyme, glutamine synthetase, that removes toxic glutamate was elevated in AD cerebrospinal fluid (D. Gunnerson and B. Haley, *PNAS, USA*, v89, 11949, 1992) and inhibited in AD brain (Butterfield et al., *J. Neurochemistry*, v68, 2451, 1997). Glutamine synthetase is rapidly inhibited by the divalent mercuric ion as it has two divalent metal ion (manganese) binding sites required for activity. It is obvious that ethyl-mercury from thimerosal would have the same effect on glutamine synthetase as mercury and methyl-mercury and

impair nervous system glutamate metabolism. Consistent with this concept is the reported ability of astrocytes (the brain cells that contain glutamine synthetase that converts toxic glutamate to non-toxic glutamine) to preferentially concentrate brain organic-mercury (*Ashner, Astrocytes as Modulators of Mercury-Induced Neurotoxicity, Neurotoxicology v17, #3-4, pp663-669, 1996*). The straight-forward conclusion is that any exposure to mercury or mercury containing compounds (e.g. thimerosal) would exacerbate any medical condition affected by the inability to metabolize glutamate.

The chemical rationale for the neurotoxicity of thimerosal is that this compound would release ethyl-mercury as one of its breakdown products. Ethyl-mercury is a well-known neurotoxin. Further, combining thimerosal with the millimolar levels of aluminum cation plus significant levels of formaldehyde, also found in these vaccines, would make the vaccine mixture of even greater risk as a neurotoxic solution. The synergistic effects of mercury toxicity with other heavy metal toxicities (Pb, Cd, Zn) has been established in the literature for many years. Further, using this vaccine mixture on infants who are ill and do not have fully developed biliary (liver) and renal (kidney) systems could greatly increase the toxic effects compared to that observed in healthy adults.

The toxic effects of exposure to thimerosal to adults and infants and always been reported to have dire consequences, including death. Similar exposures, even at lower level, in infants should have more severe consequences compared to those observed in adults made toxic by exposure to similar ethyl-mercury containing compounds. Mercury is primarily removed through the biliary system and aluminum is removed by the renal system. Inability to rid the body of these toxicants would greatly increase the damage they are capable of doing.

While one can understand the necessity of using an anti-microbial “preservative” in vaccines to prevent contamination it represents poor judgment to use a “preservative” that breaks down into a well-known neurotoxin when safer “preservatives” were available. Further, it has come to my attention through several parents that a significant number of physicians encourage mothers to have their infants receive multiple vaccinations during one visit. In one report a 13 pound baby was given 4 vaccinations. This would result in the equivalent of a 130 pound adult receiving 40 vaccinations in one day. This is quite unreasonable in my opinion, but appears to happen with a great deal of regularity in practice. Physicians do this as they are not warned of the possible consequences and are regularly informed by vaccine providers that the vaccines are totally safe. No steps were taken to recommend against this procedure.

It is very difficult to prove that mercury or organic-mercury compounds cause any specific disease that is identified by its related symptoms. This is due to the fact that mercury toxicity from various types of mercury containing materials may be considerably different and the genetic susceptibility and age of the victim would alter the response. This difficulty is further compounded due to the high numbers of confounding factors presented in the current human environment. However, since infants get autism and related disorders, and many of our aged are afflicted with AD, we know that they have

crossed the thin-red line into the neurologically diseased state. There can be no doubt that the purposeful use of mercury in medicine and dentistry, especially if it was prolonged and excessive, would significantly contribute to the onset of their disease. In my opinion, this is especially true in the case of the injection of thimerosal via vaccines in expectant mothers, day old infants and toddlers. With our current experimental results, especially the testosterone enhanced and estrogen reduction of thimerosal neurotoxicity, I strongly feel that thimerosal released ethylmercury is the most likely cause of autism and related disorders.

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DATE

Figures accompanying this presentation are on power point but consist of the following titles.

FIGURE 1: COMPARISON OF THE VIABILITY OF BRAIN TUBULIN IN CONTROL (NON-DEMENTED) VERSUS ALZHEIMER'S DISEASED BRAIN.

FIGURE 2: A COMPARISON OF THE EFFECTS OF MERCURIC ION ADDITION ON CONTROL (NON-DEMENTED) AND ALZHEIMER'S DISEASED BRAIN.

FIGURE 3: A COMPARISON OF THE EFFECTS OF THIMEROSAL ADDITION ON CONTROL (NON-DEMENTED) AND ALZHEIMER'S DISEASED BRAIN.

FIGURE 4: ZINC ENHANCEMENT OF MERCURY TOXICITY.

FIGURE 5: ALUMINUM ENHANCEMENT OF THIMEROSAL TOXICITY.

FIGURE 6: TESTOSTERONE ENHANCEMENT OF THIMEROSAL TOXICITY.