BIOMEDICAL ASPECTS OF EXPOSURE TO MERCURY AND ORGANIC MERCURY COMPOUNDS

by

BOYD E. HALEY, PROFESSOR AND CHAIR, DEPARTMENT OF CHEMISTRY, UNIVERSITY OF KENTUCKY 2005

FORWARD: Thimerosal or merthiolate is a derivative of thiolsalicylate where ethyl-mercury is attached though the sulfur or thiol group. Thimerosal was first synthesized to make a water soluble form of ethylmercury, which had potent bacterial static properties, but was very insoluble in water. Thimerosal is now used as a preservative or anti-microbial in certain medicinals. 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. Ethylmercury, due to its extreme hydrophobic nature will rapidly partition into the hydrophobic or lipophilic aspects of the human body, being concentrated in certain tissues. Further, in certain biological environments the ethyl-mercury can further break down releasing mercury cation (Hg^{2+}). Hg^{2+} is also very reactive with enzymes and proteins inhibiting their biological functions and causing cell injury or death. For example, in 1977 a report was presented on 13 infants with navel infections that were treated in a hospital with topical application of merthiolate (thimerosal). Ten of the 13 died of ethylmercury toxicity.

Ethyl-mercury, as it exists in a biological system, is more rapidly partitioned into the hydrophobic (fatty) tissues of the central nervous system and is a more potent neuro-toxin than 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 Hg^{2+}. However, it is not necessary for ethylmercury to break down to Hg^{2+} to be toxic. Ethylmercury appears to be more toxic to enzymes than Hg^{2+} in testing systems where conversion of ethylmercury to Hg^{2+} would be extremely slow.

Considerable caution must be taken when stating what is the "toxic level" of mercury and any mercury containing compounds. 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 or under oxidative stress. This is because additional stresses lower the amount of protective compounds, such as glutathione, that bind mercury and render it less harmful and aid in the excretion of mercury from the cells. If an individual is low on such protective compounds, then less mercury or thimerosal would be needed to cause a clinical effect.

Additionally, it is well known that many toxicants have corresponding compounds that will enhance their toxicity if present at the same time. For example lead is well known to enhance the toxicity of mercury. Also, the antibiotic, tetracycline,

enhances the toxicity of thimerosal in ocular tissue. We have observed that several compounds or metal ions enhance the toxicity of thimerosal when tested using neurons in culture. Included are the effects of estradiol and testosterone on thimerosal toxicity against neurons in culture that may help explain the 4:1 boy to girl ratio seen in autistic children. This latter observation could be expanded to a consideration of many complex cyclic organic compounds that may have an enhancing effect on mercury toxicity. It is the synergistic enhancement of toxicity of thimerosal-based toxicity that must be considered when evaluating children for apparent increased susceptibility to adverse effects to thimerosal exposure.

A recent publication from Denmark indicate that thimerosal present results that "do not support a causal relationship between childhood vaccination with thimerosal-containing vaccines and development of autistic-spectrum disorders." *(A. Hviid et al. Association Between Thimerosal-Containing Vaccine and Autism. JAMA v290, #13, 1763-1766).* This was preceded by another Danish study which concluded that "The discontinuation of thimerosal-containing vaccines in Denmark in 1992 was followed by an increase in the incidence of autism *(K.M. Madsen et al. Thimerosal and the Occurrence of Autism: Negative Ecological Evidence from a Danish Population-Based Data. Pediatrics v112#3, 604-606, 2003).* Overall, these papers indicate that exposure to a potent neurotoxin reduces the incidence of a neurological disease, which seems most unlikely. Looking at the data in Figure 1 of Madsen et al. gives one severe concerns regarding the thimerosal/autism issue. They show an average incidence rate of less than 0.3-0.4 per 10,000 before 1990 and a maximum incidence rate of much less than 5 per 10,000 in 1999-2000 (NOTE: This rate has been questioned by others who have looked at the Danish data). This later rate is close to what existed in the USA before 1980, before our autism rates started soaring. It is also well known that the Danes do not vaccinate their children with thimerosal vaccines on their day of birth, they do it 5 weeks later and only use half the mercury dose infants in the USA received on their first vaccination. Additionally, in the USA the first vaccination was also followed by a more aggressive vaccine scheduled that subjected infants to a much higher total exposure to thimerosal. Perhaps the autism rates in Denmark versus the USA should be considered as objective proof that earlier and increased exposure to thimerosal via vaccinations is causal for autism. It would make more sense. For certain, it is not logical to compare the effects of thimerosal on autism incidence in a country with the low Danish rate to that of the USA which is less than 5 versus over 60 per 10,000, respectively. Perhaps it should have been noted that some of the authors worked for the Stantens Serum Institute, which makes and sells thimerosal containing vaccines that are not allowed for use in their own country.

The observation of very low levels of mercury in the birth hair of autistic children in comparison to control, normal children is not to be lightly dismissed.*(L-W. Hu, J. A. Bernard and J. Che, "Neutron Activation Analysis of Hair Samples for the Identification of Autism", Transactions of the American Nuclear Society; 2003;89.)* Especially since the severity of the illness increased as the level of birth hair mercury decreased. This implies that autistic children do not biochemically or physiologically handle mercury excretion as do control children. It is my opinion, that the lack of mercury in the hair of autistic children is a reflection of the inability to excrete mercury from their cells. The observation of increasing birth hair mercury in normal children with increasing amalgam

fillings in the birth mother, and the total lack of this with autistic children, suggests that the autistic children represent a subset of the population that cannot effectively excrete mercury from their cells. This inability may be due to genetic susceptibility factors that lead to increased retention of mercury. It may also be due to the presence of synergistic factors that interfere with mercury excretion (such as certain antibiotics), or both.

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.

BIOCHEMICAL TOXICITY STUDIES: 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 12 years. To determine the relative toxicity we used two different biological testing systems: (i) brain homogenates, (ii) a mixture of four or more purified mammalian enzymes, (iii) rats exposed to mercury vapor and (iv) mammalian neurons in culture. 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. This same result was observed in rats exposed to mercury vapor. 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. This would indicate that thimerosal has the potential to be equally as damaging to neurite development than equivalent levels of Hg^{2+}.

We have calculated the concentration of ethylmercury that would be attained in a child with different levels of thimerosal exposure through vaccination. We made assumptions of 100%, 75% and 16% of body weight availability to dilute the thimerosal. These simple calculations show that thimerosal and/or ethyl-mercury certainly reaches the levels in infant bodies that could interfere with neurite growth and neuronal development. This could happen through rapid inhibition of several thiol-sensitive enzymes/proteins including actin, tubulin, creatine kinase and most recently methionine synthetase *(Waly, M. et al. and R.C. Deth, Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal)* and others not yet identified. 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 such as modified neuron structures and reduced methylation of RNA and DNA (due to inhibition of methionine synthase) that are observed in most autistics.

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 Prophylatic 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 brain neurons in culture. Our initial studies have shown that thimerosal is quite toxic to these neurons in culture with significant neuron death occurring within 24 hours at 10 nanomolar levels of thimerosal. Further, studies using vaccines with and without thimerosal present demonstrated that the presence of thimerosal accounted for most of the toxicity. The neuron toxicity studies mirror the results we observed in the enzyme toxicity studies mentioned above with the thimerosal being more toxic than inorganic mercury. Recent studies combining other agents to determine synergistic toxicities and possible protection agents have been completed and are in preparation for submission for publication. The results show a dramatic protective effect of estradiol against thimerasol toxicity. However, testosterone at non-toxic levels by itself has the ability to greatly enhance the toxicity of thimerosal.

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." I would suggest that blood mercury level measurements in infants exposed to thimerosal are not a reliable measurement of possible neurological damage. Infants that cannot excrete ethylmercury or mercury will have low blood mercury levels and still represent the infant that is most likely to be damaged.

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 another study *(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. Further, the data on the lack of mercury in the birth hair of autistic children would confound any study that that tried to correlate blood mercury (since blood mercury is the source of mercury found in hair) to any illness.

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 the mercury compound 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 Thimersal, 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. I would also point out that 200ng Hg/g of tissue wet weight corresponds to about 1.25 micromolar mercury with the assumption the entire gram is water (which it isn't). Most cells will not live in 1 micromolar mercury. Obviously, much of the mercury in this brain tissue is being tied up by proteins that provide some protection, but this protection is limited and using it up removes it from being able to protect the body from other stresses. The "3 to 9 μg/g where neurological symptoms appear" correspond to 15 and 45 micromolar levels of mercury in the brain using the same assumption. Can one really rationalize that this level of mercury in the brain is not doing damage? I cannot imagine any cellular system not being adversely affected by this level of exposure to mercury unless the experiments were extremely short or the experimenters were not

accurately measuring the “neurological symptoms”.

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 immunoglobin G samples. This mercury did not disappear or evaporate away. This decline of ethylmercury has to be due to ethylmercury reacting covalently with the protein-thiols in the medicinal preparations and is still available on injection to cause toxicity. In aged medicinal preparations, increased ethylmercury reaction with protein-thiols in the preparations would likely change the neurotoxity 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 that ethylmercury can retain its severe toxicity after a period of incubation time in a living pig, butchering and storage of the 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 a (*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, no deaths were reported in the article. However, the child who 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. For example, antibiotics that bind heavy metals, such as tetracycline, enhance thimerosal toxicity (see below in Synergistic Toxicity). Also, it has been reported that rats on certain antibiotics take over 10 times as long to excrete mercury.

It is also 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. This could change its partitioning, breakdown rate,

and may have a synergistic effect on the toxicity of any mercuric ion produced from the ethylmercury. Our research has shown that low levels of Al^{3+} greatly increase the toxicity of 50 nanomolar thimerosal against neurons in culture. Aluminum is a known neurotoxin and is known to be causally involved in macrophagic myofasciitis. The combined toxicity of the aluminum and thimerosal in vaccines remains largely untested, but commonsense indicates that a synergistic effect is to be expected.

Few of the clinical cases included in the Magos review were from vaccine exposure. However, the one that was discussed detailed problems which occurred in a 44 year old adult with a blood mercury of 0.104 μg per ml. This level was so low that Dr. Magos called the diagnosis "unconvincing". Perhaps co-administration of thimerosal with aluminum in the Hepatitis-B vaccine represents the "other aetiological 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. It could also be that this patient represents an individual that could not excrete mercury due to his genetics or health conditions and was retaining the mercury, just as we propose that autistic do, resulting in an unexpectedly low blood mercury level.

RECENT REVIEWS OF MERCURY TOXICITY RELATED TO NEUROLOGICAL DISEASES.

The most recent report that has been relied on by many to "show" the safety of vaccinations with thimerosal and dental amalgams is *"The Toxicology of Mercury—Current Exposures and Clinical Manifestations" by Thomas Clarkson, Laszlo Mangos and Gary Myers. New England J. of Medicine, 349;18 October 2003"*. This article has two fatal flaws that lead to erroneous conclusions regarding mercury toxicity and the safety of mercury exposures from dental amalgams and vaccines. Specifically, they ignore any consideration of genetic susceptibility to mercury and the numerous synergistic toxicities that exist for mercury and thimerosal. Two recent studies on mercury in the hair and birth hair of autistic children versus normal children strongly indicate that autistic children do not detoxify mercury as do normal children *(Holmes, A.S., Blaxill, M.F. and Haley, B. Reduced Levels of Mercury in First Baby Haircuts of Autistic Children. International J. of Toxicology, 22:1-9, 2003 and L-W. Hu, J. A. Bernard and J. Che, "Neutron Activation Analysis of Hair Samples for the Identification of Autism", Transactions of the American Nuclear Society; 2003;89)*. These articles emphatically show the need to consider genetics and exposures to materials that synergistically enhance the toxicity of toxicants that we expose our children to.

First, what the Clarkson et al. review is missing is that it is not the level of exposure that is the most important factor in most chronic, low level mercury induced or exacerbated diseases. Rather, it is the inability of a subset of the population to excrete mercury no matter what level they are exposed to that is causal. In this review (pg 1743) it is stated that in the "Faeroe Islands study it was found that blood pressure was increased when the blood mercury concentration was below 10 micrograms per liter, but not when it was higher." On page 1736 the make the statement "A similar study in the Seychelles found no adverse effects from fish consumption alone." However, in this

study the children with the highest levels of hair mercury did better on mental tests (e.g., the Boston Naming test) and hand-eye coordination tests than did the children with the lowest hair mercury levels. Are we to conclude that increased mercury exposure is good for you and that mercury could be used in controlling blood pressure and increasing intelligence? It seems logical that the children with the lowest blood mercury levels are the ones retaining the mercury and therefore are having the blood pressure problems. Conversely, the children with the highest hair mercury represent those that effectively excrete mercury and do better on the tests than the children with low hair mercury (ala autism) and a likely mercury retention problem. The "inability to excrete mercury hypothesis" therefore explains the "mystery" results of previous research where children with the highest "apparent exposure" to mercury (as determined by measuring blood and hair Hg levels) appeared healthier and more intellectually capable.

The second fatal flaw is the total avoidance of not only possible, but likely, synergistic toxicities such as co-exposures to other toxic heavy metals along with mercury. Presence of lead exposures would dramatically enhance mercury toxicity as presented in another section of this manuscript below. These authors also fail to point out that injection of vaccines, unlike ingestion of fish, bypasses the major toxic metal protection capabilities of the intestines where the bulk of the body's metallothionine is located (metallothionine is a protein that protects against heavy metal toxicity, especially mercury). They also fail to point out the lack of development of detoxifying organs/systems in the day old infant versus more mature humans.

The authors then make an assumption that defines the disagreement that I have with the basic logic of their analysis. "The half-life of methyl mercury in blood, which is assumed to indicate the total body burden, is usually assumed to be 50 days. In contrast, in children receiving thimerosal in vaccines, the half-life of ethyl mercury in blood was 7 to 10 days, or 1/7 to 1/5 as long as that of methyl mercury. Therefore, in the two-months periods between vaccinations (at birth and at two, four and six months) all of the mercury should have been excreted, so that there is no accumulation." They are assuming that the mercury leaving the blood is only being excreted into the feces and urine. They base this on the Pichichero et al. *(M. E. Pichichero et al. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. The Lancet, v360, Nov. 30, 1737-1741, 2002)*. They do not consider that this mercury is also leaving the blood and being taken up by the cells of the body in individuals, especially those who cannot excrete mercury. Further, any research on the half-life of an injected compound in the blood would not wait longer than 6 days to take the first sample, which was primarily done in this article---and then estimate a half-life of 7days! This just represents illogical science.

Pichichero et al. gives the level of mercury in feces from normal children after vaccination as low parts per billion (a mean of 82 ng Hg/g feces). Knowing the mean fecal level of mercury (82ng/g) and the average amount of feces produced by an average infant weighing 4 kilograms (4-12 grams/day, 8 grams average), one can calculate the approximate amount of mercury initially injected that would likely be excreted per day by this defined average size infant and how much would remain in the body after 7 to 10 days.

For example, if this average infant were given one vaccination with 12.5 micrograms (12,500 ng) mercury then after 7 days (7days X 8gm feces/day X 82ng/gm feces = 4,592ng) 12,500ng – 4,592 ng or 7,908 ng mercury would remain in this average child. If this child were given 3 thimerosal containing vaccines or 37,500 ng mercury then after 10 days (10days X 8 gm/day X 82ng/gm = 6,560ng) the amount of mercury left would be 37,500ng – 6,560ng or 30,940ng mercury. This estimate derived from the Pichichero data indicates that a substantial amount of mercury should be left in the body after 7 days, and these were normal children. In my opinion, the decrease in blood mercury in these infants is due primarily to the partitioning of major amounts of initial blood mercury into the cells of the normal infants body. The blood decrease does not justify the representation of excretion of mercury from the body as both Pichichero et al. and Clarkson et al. say it does. This logic of analysis is faulty.

Further, the Pichichero et al. research also does not represent what would happen with autistic children, who appear to be much less efficient at excreting mercury than are the normal children who were used in this study. Therefore, the amount of mercury retained would be much higher in the autistics, and the half-life in the blood would be much shorter as the mercury would be retained within the cells much longer. Yet, according to the analysis by Clarkson et al. of Pichichero et al., state that this decrease in blood half-life would imply that the mercury involvement in their illness is "remote". I totally disagree.

The 7 to 10 days exposure to thimerosal derived mercury is more than enough time for ethyl mercury to cause a toxic effect. If one compares the toxicity of methyl alcohol to ethyl alcohol one sees significant differences with methyl alcohol being more toxic and less metabolized. But ethyl alcohol, which is removed relatively quickly, can still have a toxic effect as evidenced by anyone who has become drunk---and it doesn't take 7 to 10 days to see the toxic effect on the central nervous system. Therefore, the statement by Clarkson et al. "Given the short half-life of ethyl mercury, any risks of it damaging either the brain or kidneys would seem remote." seems illogical. I would conclude the exact opposite. The shorter blood half-life is due to a more rapid uptake by cells of the infant with increasing commensurate damage.

Dr. Magos, *in a report to the IOM in 2001*, 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." What if the breakdown was caused by reactive oxygen species generated in response to an infection or oxidative stress as observed in many neurological diseases? 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*). This would make the production of toxic Hg^{2+} occur more rapidly at sites of high level of reactive oxygen species that, in the body, would be at sites of infection, 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) does not obviously make ethylmercury a less dangerous compound than methylmercury. It does indicate that

ethylmercury, more than methylmercury, releases Hg^{2+} at sites of oxidative stress or 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 or 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 was looking at one area of damage. He assumes the damage caused by methylmercury to be the same as that caused by a combination of ethylmercury and 3.4 fold extra Hg^{2+}. This is unproven and not likely as methyl and ethyl mercury adducts would partition into the hydrophobic areas of the brain whereas Hg^{2+} would most likely react in the hydrophilic aspect of the brain.

For example, the inhibition of specific brain enzymes by thimerosal (ethylmercury) compared to Hg^{2+} are markedly different. We have observed that Hg^{2+} when added to brain homogenates at low micromolar concentrations inhibit the viability of tubulin while having little effect on actin. However, thimerosal rapidly inhibits both tubulin and actin viability. In fact, our data indicates that thimerosal is more inhibitory to many brain enzymes than is Hg^{2+}. The bottom line is that until we know the entire data set for comparing methylmercury to ethylmercury toxicity we should exercise caution in calling one safe or safer by comparing its damage to the level damage at one site caused by a more toxic compound. The damage in a rat caused by thimerosal or ethylmercury injections are most likely to be less, and also different, than that caused by methylmercury. This in no way should be construed to imply that ethylmercury is not capable of causing neurological damage (autism) when injected into a day old infant.

SYNERGISTIC TOXICITY 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. 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-I level with lead at $1/20^{th}$ 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). Lead exposure is relatively prevalent in the USA. This observation alone should make physicians fight to get mercury out of the medical/dental treatment protocols.

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, pp759-7611983*, 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. 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 could enhance the toxicity of the ethyl-mercury released such as occurred with tetracycline (a commonly used antibiotic).

There is the report that autism peaks in infants born in the months of March and August and that this may be a significant hint as to the etiology of this disease. Some have postulated that this indicates an infectious vector is involved in autism as infants born in these months reach a certain age when infections, such as colds, flu, ear infections, etc. are at their highest level. If that is the case then the use of antibiotics and other medicinals are also likely to be at their highest levels. It is quite possible that the accentuation of thimerosal toxicity by increased use of antiobiotics, etc., with these children could explain this conundrum.

Since each human would likely have a level of toxicity from other mercury and non-mercury containing sources it would be impossible to determine 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.

THE EFFECTS OF AGE AND HEALTH ON THIMEROSAL TOXITICY: 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, metallothioine, selenium, lipoic acid, etc.) 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 dramatic inhibition of methionine synthase by 1 nanomolar thimerosal could also affect the level of glutathione in the body via its effects on cysteine levels. *(Waly, M. et al. and R.C. Deth, Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal)*. It should also be noted that this enzyme plays an important role in the inflammatory bowel disease (IBD) within the MTHFR gene expressed proteins *(N. Mahmud et al. Increased prevalence of methylenetetrahydrofolate reductase C677T variant in patients with inflammatory bowel disease, and it clinical implications. Gut 45: 389-394, 1999).* It is possible that inhibition of methionine synthase by thimerosal is involved in the IBD proposed to be dependent or exacerbated by the MMR vaccine when given to autistic children.

The elderly also have weakened immune systems and are more susceptible to microbial infections that are known to lower their chemical energy levels and, further, to reduce their ability to synthesize the glutathione 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 bilary 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. More recently, the importance of the genetics of

the MTHFR (5,10-methylenetetrahydrofoate reductase) or methionine synthetase/B_{12} systems has also become very important, but this remains to be worked out. I disagree with labeling those individuals who are "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 refuted by the history of the disease classified as acrodynia or Pink Disease. Some reports state that "In rare cases, thimerosal has caused systemic immune reactions including acyrodynia. *(S. Havarinasab et al. Dose-response study of thimerosal induced murine systemic autoimmunity. Toxicology and Applied Pharmacology v194, 169-179, 2004).* 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. This disease was caused by calomel, or mercurous chloride (Hg_2Cl_2), one of the least toxic forms of mercury and widely used in skin products. 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 some of the symptoms of acrodynia are similar to the clinical symptoms of children of equal age identified today as autistic. One definitely should not imply that these diseases are identical, they aren't. However, the acyrodynia situation clearly shows that exposures to mercury at levels perceived by others as safe certainly was not safe. Also, the manuscript above clearly implies that certain autoimmune factors should be observed in autistics and they are. Dr. Singh's presentation to the IOM on the immune response clearly shows this for MBP. Also, I have heard a detailed seminar by commercial antigen testing company researcher that showed that autistics make auto-antibodies to many of the normal glycolytic enzymes. This indicates that autistics are suffering from a generalized systemic autoimmunity most likely caused by a toxic insult, not a genetically inherited problem.

SUMMARY: It is the inability to see the effects of chronic, low level toxicities on human health that has been, and remains, one of our greatest failings as a highly technical society. For example, recent 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 at low nanomolar levels 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 neurofibillary 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---as usual, 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, Cahpter 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*(Duhr, E.F., Pendergrass, J. C., Slevin, J.T., and Haley, B. HgEDTA Complex Inhibits GTP Interactions With The E-Site of Brain β-Tubulin Toxicology and Applied Pharmacology 122, 273-288 (1993); Pendergrass, J.C. and Haley, B.E. Inhibition of Brain Tubulin-Guanosine 5'-Triphosphate Interactions by Mercury: Similarity to Observations in Alzheimer's Diseased Brain. In Metal Ions in Biological Systems V34, Mercury and Its Effects on Environment and Biology, Chapter 16. Edited by H. Sigel and A. Sigel. Marcel Dekker, Inc. 270 Madison Ave., N.Y., N.Y. 10016 (1996); Hensley, K., Cole, P. ,Aksenov, M., Aksenova, M., Bummer, P.E., Carney, J.M., Haley, B.E., and Butterfield, D.A. Oxidatively-Induced Structural Alteration of Glutamine Synthetase Assessed by Analysis of Spin Label Incorporation Kinetics. J. of Neurochemistry 68, 2451-2457 (1997).;David, S., Shoemaker, M., and Haley, B. Abnormal Properties of Creatine kinase in Alzheimer's Disease Brain: Correlation of Reduced Enzyme Activity and Active Site Photolabeling with Aberrant Cytosol-Membrane Partitioning. Molecular Brain Research v54, 276-287 (1998).* Recent research in our laboratory clearly demonstrates that thimerosal rapidly inhibits these same 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 excito-toxic 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 cerebrol spinal fluid *(Gunnersen, D.J. and Haley, B.E. Detection of Glutamine Synthetase in the Cerebrospinal Fluid of Alzheimer's Diseased Patients: A Potential Diagnostic Biochemical Marker. Proc. Natl. Acad. Sci. USA, 89 pp. 11949-11953 (1992)* and inhibited in AD brain *(Hensley 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, taking antibiotics and do not have fully developed bilary (liver) and renal (kidney) systems could greatly increase the toxic effects compared to that observed in healthy adults. Add in genetic susceptibility that prevents excretion of mercury and a major problem surfaces that was quite difficult to observe when looking at the general population.

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 toxic equivalent of a 130 pound adult receiving 40 vaccinations in one day as toxicity is measured as units of toxin per unit body weight. 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 without proper clinical studies 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 day old infants and toddlers.