

Thimerosal is a Developmental Neurotoxicant

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Biosketch: Dr Lucier's credentials and record of accomplishments in the field of toxicology are documented in the attached CV. He is currently a consultant in toxicology and a Senior Adjunct scientist for Environmental Defense. Dr Lucier retired from the National Institute of Environmental Health Sciences in 2000 where he was Director of the Environmental Toxicology program and Associate Director of the National Toxicology Program. In that capacity, Dr Lucier was responsible for coordinating toxicological research and testing across Federal agencies including the U.S Environmental Protection Agency, the Food and Drug Administration, the Occupational Safety and Health Administration and parts of the Centers for Disease Control. Dr Lucier was head of a research group in molecular epidemiology and risk assessment and has authored approximately 250 scientific publications. His research focused on the use of basic biology to reduce uncertainty in human risk assessments and to improve the tools used in exposure assessment. His work has made major contributions to risk assessments for dioxins, endocrine disrupters and methylmercury and he is frequently asked by Federal agencies to assist them in high visibility risk assessments. Dr Lucier chairs the Science Advisory Board for hazardous air pollutants for the State of North Carolina which makes recommendations on safe exposure levels on air pollutants of concern to North Carolina. He is also an advisor to the National Institutes of Health, a member of the NAS Committee on Toxicity Testing and a member of the Science Advisory Board for EPA. Dr Lucier was editor of the scientific journal, Environmental Health Perspectives, for 28 years.

Summary of report on thimerosal: The vaccine preservative, thimerosal contains 50% ethylmercury. Its structural analog methylmercury, is a potent and well known developmental neurotoxin and substantial evidence exists that ethylmercury is also a developmental neurotoxin. Based on studies that mercury reaches the brain after thimerosal or ethylmercury exposure, the knowledge that the amount of ethylmercury in vaccines exceeds safe levels and the results from a number of health effects and mechanism studies, it is highly probable that the use of thimerosal as a preservative has caused developmental disorders, including autism, in some children. All opinions expressed herein are based on a reasonable degree of scientific certainty using the information contained in the attached bibliography. I may also rely on my review of the testimonies of others at the trial. Illustrations used at three of my presentations on alkylmercury and thimerosal toxicity have previously been provided to the defendants.

Reimbursement: I was paid \$250 per hour for my time.

Alkylmercurial compounds are developmental neurotoxins: Organic mercury is a potent developmental neurotoxin based on numerous scientific studies in humans, experimental animals and cell systems. Much of the information is from studies on methylmercury (summarized by NAS, 2000) but there is considerable evidence that ethylmercury is also a developmental neurotoxin. Methylmercury is ubiquitous in the environment and it is known to bioaccumulate in fish. Its potency as a developmental neurotoxin coupled with its retention in fish has led the FDA and numerous state health agencies to advise women of child bearing age to limit their consumption of fish (NAS 2000, EPA 1997). Methylmercury is formed from the conversion of inorganic mercury to organic mercury by microorganisms present in aquatic environments (NAS 2000). Mercury emissions come from a number of sources including coal-fired power plants, chloralkali plants, paper pulp processes, gold mining and many others. Methylmercury is extraordinarily persistent in the environment and it is cleared slowly from the human body (EPA 1997, NAS 2000 and ATSDR 1999). Because of these properties, it is likely that every person in the United States has some amount of methylmercury in their bodies including fetuses, infants and children as well as adults. In fact the Centers for Disease Control and Prevention has estimated that 8% of the women of child-bearing age have mercury levels in their bodies higher than those considered safe by the EPA and the NAS (Schober et al 2003). Therefore, at least 60000 babies are born each year in the U.S who are already at risk for developmental disorders from mercury (NAS 2000). A recent publication provides additional data on mercury burdens of the U.S. population (McDowell et al 2004). In some regions of the U.S. the percentage of women with higher than safe mercury levels is much greater than 8% (Hightower and Moore 2003).

The developing brain is approximately 5-10 times more sensitive to the developmental neurotoxic effects of organic mercury than is the adult brain in humans and experimental animals. The first convincing evidence of the special susceptibility of the developing brain to organic mercury came in the 1950's and 1960's from reports that pregnant women exposed to methylmercury gave birth to infants with severe brain damage. These poisoning episodes occurred in Japan, Iraq and other countries (Bakir et al 1973, summarized by Risher et al 2002, NAS 2000; Clarkson 2002 and Myers and Davidson 2000). The Iraq episode also included exposure to ethylmercury. The symptoms observed in infants and children revealed a broad array of developmental disorders including visual disturbances, mental retardation, confusion, deafness, delayed achievement of developmental milestones. Autopsy samples from the Japan outbreak indicated widespread damage to all areas of the brain including altered arrangement of neural cells and changes in brain cell division and migration (Choi, 1989). Moreover, these evaluations demonstrated that more areas of the infant and fetal brain were damaged than the adult brain following equivalent exposures.

In the last 10 years a series of studies were conducted on the effects of low levels methylmercury exposures on the developing nervous system (Grandjean et al 1997, 1998, 1999a, 1999b, 2004) which demonstrated that low levels of exposure were harmful. These studies were conducted in the Faroe Islands in children whose mothers consumed

whale meat contaminated with methylmercury. Neuropsychologic testing indicated mercury-related dysfunctions in the domains of language, attention, memory, cognitive function, visuospatial and motor function. These studies demonstrate that alkylmercurials can have widespread effects on cerebral function and that several domains of brain function are affected. Some of these effects were associated with hair mercury concentrations less than 10 ug/g, a level previously considered to be safe. Some of the neurological/neurobehavioral effects caused by mercury are similar to the traits defined in autism. Evidence in support of the Faroe studies was summarized as part of EPA's risk assessment for methylmercury (Rice et al 2003)

There is ample evidence in humans and animal models to conclude that there are critical windows of vulnerability for the developing nervous system from exposure to environmental agents (summarized by Rodier, 1995 and Rice and Barrone 2000). Furthermore, various clinical disorders including autism, may be the result of interference with normal ontogeny of developmental processes in the nervous system. Unlike many organs, brain development and differentiation occurs during both the prenatal and postnatal periods. Studies in experimental animals and humans demonstrate multiple periods of vulnerability of the developing nervous system from early gestation to adolescence. The processes of cell proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis are all occurring during both prenatal and postnatal development. In addition, the blood brain barrier is not fully formed until the first year of life and there is important development of various neurotransmitter pathways during postnatal development. Therefore, infants are vulnerable to chemical insult during early postnatal development because any disruption in the carefully programmed sequence of neural development can lead to neurological disorders that are not immediately expressed. In general, adverse effects are less likely if exposure occurs before or after an organ is fully developed. Therefore, the vulnerability of the developing brain is dependent on two factors, the first is whether a toxic substance can reach the developing brain and the second is the timing of exposure (Selevan et al Environmental Health Perspectives; 108, supplement 3, 451-462, 2000). This means that the pattern of exposure can have a profound influence on developmental toxicity outcomes and that the peak exposure during a critical window of development is more important than the total or cumulative exposures spread out over time.

The principle of exposure timing for developmental toxicants has been used to explain the apparent discrepancy between studies of the developmental neurotoxicity of mercury compounds. While, Grandjean et al have reported that methylmercury is a developmental toxicant in the Faroe Islands, another group has reported that similar total mercury exposures had no effect on children in the Seychelles Islands (Clarkson 2000, Myers and Davidson 2000). This apparent discrepancy was examined in great detail by an independent panel of 28 scientists who had access to the raw data from both sets of studies (NIEHS, 1999). This panel concluded that both studies were credible and that a reasonable explanation for the different results was that exposures in the Faroes occurred in intermittent bolus doses while the Seychelles exposure were consistent over time.

Therefore, peak exposure during a critical window of brain development rather than cumulative exposure is more determinative of developmental neurotoxicity.

Comparison of ethyl and methylmercury: There is a vast amount of scientific evidence, some of it published over 50 years ago, that ethylmercury like methylmercury penetrates the brain and/or is a neurotoxicant (Warkany et al 1953, Hook et al 1954, Dahhan and Orfally 1962, Miller et al 1961, Clarkson 1972, Mukai, 1972, Tryphonas et al 1973, Derban, 1974, Yonaha et al 1975, Cinca et al, 1979, Zhang 1984, Dumitrescu 1979, Fagan et al 1979, Mukhtarova 1977, Magos et al 1985, Winship 1986, Chang and Verity 1995, Lowell et al 1996, Ball et al 2001, Eli Lilly 1999, Smith-Kline 1999 and Kramer et al 2004, Ueha-Ishibashi et al 2004). Other publications in the reference list support the notion that ethyl and methylmercury have similar toxicological properties but the ones listed illustrate that information on the neurological toxicities of ethylmercury and thimerosal are similar. Furthermore, the U.S EPA has established criteria for determining whether or not high production volume (HPV) chemicals can be grouped together for the purpose of evaluating health hazard data used in risk assessments (EPA HPV program 1999). When those criteria are applied to ethyl and methylmercury, it is clear that they would be grouped together because they have common physiochemical properties (Tan and Parkin 2000) and would be expected to cause a common pattern of toxicity operating through a common mode of action. Accidental ingestion by children of meat contaminated with ethylmercury led to severe neurological symptoms and autopsy data showed nerve cell loss, glial proliferation in the central cortex, demyelination, granule cell loss in the cerebellum and other pathologies of the central nervous system (Cinca et al 1979). Similar findings were observed in an accidental case of methylmercury poisoning (Davis et al 1994). Recent studies have demonstrated that Purkinje cell loss is a common neurological abnormality in autism and these cells are vulnerable to mercury exposures (Kern et al 2003; Sorensen et al 2000).

Both ethyl and methylmercury are dealkylated to form inorganic mercury (Magos 2003). Inorganic mercury does not pass the blood brain barrier as easily as alkylmercury but alkylmercurials once in the brain can be converted to inorganic mercury. Inorganic mercury formed in the brain cannot easily leave and therefore, it will be trapped and accumulate in the brain (Magos et al 1985, ATSDR 1999, NAS 2000 and Risher 2002). In fact, several studies have demonstrated that following ethylmercury exposure inorganic mercury is retained in the brain to such an extent that it is difficult to calculate a brain half life (Suzuki et al 1973, Platonow, Magos et al 1985, Brooks et al 1986, Clarkson 2004). These studies indicate that ethylmercury may be more neurotoxic than methylmercury although firm conclusions regarding relative potencies are difficult to make because dealkylation enhances overall clearance but at the same time it provides a mechanism for increased brain retention. The blood half life of methylmercury is highly variable among individuals, ranging from 35-190 days (al Shahrstani and Shihab 1974, EPA 1997). It is assumed that there is at least an equal variation for ethylmercury although insufficient data are available to make firm conclusions on the blood half life of ethylmercury in humans. In one case of human poisoning (Pfab et al 1996) by thimerosal,

the half life appeared to be in the range of 30-40 days. In any event, predictions of health risks from thimerosal or ethylmercury exposure must assume at least a 6-fold variation in blood and brain half life. Some data are available in rodents which indicate that the half life of methylmercury in the blood is 2-3 times longer than for ethylmercury or thimerosal (Magos 2003). However, the difference in brain half life appears to be less. Sager and Burbacher (Sager, Immunization Safety review 2004; Burbacher 2004) presented information from monkey experiments indicating that mercury from thimerosal is preferentially retained in the brain compared to methylmercury. These data indicate that the brain to blood mercury ratios steadily increase over time following thimerosal exposure and that mercury residues would still remain in the brain several months after a single thimerosal injection. Based on these data it is reasonable to conclude that ethyl and methyl are equitoxic to the brain. This conclusion is strengthened by a report that a man receiving hepatitis-B immune globulin containing thimerosal experienced significant neurological toxicity at a blood mercury level of only 100 ug/L (Lowell et al 1996). If infants are 5-10 times more sensitive than an adult male then blood levels of 10-20 ug/L could be associated with neurotoxicity in infants.

Two studies have investigated the distribution of mercury following vaccination of infants using thimerosal as a preservative (Stajich et al 2000 and Pichichero 2002). These studies demonstrate elevated levels of mercury following vaccination but the experimental design did not permit estimation of a reliable half life nor could they provide any data on brain levels. In addition to the lack of information on the brain concentrations of mercury, peak levels were not achieved and the blood mercury measurements were not made for a sufficient period of time to estimate a half life. Surprisingly, Pichichero did estimate a half life for mercury following thimerosal injections although such estimates are not supported by a reasonable scientific foundation. Moreover, the Pichichero study was based on a dose of 37.5 ug mercury although many infants received 62 ug mercury in thimerosal-containing vaccines. Nevertheless, these findings were reviewed by Magos (2003) but conclusions drawn are flawed because of data inadequacies as described above. Based on the above considerations and analyses, the Pichichero 2002 and Stajich 2000 data indicate that safe levels of mercury compounds are exceeded following the use of thimerosal containing vaccines.

Alkylmercury Risk Assessments: The EPA routinely establishes reference doses for hazardous agents under its regulatory purview. Exposure levels below the reference dose are generally considered safe. Exposure levels greater than the reference dose do not necessarily cause toxicity but such exposure levels do increase the probability of a toxic response (EPA 1997). The greater the reference dose is exceeded the greater the probability of toxicity or disease. EPA has established a reference dose of 0.1 ug/kg/day for methylmercury based primarily on the Iraq and Faroe Island developmental neurotoxicity data (EPA 1997, Rice et al 2003). No reference dose is available for ethylmercury so based on the available information ethylmercury exposures are considered equivalent to methylmercury exposures (Ball et al 2001, preceding

discussion on pharmacokinetics). The NAS convened an expert panel in 1999 to review the scientific foundation for EPA's reference dose. After deliberating for one year the NAS releases a report which reaffirmed EPA's reference dose (NAS 2000). As part of the report, the NAS conducted an exhaustive analysis of all available data and they concluded that The Faroe Islands data should be used for risk assessment. A benchmark internal dose of 58 ug/l was derived from the data which corresponds to a daily intake of 0.1 ug/kg/day. The benchmark dose is considered the blood level which corresponds to 5% of children suffering from neurological deficits as a consequence of methylmercury exposure. The reference dose was derived by dividing the benchmark dose by 10. Grandjean et al (2004) have since published data to indicate that methylmercury risks may have been underestimated due to exposure misclassification. In addition, Gilbert and Grant-Webster (1995) concluded that the safe exposure level should be 0.06 ug/kg/day. This means that many more infants than previously thought may be already at risk and that any additional alkylmercurial exposure from thimerosal or other sources would enhance their probability of a developmental neurotoxic response.

Risk assessments for thimerosal in infants must reflect the knowledge that peak exposures are more relevant to toxic outcome than are cumulative exposures (described earlier in this document) and that every infant has an existing body burden of alkylmercury compounds (NAS 2000, EPA 1997, Schober et al 2003, McDowell et al 2003). Many infants already have blood mercury levels in excess of 1 ug/l. Thimerosal-containing vaccines have significantly added to the organomercurial burden of infants. On vaccination day, a 2-monthold infant received 3-18 ug/kg ethylmercury. This number is 30-180 times greater than EPA's reference dose for methylmercury. For developmental neurotoxicants, the most scientifically credible measure of exposure is the amount of chemical in the body during critical windows of neural development. Therefore, it is not appropriate to average ethylmercury exposures over a 6 month period as was done by the FDA (Ball 2001). Even if this was done, the average daily exposure still exceeds the safe level established by EPA.

The ATSDR established a safe dose of 0.3 ug/kg for methylmercury (ATSDR 1999) but this assessment was based on the Seychelles not the Faroe Islands data. The Faroe Island-based approach is more relevant for risk assessment (NAS 2000) because it is a positive study that was judged as sound after a rigorous review by an independent panel of 28 scientists who had access to the raw data from both the Seychelles and Faroe Islands (NIEHS 1999). Even if the ATSDR risk assessment is used, thimerosal exposures on vaccination day still exceeded safe levels by 10-60 fold. The magnitude of alkylmercury exposure from thimerosal-containing vaccines placed in the context of well-founded risk assessments strongly indicates that ethylmercury exposures from thimerosal caused neurodevelopmental disorders in some children. The precise nature of the developmental disorders would likely vary between individuals and the array of expected disorders includes some symptoms associated with autism. It is difficult to establish a firm quantitative measure of probability for an adverse outcome but it is likely between 1 and

10% for infants injected with thimerosal-containing vaccines would have neurological disorders.. Moreover it is very likely that some individuals are at greater risk than others for the reasons described in the following section.

Susceptible Individuals: It is generally accepted that not all individuals react alike to the toxic properties of chemical exposure; some are highly sensitive whereas others are resistant. Dramatic differences among individuals in their response to chemical toxicants are commonly observed in day to day living. For example, not all people who smoke get lung cancer although cigarette smoke is a known human carcinogen. Likewise, some individuals experience adverse side effects from specific pharmaceuticals while most do not although the dose is the same for everyone. Some of the factors responsible for differing sensitivities are genetic predisposition, age gender, diet, co-exposure to other chemicals and different sources of exposure to the same class of chemicals (Lucier,1996). These factors are also described in various documents and risk assessments on alkylmercurials (NAS 2000, EPA 1997, ATSDR 1999). This means that thimerosal could cause severe toxicity in some individuals and not others. For example, individuals with the same blood levels of organic mercury in the Iraq poisoning episode exhibited vastly different outcomes (Bakir et al 1973)

Merthiolate and thimerosal are well known sensitizing agents in adults and children and it has been shown that thimerosal causes delayed hypersensitivity (Forstrom et al 1980, Wohrl et al 2003). These findings confirm that some individuals are more sensitive than others to adverse reactions from thimerosal and they suggest that background exposures to methylmercury may have sensitized some infants to ethylmercury in vaccines. Various susceptibility factors for thimerosal are summarized below:

- 1. Background exposures to alkylmercury:** The levels of mercury in infants and children vary at least 10-fold (Schober et al 2003, McDowell et al 2004). This is likely a consequence of different levels of exposure and different clearance rates (al Shahrstani and Shihab, 1974) between individuals. This means that some infants will be more vulnerable to additional mercury exposure from vaccines than others simply because of their preexisting mercury body burden.
- 2. Gender:** Several articles including the assessments of Gerlai and Gerlai (2003), Yeargin-Allsopp et al (2003) and the Institute of Medicine (Immunization Safety review 2001) have concluded that the prevalence of Autism in boys is approximately four times the prevalence in girls. The mechanisms responsible for the gender difference are not clear although there are indications that the presence of testosterone may be a risk factor (Harber 1965) and Sager (1984) demonstrates sex differences in response to alkylmercurials.

- 3. Genetic predisposition:** There is a growing recognition by pharmaceutical industries, regulatory agencies and the scientific community that human disease is caused by gene-environment interactions and that by understanding how chemical substances interact with molecular and biological pathways we will be better able to predict the consequences and potential for adverse side effects from exposure to pharmaceuticals and chemicals. In the case of autism Gerlai and Gerlai (2003) have provided a long list of molecular mechanisms suspected to play a role in autism but the interactions of organomercurials with those pathways is unclear. However, studies on one pathway have implicated the glutathione pathway which is involved in the detoxication of organomercurials (Westphal et al 2000, Muller et al 2001, Bradstreet Immunization Safety Review 2004, James et al 2004, James et al 2005). Taken together these studies indicate that individuals with deficiencies in the glutathione transferase pathways are at increased risk for the neurotoxic actions of thimerosal. There is a vast body of scientific literature to support the contention that the glutathione metabolism plays a key role in wide variety of chemically-induced toxicities in numerous organ systems. This knowledge is consistent with the results of Holmes et al (2003) which showed reduced levels of mercury in hair of autistic children and the results of Bradstreet et al. (2003) on urinary mercury levels in autistic children. A recent study (Hornig et al 2004) has demonstrated a link between autoimmunity and autism based on the effects of thimerosal on inbred strains of mice. This study along with the results of Voldani et al (2003) provide evidence for genetic susceptibility to thimerosal-mediated neurodevelopmental disorders. In fact, Havarinasab et al (2004) has demonstrated that thimerosal is more effective in inducing autoimmune reactions than is methylmercury.
- 4. Diet:** The rate of mercury elimination from the body is significantly influenced by diet (Rowland et al 1980, Rowland et al 1984), gut flora and biliary excretion (Ballatori and Clarkson, 1982). The key event appears to be the role of the gut flora in dealkylation of organomercurials. Thus, differences in diet would likely exert an influence on levels of mercury in the brain and since the human diet is highly varied there should be corresponding differences in susceptibility based on those dietary differences. Likewise, dietary differences in selenium are likely to cause variation among children in brain mercury levels (Brzezniczka and Chmielnicka 1985). Infant weights vary considerably depending on diet and other factors. If each infant received the same absolute amount of mercury in vaccines regardless of weight, some infants received higher doses than others on a ug/kg body weight basis.
- 5. Co-exposure to other chemicals:** Humans are never exposed to chemicals in isolation and different chemicals can interact to potentiate their toxic effects. Therefore, another source of susceptibility is the nature of the chemical world in which we live. In relation to thimerosal, studies have shown that aluminum (Jones 1972) and antibiotics (Crook and Freeman 1983) can dramatically increase toxic

reactions from thimerosal exposures. Therefore, if infants are exposed to aluminum or antibiotics they may be more susceptible to the toxic effects of thimerosal.

The above information on susceptibility demonstrates that some infants would be at far greater risk than others for developmental disorders as a consequence of thimerosal exposure in vaccines. In general, the greater the number of risk factors the greater the risk.

Effects of Thimerosal in cells: The use of mechanistic data in risk assessments is increasingly recognized by regulatory agencies around the world. The reason for this is that mechanistic studies help us understand the basic biology responsible for chemically-mediated toxicities and to better determine if observed results are biologically plausible. The utility of mechanistic studies is enhanced when the molecular/biochemical event being measured is relevant to the toxic endpoint in question and the amount of chemical needed to alter the biochemical event is consistent with the amount to which people are exposed. Based on these criteria molecular/biochemical events were selected for comment in this report only if they are relevant to mercury exposures from thimerosal-containing vaccines. In general, treatment of cells with thimerosal concentrations of 1 μM or less provides data relevant to estimated safe exposure levels for thimerosal.

1. Leong et al 2001 have reported that inorganic mercury at low concentrations (0.1 μM) is capable of altering nerve development and increasing the rate of neurodegenerative processes. The degeneration of nerves caused by mercury is visible on video (commons.ucalgary.ca/mercury). Since inorganic mercury is retained preferentially in the brain following thimerosal or ethylmercury exposure this finding indicates that infants receiving thimerosal are at risk for neurotoxicity.
2. Waly et al (2004) have reported that concentrations of thimerosal as low as 0.001 μM inhibited dopamine-stimulated methylation activity in human neuroblastoma cells. This inhibition could lead to adverse consequences on gene expression essential for normal brain development and therefore increase the risk of neurodevelopmental disorders.
3. Makani et al (2002) demonstrated that thimerosal induces apoptosis in human T cells via a mitochondrial pathway involving oxidative stress and glutathione depletion. This effect was evident at all concentrations tested, the lowest being 0.5 μM . Apoptosis is an essential pathway for the progression of normal brain development so this study enhances the biologic plausibility that thimerosal in vaccines increase the risk of developmental disorders. In a related study Baskin et al (2003) reported that thimerosal can induce DNA damage, cell membrane damage and programmed cell death pathways at low concentrations in human neurons.

4. Comparative studies on the ability of thimerosal and methylmercury to disrupt normal cellular calcium levels indicated that thimerosal and methylmercury were equipotent and effects occurred in rat cerebral neurons at concentrations below 1 uM (Ueha-Ishibashi et al 2004). This effect is related to cytotoxicity and like the above studies it enhances the plausibility that thimerosal causes neurodevelopmental disorders.
5. Several other mechanism studies demonstrate that thimerosal is capable of interfering with cellular pathways critical for normal development of the brain (Brunner et al 1991, Wallin and Hartley-Asp 1993, Song et al 2000).

Vaccine manufacturers ignored scientific data on the toxicity of thimerosal for 50years: In 1999, when I was Chair of the White House-directed interagency review of methylmercury toxicity and exposure, it was revealed that ethylmercury was used as a preservative in vaccines injected into infants. It seemed unbelievable to me and many of my colleagues that infants would be deliberately injected with alkylmercury, known for decades to be a developmental neurotoxin. It is very troubling that this practice could continue year after year and that parents had no knowledge that the vaccine program was unnecessarily placing their children at risk. The vaccine program has made immense contributions to public health but it clearly would have been better if thimerosal was not used as the preservative. I have reviewed some of the early literature used to justify the use of thimerosal as a preservative and subsequent publications on the toxicity of merthiolate, ethylmercury and thimerosal. Based on this information, I conclude that the justification for considering thimerosal or merthiolate as safe was inadequate and flawed, information on alternative preservatives was ignored, the vaccine manufacturers ignored a significant body of knowledge on health effects for at least 50 years and that the vaccine manufacturers did not conduct necessary toxicology studies to establish safety. The basis for these conclusions are as follows:

1. The key publication cited by Lilly in their statements of safety for thimerosal was a 1931 publication (Powell and Jamieson 1931). This paper reported studies in which adult experimental animals were injected with merthiolate and followed for up to seven days. Some animals lived and some died. No attempt was made to follow the animals longer than seven days and no attempt was made to determine if merthiolate caused neurological or developmental toxicity. Limited data were presented from studies in dogs but these data were inadequate for toxicity assessment. This paper falls miles short of a scientifically-justifiable claim of the safety of thimerosal use in vaccines.
2. Another paper (Smithburn et al 1930) used to claim safety of thimerosal and merthiolate reported on the injection of merthiolate to 22 people suffering from meningitis. Many of the people died. The ones that lived were followed for a brief

- period of time although the scope of the clinical evaluations was not documented in the paper. This paper falls far short of a scientific justification that thimerosal was safe for use in infant vaccines.
3. In a letter to Lilly Company (Pitman-Moore Company 1935) concern was expressed that merthiolate was more toxic to dogs than claimed by Lilly and that other preservatives were more effective. This letter was apparently ignored as no additional studies were conducted.
 4. Herrell and Heilman (1943) stated that that merthiolate might be too toxic for use as a preservative and that other preservatives appeared to more efficacious. Similar conclusions were made by Engley (1953)
 5. Two papers in the 1940's (Ellis 1943, Cogswell and Shoun 1948) reported on the dangers of using merthiolate. Other early papers (Epstein 1963, Nelson and Gottshall 1966) also reported that merthiolate was toxic.
 6. Over 50 years ago, several publications (Hunter et al 1940, Hunter and Russell 1954, Hook et al 1954) reported that alkylmercury compounds crossed the blood brain barrier and caused neurological toxicity yet Lilly did not conduct additional studies nor did they change their claim that mercury containing preservatives were safe.
 7. From 1950 to the mid 1970's numerous studies in Iraq, Japan and other countries (already referred to in this document) plus others (Jalili and Abbasi 1961, Damulji 1962) reported on the neurotoxicity of ethylmercury and alkylmercurials. These studies demonstrated that alkylmercurials were potent neurotoxins and that the developing brain was more sensitive than the adult brain. In the face of such overwhelming scientific evidence to the contrary, how could vaccine manufacturers persist in their claim that the use of mercury-containing preservatives was safe? It wasn't until 1967 that Lilly removed the non-toxic (Lilly 1967). They did not acknowledge until much later that mercury-containing preservatives were neurotoxic to the developing brain.
 8. In 1971 (Lilly 1971) Lilly said in a letter to Dr Sigel that merthiolate was cytotoxic and must be diluted to less than 1/1000000 in order to be non toxic to cells. Knowing this how could thimerosal be marketed as a preservative in infant vaccines?
 9. In 1972 (Axton 1972) reported on six cases of merthiolate poisoning in which 5 of the 6 patients died yet Lilly claimed in a 1976 letter to a drug company (Lilly 1976) that the ethylmercury in preservatives does not pose a toxic risk. Moreover, Gasset et al (1975) had previously reported that after topical application of thimerosal, mercury was detected in the blood and tissues of rats and their

- offspring demonstrating that mercury from thimerosal crossed the placental and brain barriers.
10. Blair et al (1975) reported that mercury was detected in the brains of Squirrel monkeys receiving thimerosal and the authors warned that thimerosal might pose a risk when used in vaccines.
 11. Fagan et al (1977) reported that the application of thimerosal to infants with omphaloceles had blood and organ levels of mercury well in excess of minimally toxic levels. This publication stated that organic mercurial antiseptics should be heavily restricted or withdrawn from hospital use. Similarly, Heyworth and Truelove (1979) said that merthiolate treatments might lead to mercury accumulation and toxic effects.
 12. In the 1980's numerous publications questioned the safety and efficacy of merthiolate and thimerosal including reports of delayed hypersensitivity and additional studies on neurotoxicity (Forstrom et al 1980, Sheth et al 1983, Rohyans et al 1984, Stetler et al 1985, Winship 1986, Cox and Forsyth 1986, Sunderman 1988).
 13. In 1982 the FDA (Federal Register 1982) in an advance rule-making document proposed to classify mercury-containing drug products for topical antimicrobial use as neither safe nor effective.
 14. In 1989 a European working group reviewed thimerosal and concluded that ethyl and methylmercury were equitoxic and that the use of thimerosal-containing vaccines in infants and toddlers should be discouraged.
 15. In the early 1990's European countries banned the use of thimerosal (Madsen et al 2003, Hviid et al 2003) because it was considered neurotoxic. A strong case against thimerosal use was made in 1991 by Seal et al (1991) who was concerned about both safety and preservative efficacy.
 16. The vaccine manufacturers admitted in 1991 (Merck Exhibits 285 and 286) that the mercury dose in vaccines was 87 fold over the safe value yet they continued to market thimerosal. This information was not made public until FDA was forced to release it because of the FDA Modernization Act of 1997. Moreover, the VAERS program documented 83 cases of autism related to thimerosal exposure between 1990 and 1999 (Exhibits 171 and 172)..

Autism trends, epidemiology studies on associations between autism and thimerosal and conflict of interest issues: Several studies provide strong evidence that the prevalence of autism has increased dramatically over the last 20 years (Gerlai and Gerlai 2003, Gurney et al 2003, Yeargin-Allsopp 2003, Bertrand et al 2001, Yazabak 2003, Bernard 2001). The increase in trends provides convincing evidence that a significant proportion of autism cases are caused by environmental factors and that although genetic factors may predispose to autism there must be an environmental (diet, pharmaceutical and/or chemical) trigger. The only plausible explanation for the rise in autism supported by a strong scientific foundation is that thimerosal causes some cases of autism. The scientific foundation for this statement is described earlier in this report. Autism spectrum disorder is likely caused by more than one factor because of the wide array of symptoms used to classify the disease and not all of these symptoms are completely shared by all individuals diagnosed with autism.

During the last two years numerous studies have been published on the relationship between exposure to thimerosal-containing vaccines and autism (Verstraeten et al 2003, Stehr-Green et al 2003, Hviid et al 2003, Madsen et al 2003, Geier and Geier 2003a, Geier and Geier 2003b, Geier and Geier 2003c, Geier and Geier 2004a, Geier and Geier 2004b, Andrews et al 2004, Heron et al 2004). Many of the results presented in these papers were discussed at the Institute of Medicine (Immunization Safety Review transcript 2004). Each of the papers has serious methodological flaws that limit their use in risk assessment. Flaws identified include inappropriate use of trend data, questionable criteria for exclusions, questionable designation of control populations, questionable characterization of doses, inclusion of doses much lower than experienced in the U.S. population, implied assumptions that all autism cases are caused by thimerosal, use of different diagnostic criteria in the same study, confusing use of hospital records and inappropriate comparisons across studies. It is important to note that a recent publication (Geier 2004b) prior analyses were updated using the VAERS database and methods developed by the National Immunization Program. This publication found statistically significant effects of thimerosal on the incidences of autism, speech disorders, mental retardation, personality disorders and thinking abnormalities. Taken together, the Geier publications demonstrate that thimerosal causes increased incidences of a number of neurodevelopmental disorders, including autism, in a dose dependent manner. These studies have been published following peer review in five separate scientific journals and they paint a picture consistent with other experimental studies on the neurotoxic actions of ethylmercury and thimerosal.

Several of the studies conclude that there is no association between thimerosal exposure and risk for autism (Stehr-Green 2003, Hviid et al 2003, Madsen et al 2003, Andrews et al 2004, Heron et al 2004). This seems like a rush to conclusion given the weakness of the data and the presence of serious confounders. None of the papers indicated disclosed either a financial conflict of interest nor an appearance of conflict of interest. Such disclosures are common practice for scientific journals because of concerns that industries might have influenced outcome of the studies or experiments (Bekelman et al

2003, Johns et al 2003, Melander et al 2003, Lexchin et al 2003, Bhandari et al 2004, Lancet 2004). The U.S House of Representatives Committee on Government Reform (2000 and 2003) has determined that the FDA and CDC routinely allow those with conflicts of interest to influence vaccine policy making. Because, the thimerosal issue is of great public health interest, the authors of the so-called negative studies should reevaluate their lack of disclosure statements. The authors of the papers that reported a positive association between thimerosal exposure and autism did make a full disclosure of financial interests and this should be done by the others. In addition, the Pichichero 2002 paper indicated that the authors did not declare any conflict of interest although there clearly was the appearance of conflict (New York Times 2002).

The Verstraeten paper, representing the United States population, was essentially neutral in its conclusions regarding thimerosal exposure and autism risk. However, the paper must be questioned for several reasons. First, the author presented a draft of his results in 2000 at a meeting in Georgia (Simpsonwood Transcript 2000) which indicated a high risk of neurodevelopmental disorders from thimerosal exposure. However, in subsequent drafts the strength of the association diminished and the paper eventually published in Pediatrics (Verstraeten 2003) was neutral. Dr Verstraeten left CDC in 2001 and went to work for a vaccine manufacturer yet his employment with the vaccine manufacturer was not disclosed in the 2003 publication; it appeared as he was still working for CDC. This situation is a serious appearance of conflict and raises the possibility that the data might have been manipulated to make the relationship between autism and thimerosal in the 2000 draft disappear. Dr Robert Davis, who worked for vaccine companies (Immunization Safety Review 2004), advised Dr Verstraeten on the CDC paper between 2000 and 2002 when many of the changes were made that diminished the relationship between thimerosal and neurodevelopmental disorders. Some of these issues with the CDC study are discussed by Halsey and Geier in their letters to the Editor of Pediatrics (2003).

The IOM 2004 report on thimerosal-containing vaccines was funded by the CDC. The record shows that the IOM was inappropriately influenced by the CDC. Transcripts of an early organizational meeting in (IOM transcript Jan 2001) reveal statements to the effect that the Committee would not conclude that thimerosal caused neurodevelopmental disorders because the CDC did not want the IOM to conclude a causal association. This kind of prejudicial push to a particular conclusion is totally inappropriate for as funding agency requesting an objective evaluation by an IOM Committee or any Committee for that matter. Moreover, the Committee Chair stated, before any evidence was presented, that the Committee would never determine that autism was a true side effect. Statements like this would not be made if the deliberations were intended to be objective and based on scientific facts. The IOM concluded in 2004 that thimerosal does not cause autism but this conclusion is tainted because of the prejudicial statements made by the Committee at the onset of deliberations and the undue reliance on research conducted by scientists who did not disclose conflicts of interests in their publications. Inexplicably, the IOM Committee seemingly ignored a vast body of science, including epidemiology studies,

indicating that thimerosal causes neurodevelopmental disorders. In fact, Congressman Weldon (Weldon 2004) expressed concern that the committee members were biased and had conflicts of interests and that the IOM report did not constitute an objective evaluation of the facts.

The 2003 Mercury in Medicine Report by the U.S. House of Representatives noted that CDC and the National Immunization Program are conflicted in their ability to monitor the safety of vaccines because they are also trying to increase immunization rates. The report criticized FDA and CDC for being asleep at the switch regarding safety data for thimerosal and for having a misplaced protectionism for the pharmaceutical industry. CDC ranks its priorities as follows (Plaintiffs exhibit 82):

1. disease prevention
2. immunization coverage
3. partnerships
4. science
5. systems
6. vaccine safety
7. NIP work environment

Clearly, the CDC is more concerned with immunization coverage and partnerships with industry than it is with vaccine safety. In response to the growing criticism concerning CDC's ability to monitor vaccine safety, CDC recently separated the National Immunization Program which advocates vaccination from the Vaccine Safety Branch which monitors the potential risks from vaccines (New York Times 2005). This step appears to recognize that vaccine safety has received short shrift in the past.

Unfortunately, the CDC has not made all the data available that was used to assess the relationship between thimerosal exposure and autism. The withholding of non-classified scientific information obtained from the expenditure of public funds and used in public health policy is not defensible in this case (Weldon 2004) especially in light of the circumstances described above. Independent scientists must have the opportunity to analyze all the datasets used by Verstaeten and CDC in their investigation of the relationship between autism and thimerosal use before the 2003 paper can be considered of use in public health policies and assessments. Availability of raw data to anyone who wants it is common practice in many Federal research agencies including components of the National Institutes of Health when such data has any impact on public health policy.

Thimerosal and Causation of Neurodevelopmental Disorders: The criteria for evaluating whether or not a chemical exposure causes a particular disease in humans is often contentious. In 1965, Bradford Hill articulated guidelines for assessing causation and those guidelines are still used today. The guidelines are comprised of nine considerations; strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy. These are not meant to be strict criteria for determining causality but they do provide a framework for such assessments. I will briefly discuss these considerations based on the information and references already referred to in my report;

1. **Strength:** There are numerous publications and exposure circumstances that clearly demonstrate that alkylmercury compounds are potent developmental neurotoxins. Developmental neurotoxicity is a sensitive endpoint for alkylmercurials, effects occur following low doses in humans, experimental animals and in isolated cells and ethylmercury and methylmercury have similar potencies. The evidence is strong that thimerosal is a developmental neurotoxin including several positive epidemiological studies.
2. **Consistency:** There are several positive epidemiological studies in the peer-reviewed scientific literature demonstrating that thimerosal-containing vaccines in the U.S. have caused increased incidences of neurodevelopmental disorders. Several studies have not shown an association but they were not conducted in the U.S., doses employed were less than in the U.S., the so-called negative studies were conducted by scientists who did not disclose conflict of interests and they are fraught with methodological problems. One U.S study conducted by the CDC was neutral but it was seriously confounded by conflict of interest issues.
3. **Specificity:** Episodes in Japan, Iraq and other countries have shown that alkylmercurials cause neurological disorders and children, infants and the unborn fetus are at special risk. The array of developmental disorders caused by alkylmercurials is very wide and symptoms vary among individuals exposed to the same dose. The pattern of developmental neurological symptoms caused by alkylmercurials are likely influenced by timing of exposure, preexisting mercury levels in the body, genetic factors and dietary factors.
4. **Temporality:** The developing nervous system is at risk and the reported effects of thimerosal on neurological functions occurred after injections of ethylmercury-containing vaccines in a manner consistent with the principles of developmental neurobiology including latency of effects.
5. **Biological gradient:** Alkylmercury compounds cause developmental disorders in a dose dependent manner in humans, experimental animals and in isolated cells and effects occur at low doses. In fact, the CDC estimates that 8% of the women of child bearing age have mercury levels that are considered unsafe.

6. Plausibility: Ethylmercury-containing vaccines were administered to infants at doses much higher than those considered safe during a time when the central nervous system is especially vulnerable to developmental neurotoxins. There is clear biologic plausibility that thimerosal has caused neurodevelopmental disorders in some children.
7. Coherence: The evidence that thimerosal causes neurodevelopmental disorders is coherent based on assessment of human studies, the known exposures to ethylmercury and expectations based on dose, neurological damage and mechanistic considerations. It would be reasonable to predict that the use of thimerosal-containing vaccines would cause developmental disorders.
8. Experiment: The positive epidemiology studies on thimerosal are supported by a consistent and convincing scientific literature in experimental animals and in in vitro systems.
9. Analogy: There is a strong scientific foundation for the contention that ethylmercury and methylmercury are similar in their neurotoxic properties and that data from studies on methylmercury can be used to assess the risks from ethylmercury exposures.

It is important to note that EPA (EPA revised cancer risk assessment guidelines), the National Toxicology program (NTP 2002) and the WHO (WHO 2005) have established criteria for determining chemical causation of disease for cancer and other diseases. These criteria require that data from human studies, mechanistic studies and animal studies be considered in determining causation.

The CDC has published (CDC 2005) case definitions for chemical poisoning. They state that laboratory criteria for the diagnosis of organic mercury poisoning is a case in which blood mercury levels exceed 10 ug/L. They do not distinguish between ethyl and methyl mercury in this case definition. A case classification of probable poisoning is defined as *“a clinically compatible case in which a high index of suspicion (credible threat or patient history regarding location and time) exists for organic mercury exposure, or an epidemiologic link exists between this case and a laboratory confirmed case.”* Based on these criteria it appears that use of thimerosal in infant vaccines would constitute mercury poisoning as defined by the CDC if consistent with clinical findings.

The vaccine industry has also established criteria for determining if adverse events are related to vaccine administration (Plaintiffs exhibit AP 218). According to these criteria an adverse event is considered to be related to vaccine administration with a high degree of certainty if the adverse event followed a reasonable temporal sequence after administration of the vaccine and it could not be reasonably explained by the known characteristics of the patients clinical state, environmental or toxic factors or other modes of therapy administered to the patient. It follows that many neurodevelopmental disorders

in children who had received thimerosal-containing vaccines were caused, in part, by the mercury in vaccines unless another cause is demonstrated.

Conclusions: Companies using thimerosal in their products failed to conduct adequate studies on the toxicity of thimerosal and they did not use or develop safe substitutes for preservatives in vaccines. They failed to warn that thimerosal was unreasonably dangerous because of its neurotoxic properties. The manufacturers failed to heed a vast body of scientific information indicating that thimerosal was toxic. Therefore, thimerosal was more dangerous than would have been anticipated by pregnant parents bringing their child to the doctor for vaccinations because of the negligence of the manufacturers.

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