Respondent's Exhibit AA

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EXPERT REPORT OF RICHARD B. MAILMAN, Ph.D.

I. BACKGROUND AND QUALIFICATIONS

My training and experience are particularly relevant to evaluate and comment on the expert report of Dr. Richard Deth. As background, I am a tenured Professor with appointments in the Departments of Psychiatry, Pharmacology, and Neurology in the School of Medicine, and in the Division of Medicinal Chemistry and Natural Products in the School of Pharmacy at the University of North Carolina at Chapel Hill ("UNC"). I am also a member of the Curricula in Toxicology and Neurobiology. I received a Ph.D. in Physiology with a minor in Toxicology in 1974 from North Carolina State University ("NCSU"), and received subsequent post-doctoral training from both NCSU and UNC in toxicology, neuropharmacology, and neuroscience. I have been working in the field of neuropharmacology, neurotoxicology, and neuroscience for over 30 years. I have published more than 170 peer-reviewed research papers, more than 50 chapters and reviews, two books, and hold patents for the discovery of novel potential drugs. I have had funding for my research from the National Institutes of Health for nearly 30 years.

I regularly teach courses in toxicology, pharmacology, and neuroscience to advanced graduate students, as well as to medical students, medical residents, and fellows. I have trained more than two dozen doctoral students and more than a dozen post-doctoral fellows in the fields of pharmacology, neuroscience, toxicology, and medicinal chemistry, and have mentored numerous young faculty members.

I am a Fellow of the American College of Neuropsychopharmacology, and a member of the American Society for Pharmacology and Experimental Therapeutics, the American Chemical Society, the Society for Neuroscience, the American Society of Neurochemistry, the International Society for Neurochemistry, the American Association for the Advancement of Science, and the Society of Toxicology. I have served on several national committees for some of these professional societies. I have won several scientific awards, including the Burroughs-Wellcome Scholar in Toxicology Award from the Society of Toxicology. Over the past 20 years, I have regularly served as a consultant to the National Institutes of Health, and at intervals to the US Environmental Protection Agency, the US Army, and several pharmaceutical companies and non-profit foundations.

I have served or am currently serving on the editorial boards of many journals, including Current Opinion in Central and Peripheral Nervous System (CPNS) Drugs, the Journal of Molecular and Biochemical Toxicology, Neurotoxicology, Synapse, Neurochemistry

International, Neurotoxicology and Teratology (Neurochemistry Field Editor), Fundamental and Applied Toxicology, Brain Research Bulletin, Psychopharmacology Bulletin (Associate Editor), and the Journal of Molecular Neurobiology. In addition, I am a regular reviewer for scientific and medical journals. For example, in the last twelve months I have reviewed manuscripts for the Journal of Pharmacology and Experimental Therapeutics, Molecular Pharmacology, the American Journal of Psychiatry, Neuropsychopharmacology, Neuroscience Letters, Brain Research, European Journal of Pharmacology, Expert Opinion in Investigational Drugs, Expert Opinion in Pharmacotherapy, FASEB Journal, Journal of Neurochemistry, Journal of Medicinal Chemistry, and Psychopharmacology, among others.

II. DR. DETH'S THESIS

Dr. Deth proposes that thimerosal plays a significant role in causing and/or contributing to autism and other neurological disorders and that, in his words, this is "well-supported by studies of its distribution and elimination and by studies of its metabolic and neurological actions." He then provides evidence, comprised in large part of data from his own laboratory, that purportedly supports his thesis that effects of thimerosal closely parallel metabolic abnormalities found in autistic children, and that thimerosal induces a state of oxidative stress and impaired methylation status. He believes that these metabolic abnormalities interfere with mechanisms critical for normal attention and cognitive abilities, and therefore account for key symptoms of autism. He also proposes that thimerosal exposure is of particular importance in individuals possessing what he terms "risk-inducing genetic variants in thimerosal-sensitive pathways" that impact "the capacity for mercury detoxification or elimination, the ability to sustain redox or methylation balance, and/or the ability to synchronize activity of neuronal networks."

A. Perspective on approaching this matter:

The evaluation of Dr. Deth's thesis will differ depending on the forum in which it is considered. For example, I am aware that lay individuals may accept or reject such a thesis based on their immediate experiences, on information available in the popular press, on material from blogs or websites, or simply on word-of-mouth. In the world of science, evaluations are based on the source and quality of data, the logic of how that data is assembled into a thesis, and the possible alternate explanations for any of the data.

B. Untoward consequences of substituting advocacy for the scientific method:

The failure to adhere to a rigorous scientific approach can have deleterious results. The value of a proper scientific approach, even in the absence of strongly held popular views, was taught to me as a young scientist by the controversy regarding neonatal retrolental fibroplasia (Silverman, 1977). Soon thereafter, I became involved scientifically in two major public controversies: one relating to the purported benefits of megavitamin therapy, the other, the "Feingold hypothesis," that claimed that most ADHD was a result of artificial food colors. I was a coauthor of major scholarly reviews on both questions (Lipton et al., 1979a; Lipton et al., 1979b) that came to tempered conclusions based on the approach I advocate (*vide infra*). These conclusions have withstood the test of time. In the case of the food color-ADHD controversy (Lipton et al., 1979b), there were data published in the prestigious journal SCIENCE suggesting that food colors (and one food color in particular, Red #3) affected dopamine function and

thereby caused ADHD. My laboratory played a major role in resolving this controversy (Mailman et al., 1980), and showed that with the use of proper controls, there was no evidence for dopaminergic toxicity from Red #3, as had been suggested. The more general lessons taught by this controversy (Mailman and Lewis, 1981; Mailman et al., 1981) are relevant to these proceedings as well.

In reviewing a prior paper of mine in preparation for this expert report, I was struck by two sentences (Mailman et al., 1980): "Although hyperkinesis is a medical problem, the suggestion that it may be due to synthetic food additives has given it social and political dimensions that increase the need for sound clinical and basic data upon which to make policy judgments. Whatever the outcome of future scientific and clinical experimentation, cautious presentation and interpretation of data will prevent expensive and spurious perturbations of the public and scientific consciousness." If one substitutes "thimerosal" for "synthetic food additives", and "autism" for "hyperkinesis," these words are as valid today as in 1980. I have reviewed the aspects of thimerosal toxicity as it relates to Dr. Deth's expert report and scientific approach, and feel he has failed to execute this responsibility. I shall illustrate this by focusing on examples from Dr. Deth's own research that relate to my specific scientific expertise.

III. THE PROPER APPROACH TO ADDRESSING DR. DETH'S THESIS

Prior to addressing some of the specifics in Dr. Deth's expert report, I would like to provide a perspective of how I believe problems such as the purported thimerosal-autism connection should be approached. For the past thirty years, one of my particular interests was in how one does what is now called translational research, specifically, the process of taking a clinical observation to the lab bench, or a lab observation to the clinic. My direct experiences in the translation of basic scientific findings to the clinic have provided me an in-depth understanding of many of the issues that are essential to consider in making such extrapolations. This experience has come from the study of drugs, nutrients, and toxicants. I would like to review the lessons I have been taught or that I have drawn from my experiences. Unfortunately, I believe that Dr. Deth has violated many fundamental rules on how to approach such problems, and has therefore reached conclusions that are likely to be spurious.

A. It is critical to understand the scientific method:

The scientific method that is accepted by most reputable practicing scientists is directly relevant to Dr. Deth's report. The term "hypothesis" is particularly critical, and describes a tentative explanation of a phenomenon that is subject to further testing. The hypothesis can be supported by some preliminary evidence, or it can be a totally speculative formulation with no direct evidence. To advance beyond a hypothesis, the idea must be subjected to rigorous testing. What is often forgotten is that it is the job of the scientist to attempt to <u>disprove</u> the hypothesis - not to prove it, as the lay public might believe. This distinction is critical, and will be clearer when I discuss both Dr. Deth's approach to the autism-thimerosal link, and his own experimentation.

When a scientist attempts to challenge the hypothesis under study, two things can happen. First, an experiment can produce data that are inconsistent with the hypothesis, meaning that the idea is shown to be false. Second, the experiment can challenge the hypothesis, yet

produce data that are consistent with it. When repeated and independent challenges to a hypothesis fail to disprove it, the idea can then rise to the status of a "theory." A theory, contrary to its use in the popular realm, is an idea that has repeatedly been challenged and which has survived intact these attempts to invalidate it - the term theory does not refer to an off-the-cuff idea that might explain something.

B. Skills of the scientific specialist: relevance to Dr. Deth's research on thimerosal:

A scientist conducting experiments, whether in humans, animals, cells, or subcellular preparations, must follow the accepted practices of the relevant discipline. For example, in clinical studies, the use of placebo controls and double-blind designs are critical to avoiding false conclusions. Similarly, the disciplines of pharmacology and toxicology have at their foundation additional principles, including theory related to dose-effect relationships. It is critical to understand and judiciously apply these principles when extrapolating from in vitro experiments to the clinic. When experiments have social or political consequences, scientists bear a particular responsibility to adhere to proper methods and dispassionate interpretation. In my review of Dr. Deth's original research, which forms the basis of many of his opinions, I find he has failed to comport himself in this way, as I shall exemplify below.

IV. OVERVIEW OF THE SCIENTIFIC ISSUES IN DR. DETH'S REPORT: ADVOCACY OVERRULES SCIENTIFIC RIGOR

In his expert report, Dr. Deth fails to employ critical and skeptical approachs to ideas and data. In the Summary of his expert report, he states that a "*significant role for thimerosal in causing and/or contributing to autism and other neurological disorders is … well-supported by studies of its distribution and elimination and by studies of its metabolic and neurological actions*" [Deth, p. 2]. As an expert in neurotoxicology, I find that the available evidence does not, in fact, support this statement, and that Dr. Deth neither critically evaluates the evidence, nor considers alternate hypotheses and data. This lack of analytic rigor is quite at odds with the scientific method, and seemingly betrays a bias that is inconsistent with the objectivity demanded in science. Dr. Deth states, for example, that "*[m]ethylmercury is considered to be one of the most toxic non-radioactive substances and a general threat to human health (1).*" Although methylmercury is a well-known toxicant, and has been a major cause of environmentally-caused neurodevelopmental disorders, there are many other compounds that are far more toxic (from anthropogenic compounds like nerve gases to natural toxins like botulinum toxin, cycad alkaloids, and ricin). While methylmercury is not directly involved in the thimerosal debate, Dr. Deth makes the statement, "[t]here is no *a priore [sic]* reason to assume that ethylmercury does not share a similar level of toxic risk as methylmercury, since they are close chemical analogs sharing many physical and chemical properties." It is common knowledge that small changes in chemical structure can dramatically change the biological effects of a given compound. There are many toxicological examples, such as the markedly different effects of trimethyltin and triethyltin or the different effects of arsenic species. Thus, Dr. Deth's position is poorly founded scientifically.

Dr. Deth also makes the statement that, "[a]utistic children possess a higher frequency *[sic]* risk-inducing genetic variants in thimerosal-sensitive pathways, making them more vulnerable than others to its neurotoxic effects….Congruence between attributes of thimerosal

and the features of autism provide convincing evidence that thimerosal exposure represents an important risk factor for autism, particularly in individuals possessing certain risk-inducing polymorphisms." This would suggest to one not conversant with the facts that there is a clear mechanistic link between thimerosal neurotoxicity and autism. Neither the information or references provided later in Dr. Deth's expert report, nor the scientific literature of which I am aware, support such speculation. In my expert opinion, the total of Dr. Deth's report is based on selective and non-critical review and interpretation of the available scientific and medical evidence.

A great deal of Dr. Deth's expert report is based on the role of dopamine and dopamine D_4 receptors as they relate to autism. This is also one area of my expertise.

V. WHAT IS THE EVIDENCE FOR D_4 DOPAMINE RECEPTOR-MEDIATED CHANGES IN METHYLATION AND AUTISM?

A. Attempts to capture Dr. Deth's working hypothesis:

One of the great difficulties I had in evaluating the specific science in Dr. Deth's report was that he covers such a broad range of issues and mechanisms that a thorough critical review becomes impractical within the context of my expert report. As an example, he discusses four areas that he believes are affected by thimerosal (1. Maintenance of cellular reduction/oxidation (redox) status. 2. Support of methylation (single-carbon transfer) reactions. 3. Detoxification and elimination of heavy metals and xenobiotics. 4. Formation of sulfate). I have chosen to focus on the second major area (support of methylation), because Dr. Deth has published in that arena and cites his own work in support of this hypothesis. Indeed, I believe much of Dr. Deth's expert report is based on data from his laboratory, as there is little else that provides support for his thesis. I would like to address both his experiments and the extrapolations he made from them.

B. Dr. Deth's hypothesis regarding the effects of thimerosal on methylation:

As Dr. Deth states, there are many homeostatic mechanisms in cells, including those that regulate what I term sulfide-thiol potential. Dr. Deth proposes that regulation of the enzyme methionine synthase is affected by thimerosal. There is a large literature on methionine synthase, and it clearly plays a role in intermediary metabolism: it is related to nutrients like folate and vitamin B12, as well as the pathways in which they are involved. Inhibition of methionine synthase activity is not regarded as an underlying biologic process causing autism by the general scientific community studying methionine mechanisms, despite the breadth of work in this field. Dr. Deth notes that oxidative stress can inhibit methionine synthase activity, and then reports that studies from his laboratory showed potent direct inhibition of neuronal methionine synthase by thimerosal at concentrations of 1 nM. This is far below what he believes were produced by thimerosal-containing vaccines.

In addition to its other roles, Deth proposes that methionine synthase is required to transfer folate-derived methyl groups to the dopamine D4 receptor. He proposes that D4-mediated phospholipid methylation allows dopamine to affect the fluid properties of the membrane of neuronal cells. He hypothesizes that this would in turn affect dopamine-dependent

synchronization of neuronal networks during attention and cognition. Since this mechanism is absolutely dependent upon activity of methionine synthase, he proposes it is highly sensitive to oxidative stress. He then states that his lab has found that thimerosal potently inhibits dopamine-stimulated phospholipid methylation at half-maximal concentrations, well below plasma levels produced by immunization with thimerosal-containing vaccines. I shall focus in detail on this aspect of his report - in part because it is one of the mechanisms he proposes as being important, and in part because it is the only area where he contributes data to this controversy.

C. Critical evaluation of the thimerosal-methylation hypothesis:

One question is whether the proposed mechanism is of the importance assigned to it by Dr. Deth. A search of the medical literature via PubMed shows more than a thousand citations to research with the D_4 dopamine receptor. Of these, only six discuss D_4- mediated phospholipid methylation, and all of these originate from Dr. Deth's group. While in rare instances important conceptual ideas are ignored by other scientists, far more often the failure of an idea to catch on over many years reflects upon the idea itself. The failure of other labs to work on this mechanism may suggest that Dr. Deth's view of the importance of this mechanism is not widely shared. Based on my own knowledge of this field, this is not a primary mechanism that is accepted for this receptor.

More importantly, however is the actual examination of the research from Dr. Deth's laboratory. The mechanisms put forth on pages 4 & 5 of Dr. Deth's expert report originate from data from his laboratory related to the mechanisms of action of thimerosal (Waly et al., 2004). Waly concludes that the effects of thimerosal on the function of dopamine D_4 receptors supports the notion that thimerosal plays a role in neurodevelopmental disorders. I have serious reservations about the conclusions that were drawn and aspects of the experiments that underlie them. I would therefore like to discuss some of the most important issues.

First, Waly used SH-SY5Y (SK-N-SH-SY5Y) cells, a clonal line originating from a human neuroblastoma tumor. Although this cell line is of nervous system origin, it is not of brain origin, and has an unusual phenotype that is not typical of either brain neurons or glia. One critical missing experimental direction was that more physiologically relevant systems using mammalian neurons should have been used.

Second, the Waly paper also failed to use experimental controls that are the common standard in such studies. As an example, when attempting to relate a specific receptor to a function, it is customary to use the most selective pharmacological agonists and antagonists that are available. Waly chose as their agonist dopamine. Dopamine is non-selective for the dopamine and other monoamine receptors, and SH-SY5Y cells are reported to express not only D_4 receptors, but also D_1 and D_2 receptors, as well as many other receptors that could be affected by dopamine (including $alpha_2$-adrenergic receptors and several serotonin receptors). The experimental design did not control for these possible effects. Moreover, dopamine can readily oxidize to products that can be biologically active, and the oxidation products of dopamine are themselves active in a redox sense. The methods as described did not control for this potential concern, either.

Third, Waly claimed that the D_4 nature of the labeling was confirmed by its blockade of clozapine. Clozapine is a very non-selective drug that also binds to many of the other receptors

expressed by these cells, including not only D_4 receptors, but also other dopamine receptors, several cholinergic receptors, adrenergic and histamine receptors. The cell line used is known to express a host of other cell surface receptors including nicotinic and muscarinic cholinergic receptors, mu-, delta-, kappa-opioid receptors, α2-adrenoreceptors, NMDA glutamate receptors, sigma receptors, NPY receptors, somatostatin (SRIF) receptors, endothelin (ET) receptors, several serotonin receptors, and low levels of D_1 and D_2 dopamine receptors. Waly failed to utilize other typical controls that would have confirmed their speculation, such as use of selective pharmacological antagonists and RNA interference approaches.

Finally, Waly concluded that there was direct D_4 receptor involvement in folate-dependent PLM by labeling cells with [^{14}C]formate for 30 min, and then determining what molecules were radiolabeled at the end of that time. They claim a single 41 kDa protein was found that they said corresponded to the D_4 receptor. Such a conclusion is unsubstantiated without additional controls as other dopamine receptors are expressed in these cells, and the specificity of the antibody was not adequately controlled. Indeed, in primates, there are several polymorphic variations in the coding sequence of the human D_4 receptor due to repeats of a 48-base-pair sequence in the putative third cytoplasmic loop of this receptor. It was essential to determine which of these species (e.g., $D_{4.2}$; $D_{4.4}$; $D_{4.7}$ etc.) were expressed, if one is seeking to relate this to humans. The paper, however, does not make this determination. This is especially important, given Dr. Deth's statements regarding specific genetic polymorphisms affecting responses to thimerosal.

The concerns that I have with Dr. Deth's research lead to the conclusion that this is not a valid scientific basis to suggest that thimerosal is a causative agent in autism. It is my expert opinion that it is pure speculation to believe that even the earliest hints of causation have been found.

D. Lack of consideration regarding the same mechanism being involved in other disorders:

A major concern throughout Dr. Deth's expert report is the failure to follow logical and critical thinking in forming his views. I have noted this as it regards thimerosal, but I also came across another article of Dr. Deth dealing with the same biochemical mechanisms as it relates to a different agent, the gaseous drug nitrous oxide. I believe this example underscores the concerns that I expressed earlier.

The abstract of this paper (Culley et al., 2007) begins by stating, "[n]itrous oxide is a commonly used anesthetic that inhibits the activity of methionine synthase, an enzyme involved in methylation reactions and DNA synthesis and repair. This inhibition triggers vacuole formation and degeneration of neurons in areas of the developing and mature brain that are important for spatial memory, raising the possibility that nitrous oxide might have sustained effects on learning." Most readers would assume that toxicity of nitrous oxide is mediated by the inhibition of methionine synthase, much as was proposed for thimerosal.

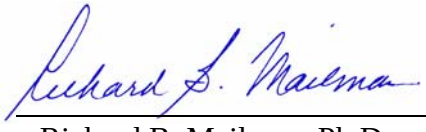
Indeed, the authors go on to state, "[n]itrous oxide inhibits methionine synthase, an enzyme important for methylation reactions and DNA synthesis and repair. Decreased methionine synthase activity produces a myelopathy and is implicated in dementing illness,

possibly related to accumulation of homocysteine, a cytotoxic amino acid normally remethylated to methionine, an essential amino acid, by methionine synthase. In addition, high concentrations of nitrous oxide produce a reversible neurotoxic reaction in the mature brain characterized by mitochondrial swelling and vacuolization of neurons. With prolonged administration, the toxicity is irreversible and associated with massive apoptotic neurodegeneration and neuronal death."

Although it may be that most of these statements are true in isolation, what would not be clear to a non-scientist is that they have not been causally related to each other. Effects of profound loss of methionine synthase activity may have far different effects than transient small decrements. Moreover, the relationship of homocysteine to "dementing illness" is not causal. While it is true that nitrous oxide can be toxic, this in no way shows it is related to the transient inhibition of methionine synthase shown in this paper. Indeed, a host of other mechanisms may be involved. For example, cobalamine (vitamin B12) can be destroyed oxidatively by nitrous oxide, and in people with subclinical vitamin B12 deficiency (e.g., older people), symptoms of B12 deficiency occur. The Culley paper does note that nitrous oxide neurotoxicity was reported to be related to the drug affecting the NMDA class of glutamate receptor, as it is well known that drugs that affect this receptor can be neurotoxic. To my knowledge, the mechanisms of toxicity of NMDA receptors do not prominently include effects on methionine synthase. Thus, it appears to me that there is an attempt to establish "guilt by association" that is not scientifically appropriate. It would appear that Dr. Deth and his collaborators are attempting to implicate methionine synthase activity as the primary mechanism of nitrous oxide toxicity, which could then be argued lends support to their thimerosal hypothesis. They have failed to establish the links between the isolated points they make concerning nitrous oxide, rendering their conclusions invalid. Again, their approach is at odds with established scientific method.

VI. SUMMARY

After careful review of Dr. Deth's expert report, I find that it is a selective and non-critical review of the available scientific data. Scientists frequently disagree on the interpretation of the available evidence, but honest disagreements must come from considering and weighing all evidence. Dr. Deth has taken preliminary studies that were suboptimally designed, and created a hypothesis that superficially provides support for the notion that thimerosal is a causative factor in autism. One must consider the complexities of brain development, proper pharmacological and toxicological controls, and alternate hypotheses. Dr. Deth's expert report fails to do this, and I find his overall thesis unconvincing. Based on my review of the available scientific literature and Dr. Deth's report, I do not believe there is any link between thimerosal and autism.



February 24, 2008
Date

Richard B. Mailman, Ph.D.

VII. REFERENCES:

The following specific material was used in the preparation of my report in additional to my general personal expertise and standard references of the field.

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