THE NATIONAL ACADEMIES

Immunization Safety Review Committee

Vaccines and Autism

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Table of Contents

P R O C E E D I N G S

Agenda Item: Welcome and Opening Remarks

DR. MCCORMICK: Good morning. As chair of this study, I would like to welcome you to this meeting of the Immunization Safety Review Committee.

This project is sponsored by CDC and NIH as a means to help them address vaccine safety concerns. The official charge to the committee is included in the handouts you have received.

I am joined by the committee members, who are sitting in the first two rows in the audience.

This meeting is the ninth in a series of meetings of over three-plus years that focus on the hypothesized relationship between an adverse event and a vaccine. Today's meeting will focus on vaccines and their potential link to autism.

In addition to the presentations we will hear today, the committee has received an extensive amount of information. We have convened two prior scientific workshops in 2001 on this topic. This is one of five notebooks of material that we have on this topic that we have accumulated. I might add, they are double-sided. I will also say that the committee has read them. Believe

me, they have read them.

Based on its assessment of evidence of causality, biologic-mechanisms-of-adverse-events hypothesis, and the significance of the issue in the broader societal context, the committee will recommend the appropriate public-health response -- for example, surveillance, research, communication, or policy review.

Today's presentations will cover a variety of topics relevant to the issue of vaccines and autism, including background on etiology and pathophysiologic factors in autism, an update on new studies addressing the association between MMR vaccine and autism, as well as those with thimerosal-containing vaccines. Additional presentations will address biologic mechanisms. Each presentation will be followed by a brief question-and-answer period, primarily focused on the clarification of fact. Because the meeting was called to assist the committee in its research, committee members will ask the questions first, followed by participants, as time permits. All audience members should identify themselves and their organizations before asking questions and should limit themselves to a single question.

We have a very long day. We have a timer system

that we will use to help us adhere to the agenda. A yellow light will blink when you have two minutes left in your formal presentation; a red light will appear at the end of the allotted time to indicate the beginning of the question-and-answer period. Out of respect for all our speakers, I will have to ask you to keep to the schedule.

For the record, the speakers will be asked to disclose whether they have received funding from vaccine manufacturers or a federal agency to conduct vaccine-safety research, and whether they have otherwise received financial remuneration in any way for activities related to the topic of vaccine safety.

The day will then conclude with a public comment session, in which the members of the audience are welcome to provide comments related to today's topic. A sign-up sheet is available at the registration table for people interested in commenting during this session. Given the number of people at this meeting, comments must be kept brief. I will ask you to wrap up at two minutes. Those who are unable to provide comments today or would like to make additional comments may submit those in writing to the committee by February 16, 2004.

This is an open, on-the-record session. Today's

meeting is also being webcast in real time. For those of you listening, most of the PowerPoint presentations are available through the project website at www.iom.edu/imsafety. A verbatim, unedited transcript of this public session will be available to the public through the committee's public-access file system. In addition, the audio version of this meeting will be posted on the Web after the meeting.

I also would like to remind everyone that this is an information-gathering session. That is, the committee is in the process of assembling materials that it will examine and discuss in the course of making its findings, conclusions, and recommendations. Therefore, I ask everyone here today to be extremely mindful of the fact that the committee has made no conclusions, and it would be a mistake for anyone to leave here today thinking otherwise. Comments made by individuals, including members of the committee, should not be interpreted as the positions of the committee or of the National Academies. In addition, committee members typically ask probing questions in these information-gathering sessions, which may not be indicative of their personal views.

In addition to the auditorium, the audio from

this meeting can be heard in the Lecture Room of this building if anyone needs to do so.

The committee's report on vaccines and autism will be released approximately three months from today. The committee will deliberate thoroughly before writing its report. Moreover, once a draft report is written, it must go through a rigorous review by experts who are anonymous to the committee. The committee then must respond to this review with appropriate revisions that adequate satisfy the Academy's Report Review Committee and the chair of the NRC before it is considered a National Research Council report.

Now I would like to really start the meeting by introducing our first speaker, Congressman Dave Weldon of the U.S. House of Representatives. Welcome and thank you for taking time from your schedule to address the committee.

Agenda Item: Congressional Speakers - Dave Weldon

CONGRESSMAN WELDON: Good morning. I appreciate the opportunity to address you again.

Since I last addressed you, I continue to be the focus of a great number of phone calls, inquiries, from parents, scientists, autism interest groups, and, more

recently, members of the media. All are seeking information and answers to the questions before you.

My desire remains one of getting at the truth to these matters, and I continue to believe passionately that we need to protect the integrity of our national vaccine program. In my clinical practice, I administered thousands of vaccines. I know the tremendous benefit to humanity of vaccines and the serious risks associated with undermining public confidence.

The failure, however, to get answers to these questions on vaccine safety is beginning to undermine public confidence.

I must begin by sharing my disappointment at the number of reports that I continue to receive from researchers regarding their difficulties in pursuing answers to these questions. It is past time that individuals are persecuted by asking questions about vaccine safety. We have recognized error in vaccine policy before with live polio, wholesale pertussis, and rotavirus. I am repeatedly informed by researchers who encounter apathy from government officials charged with investigating these matters, difficulty in getting their papers published, and the loss of research grants. Some report

overt discouragement, intimidation, and threats, and have abandoned this field of research. Some have had their clinical privileges revoked, and others have been hounded out of their institutions.

An example of the latter is Dr. Andy Wakefield, who has described to me personally how the intellectual climate at Royal Free Hospital in London became intolerable for him, and he was forced to depart. Virtually all of his ongoing research now has been privately funded, while those seeking to disprove him receive government funding.

I witnessed some of this firsthand at a hearing, when a Dr. Brent Taylor made repeated inappropriate comments about Wakefield and his work, causing me to seriously question Dr. Taylor's objectivity and motives. Mind you, half of Dr. Wakefield's theory has been proven correct and widely accepted in the medical community. Hundreds of children with regressive autism and GI dysfunction have been scoped, and clinicians are seeing the same inflammatory bowel disease that Wakefield originally described.

The NIH is now finally funding an attempt to repeat Dr. O'Leary's findings of measles RNA in Wakefield's biopsy specimens, though I am disappointed that it has

taken this long. A clinician in New York was poised to attempt to repeat O'Leary's findings two years ago, but ultimately was refused by his IRB, and then subsequently had his clinical privileges withdrawn.

This atmosphere of intimidation and concern, to a certain degree, even surrounds today's hearing. I have received numerous complaints that this event is not a further attempt to get at the facts, but rather a desire to sweep issues under the rug. I have the utmost respect for the Institute. Nonetheless, I shared these concerns with Dr. Gerberding. Last week, I was pleased when she called me to assure me that these concerns are unfounded and this is not the case. She informed me that she wants to meet with me, with parents, clinicians, and researchers, and really work to get answers to these questions in the years ahead.

I understand that such outreach was attempted prior to her arrival, but those efforts turned out not to be serious. Perhaps with her new leadership, we can get better results.

I certainly stand ready to help with funding issues, though I must say that in recent years both NIH and CDC have received dramatic increases in their funding,

which, unfortunately, has not been applied adequately to this area of concern. I have the utmost respect for and confidence in Secretary Thompson, Dr. Zerhouni, and Dr. Gerberding. However, I believe they have not been served well by the people under them. Dr. Gerberding assured me over a year ago that she would welcome outside researchers into the Vaccine Safety Datalink. It then took me and my office over a year to get independent researchers access to this information.

Once in, it was quickly discovered that if you sort the VSD data to compare the children who, in 1997 and later, received thimerosal-free DTaP versus those who received thimerosal-containing DTaP, there was a dramatic, statistically significant increase in autism for those who received the thimerosal-containing preparation. Unfortunately, the NIP has hampered further research by refusing to make available post-2000 data.

It is extremely important that outside, independent investigators be given ample opportunity to review these data sets and that they not be reserved exclusively for government-employee researchers, who may have conflicts of interest.

In 2001, you concluded that exposure to

thimerosal-containing vaccines could be associated with neurodevelopmental disorders. I urge you not to retract from this conclusion, but to build on it. Your recommendation in 2001 that there be an immediate effort to end the administering of thimerosal-containing vaccines to infants was wise. Unfortunately, almost three years later, infants are still receiving some thimerosal-containing vaccines. Furthermore, federal officials seem poised to recommend possibly administering thimerosal-containing flu vaccines to children 6, 7, and 23 months old.

Some recent literature gives me further reason for concern. Bradstreet and others have found that chelation therapy in autistic children shows significant levels of excreted mercury in their urine when compared to age-matched controls. When one couples this finding with the very low levels of mercury in hair-analysis specimens of autistic children when compared to controls, reported by Holmes, it begins to paint a picture that autistic children may indeed handle mercury differently.

Certainly, the recent findings of Deth reported recently in *Molecular Psychiatry* are of tremendous interest. Concentrations of thimerosal as low as 1 nanomole were inhibitory of critical enzymes believed to be

involved in neurodevelopment. Both Staitchiech(?) et al., in *Pediatrics*, and Pichichero et al., in *The Lancet*, showed that there were blood levels far in excess of 1 nanomole in infants, both acutely and for many days after a single thimerosal-containing vaccine. Certainly, as I stated earlier, the review, initially, of the Vaccine Safety Datalink information by an independent researcher shows a statistically significant increase in autism as compared to the DTaP group that did not receive thimerosal-containing DTaP after 1997.

Even the much-maligned Verstraeten study found an association between higher exposures to thimerosal and neurodevelopmental disorders in some HMO populations.

Some have argued that there is no need for concern because methyl and ethylmercury react very differently in the body and that ethylmercury exposure levels were too low to cause harm. There is very little science to back up this claim of no harm. In fact, a review of the medical literature by me and my office shows information suggesting that ethylmercury may be as harmful as methylmercury.

In 2001, you recommended studies to compare children receiving thimerosal with those who did not. You

urged the monitoring of the prevalence of neurodevelopmental disorders as thimerosal was removed. Unfortunately, government officials have done neither. Outside researchers have made some progress, but they have been hampered in gaining adequate access to the VSD.

With regard to MMR and autism, I urge the committee to build upon its 2001 conclusions and recommendations. A strong signal from you could lessen the intimidation obstructing this research. You concluded that, since the MMR was mandatory, it was the responsibility of the government to ensure its safety, even if hypothesized adverse outcomes are rare. I concur with your conclusions then.

As with thimerosal, my concerns about MMR have not subsided. The NIH is presently funding an effort to duplicate the work of Drs. O'Leary and Wakefield.

Vaccine-strain measles virus has been identified in the inflamed GI tract of children with regressive autism. Cerebrospinal-fluid analysis of many of these autistic kids with inflammatory bowel disease and measles RNA in their guts is showing the presence of measles RNA in the CSF and high levels of anti-myelin basic protein antibodies. Rechallenged cases of children with regressive

autism have been observed and documented.

The medical community has largely accepted, of course, this new form of bowel disease in children with regressive autism.

Federal research funding has not been directed to investigating many of your MMR research recommendations. When I shared some of these findings with CDC and NIH officials regarding measles RNA being found in the CSF in these children, the response I received back was a blank stare.

If I were charged with the responsibility of protecting the safety of our vaccine program, I would begin an immediate investigation to see if this information were true. All that it has elicited so far has been a collective yawn.

While I have considerable respect for Dr. Gerberding, I am concerned about the ability of the CDC's national immunization program to objectively investigate this matter. The CDC has a built-in conflict of interest that is likely to bias any views. CDC is tasked with promoting vaccination, ensuring high vaccination rates, and monitoring the safety of vaccines. They serve as their own watchdog -- neither common nor desirable when seeking

unbiased research. This has been a recipe for disaster with other agencies. Congress recently saw the wisdom of splitting the FAA because its dual functions left it conflicted between promoting flying and regulating the flying public. In the aftermath of the space shuttle *Columbia* accident, the Gehman Commission found that a critical problem in the shuttle program was that some individuals who were responsible for getting the shuttle off on time were also responsible for flying it safely. The Gehman Commission recommended separating these functions.

This same conflict is inherent in the CDC. Unfavorable safety reports lead to lower vaccination rates, and association between vaccines and autism would also force CDC officials to admit that their policies irreparably damage thousands of children. Who among us would easily accept such a conclusion about ourselves? Yet this is what the CDC is asked to do.

Also, the relationship between the CDC and the vaccine manufacturers has become extremely close. If a conflict of interest does not exist here, then we certainly have the appearance of one.

Given these facts, studies conducted for or by

the CDC should be evaluated within this context. Evaluating how best to eliminate this conflict of interest would be a worthwhile endeavor of the IOM. I urge the IOM to take this matter under review.

Further undermining my confidence in the CDC's ability to monitor safety is the experience I had in assisting an independent researcher gain access to the VSD, and what we have discovered subsequently. The CDC erected excessive barriers and has imposed severe limits on access to this data. Researchers are not provided data collected beyond the year 2000, seriously limiting the ability to provide for independent research to observe the effects of the removal of thimerosal.

The IRB approval process forces researchers to receive approval from as many as seven IRBs, each with its own requirements. CDC places strict limits on what data is available to researchers. Access to the complete database is virtually impossible, and the data is made available on an inadequate PC. Raw data sets used by the CDC to conduct their studies are not made available to independent researchers. Only altered data sets are provided. Thus, the CDC's work cannot be evaluated by outside researchers, and validated.

To summarize: Last week, Dr. Gerberding shared with me that she would be devoting additional time, personally, to this issue, that she believes the research should not end with this meeting. She indicated her desire to see this research continue and emphasized that we should let the truth prevail, regardless of the consequences.

I again urge you to build on your recommendations and findings of possible associations established in your 2001 report regarding MMR and thimerosal. There are increased reasons for concern. The evidence of persistent measles infection in the GI tract and CSF of children with regressive autism continues to expand, and further research must be done. Many of the research recommendations you set forth in your 2001 report have been ignored by the federal agencies charged to investigate it.

Studies conducted by or in conjunction with the CDC should be considered in the context of the CDC's inherent conflict of interest. More investigation is needed to answer these questions with the degree of certainty that science demands.

In closing, I would like to quote from the Verstraeten study. While I have serious concerns about some of the findings in that study, I do concur with one of

their closing recommendations. The authors stated, "We believe that additional investigation is required because of the widespread exposure from vaccinating virtually the entire birth cohort of the United States and the importance of speech and language disorders among children and adolescents. For elucidating further whether a causal association exists between thimerosal exposure and neurodevelopmental conditions, additional studies, with different designs, will be needed."

I concur fully with these remarks and encourage you to adopt these recommendations by calling for a redoubling of these research efforts.

Thank you very much, and thank you for your time.

DR. MCCORMICK: Thank you very much, Representative Weldon.

The next presentation will be given by Dr. Mady Hornig, associate professor at Columbia University Mailman School of Public Health. She will discuss etiologic factors and pathogenesis of autism, including a discussion of immune dysfunction in people with autism. Because Dr. Hornig is addressing two separate issues on the agenda, she has been allotted 50 minutes for her presentation and 10 minutes for questioning.

Etiologic Factors and Pathogenesis of Autism: Evidence from Clinical Studies and Animal Models - Mady Hornig

DR. HORNIG: Thank you very much.

I have been charged today with addressing the etiologic factors and pathogenesis of autism, and drawing examples from recent clinical work, and also animal-model research.

The idea that environmental factors may have had a play in a variety of neuropsychiatric disorders is not a new one. Here we see it back even in the 1850s, with Escarole(?), 1845: Many authors assure us that mental alienation is epidemic. It is certain that there are years when, independently of moral causes, insanity seems to extend to a great number of individuals.

We can provide a perspective that I believe has a very important role in understanding the pathogenesis of these disorders. We are very interested in the interplay in the development of the human organism, the interplay of genetic factors, in terms of the timing of events, the precision of the steps that allow for human development, and their timing and the developmental status of immune and nervous system, as well as environmental factors of a very

wide range, beginning with infections, both bacterial and viral, toxic agents, malnutrition, and even mediators of the stress response. We believe that these factors can interact with one another at multiple points, both prenatal and postnatal, with development of disorders such as autism and ADHD; in early adulthood, psychiatric and demyelinating disorders such as multiple sclerosis; and even in late adulthood, with degenerative disorders perhaps having their beginnings laid down during the prenatal time course, for disorders such as Parkinson's and Alzheimer's disease.

What clues do we have to the neurobiology of autism? Let's begin with its beginning in the literature. Certainly, there may have been disorders that had autistic features that may have gone by other names and may be associated with mutations or toxic events, et cetera. But despite our wonderful descriptive physicians looking at the whole panoply of disorders that are out there, there were no descriptions until the 1940s, first, of course, with Kanner's description in 1943, followed soon thereafter by Asperger's in 1944.

There are clues there, perhaps. Without appropriate research, it is difficult for us to know which range of environmental factors might be at play. However,

that is also a time point shortly after the introduction of diphtheria vaccine, which also had thimerosal.

The prevalence of this disorder has increased up to tenfold since 1985. I am not an epidemiologist per se. There are many who are much more expert in that subject and can shed more light on the epidemiologic associations for the disorder. But there are clinical associations, including the male-to-female ratio, suggestive, perhaps, of everything from X chromosome inactivation, X-linked disorders, to susceptibility relating to testosterone *in utero*. We have regressive subsets reported. These are reported both on a typical and an atypical developing context. Some children will develop a regression after having normal development. The regression does not necessarily have to be only in language, but can also include a variety of other skills and so-called apraxia, difficulties in completing motor movements.

There is a seasonal association. Again, there are some studies -- it is controversial -- where March and August births are very prevalent.

We know also, if you look at some of the features of the children, a finding as simple as head size, macrocephaly -- there are some reports now that maybe heads

are longer than they are tall. What this can tell us about the embryologic origins and the developmental origins of head growth and the metabolism is important to understand.

A developmental lesion is suggested by the neuropathology. But as I will show you shortly, there is very old literature on which to base neuropathology, and only a newly growing group of studies that are using modern techniques that can look at issues like microglial activation and apoptosis. We know that disturbances range from dopamine to serotonin to glutamate. But this also is a disturbance collection that would occur in a wide variety of other disorders. Viral and immune links are suggested.

We also know that, although there are strong genetic contributions, they are quite complex, and we need to understand and leave room for the heterogeneous manifestations of the disorder, the possibility that there are multiple causes, and that there are likely to be epigenetic contributions.

So how do we approach the idea of implicating microbes in disease or immune changes in disease? First of all, we can go back again to Koch's postulate that a microbe should occur in every case of a disease, that it should be specific to that disease, and then that it can be

isolated, grown in the laboratory, and cause disease even after inoculation in animals.

However, there are many, many problems with invoking this postulate. Some microbes cannot be grown in the laboratory. We know that host factors, environmental factors, and the timing of the insult may influence the expression of disease, and also that long-term sequelae and sequelae distant to the actual physical introduction of the agent may play a role. Suitable animal models may not exist.

Some examples of this wide variety of ways in which agents can cause disease: Replication can cause direct cell damage. Anterior spinal horn cells, motor neurons, are killed by poliovirus. A toxin can have a local effect, such as in cholera toxin, which will alter the ion transport in intestine and cause diarrhea. You can have a distant effect from a toxin, such as botulinum toxin, which will cause an effect at the nerve-muscle junction through interruption of neurotransmitter function. Hepatitis B can kill liver cells through the immune response generated to the agent. This example of measles virus is an example of immunosuppression, causing tuberculosis exacerbation. We know today, with HIV, that

that is certainly there, susceptibility to immunosuppression from a wide range of agents.

Differentiated cell function can be affected as well. LCMV can cause a persistent neurotransmitter or hormone-production abnormality. Molecular mimicry is also another mechanism. With streptococcal infections, we will be looking at an animal model briefly that has a central-nervous-system effect, which has some relevance for autism.

Very complicated factors must be allowed for. We know that in multiple sclerosis, where you live before puberty has an effect on your risk for multiple sclerosis. The actual mechanism that creates that risk is still not clear. But we have to think broadly in terms of the potential for a variety of mechanisms in complex disorders of this nature.

Psychiatric diseases also may have effects from viral insult. Probably a variety of prenatal insults can lay the foundation for the development of schizophrenia in early adulthood.

So the challenge is to find footprints of agents that are present in low levels in the affected tissues, that defy the traditional methods for isolation and characterization, or have even left the scene -- a hit and

run -- by the time the disease is manifest.

In the epidemiologic investigations that have reported this tenfold increase, at least, we know that if you look before 1985, the broad autism phenotype was about 4 to 5 per 10,000, and classic autism, around 1 to 2; after 1985, as high as 67 per 10,000 for the broad autism phenotype, and about 40 for classic autism. There are a variety of events that have occurred besides vaccine changes that, of course, can be responsible for these types of changes. We also need to ensure that diagnostic substitution and the wider case definition and awareness are also controlled for in these analyses. Certainly, the wider case definition and awareness have had some role. The question is, how much of a role? It is unlikely to be the answer for all of the increase.

A variety of environmental exposures are becoming increasingly likely to be considered in the pathogenesis of autism. Infectious agents in generalized immune challenge, toxins, including cumulative mercury burden -- not just that from thimerosal in vaccines, but also from methylmercury in industrial sources of mercury -- PCBs and even PBDEs, these flame retardants, are beginning to be associated with central-nervous-system damage. The role

that they play in autism is as yet unclear.

Vaccines and vaccine components, of course, are the charge to this committee. We know that there are a variety of epigenetic effects that can occur from wide classes of xenobiotics. We need to know which xenobiotics can have these effects, which ones are most important during development. As recently reported by Richard Deth and colleagues, there are effects of thimerosal directly on enzymatic processes relevant for the methylation of DNA and for gene expression.

We know also that there are changes -- 1985 was when the association became known of Reye's syndrome with aspirin. The entirety of the pediatric suggestion was to use acetaminophen as opposed to aspirin. It wasn't until later, when ibuprofen became available in pediatric form, that there was the beginning of introduction of acetaminophen. Acetaminophen has, in at least 20 percent of the population, a glutathione or an antioxidant stressor effect that can occur. So population-based differences could indeed interact with that type of effect.

We need to survey the wide variety of changes that have occurred in order to understand these.

Although this study by Croen and Grether was

really based on a data set from the California Developmental Disability Services and was not really meant as a well-designed epidemiologic study -- but we don't have well-designed epidemiologic studies to give us real data regarding prevalence -- this is showing, on top here, between 1987 and 1994, that the diagnoses of mental retardation remained quite stable, and there was an increasing report of autism in the California Services population, again suggesting that there is not a diagnostic substitution, at least not as a full answer to the suggestion of an increase in prevalence.

Other environmental factors: Geographic clusters have been looked at. Most of these have not had a high linkage. In Brick Township, there was a suggestion that the proximity to the industrial cesspool perhaps might have been associated, but it really turned out to be simply one of the first reports that there was a far higher prevalence of autism than we had previously appreciated in our country.

There are a variety of links, loose links, through epidemiologic studies, to infection and antigenic challenge, the seasonal representation of March and August births. There are viral associations ranging from rubella

to herpes. Vaccines, as well, have been suggested. We know that immune disturbances are higher in children with autism. There is a Th1 to Th2 shift, and also a strong family history of autoimmune disease, particularly through the maternal lineage.

Neuropathologic and brain-imaging studies support a developmental lesion. Most of the older literature suggests a pre- or perinatal lesion. However, there is increasing evidence to support the possibility of postnatal influences as well. The findings are not consistent. We desperately need more brains to study, as well as more studies using brain-imaging techniques, both structural and functional.

So our hypothesis is that gene-environment interactions are critical determinants of adverse outcomes.

Clinical features can be very telling. This is our Chinese menu -- two from column A, one from column B -- from the DSM-IV method of diagnosis. We know there is a plethora of symptoms in this disorder. However, there are features that may actually be instructive in helping us to understand the causes and complexity of this disorder. They are not all psychiatric. Even within the psychiatric, there is a wide range. There are motor symptoms that

include activity-level differences, reduced motor tone, postural abnormalities, coordination, motor programs, and even autonomic reflex disturbances; eye gaze, peripheral vision, and ocular motor disturbances amongst the visual abnormalities. GI disturbances, elimination disorders, inflammatory bowel disease have been reported. Cognitive disorders include mental retardation. The rate of mental retardation in diagnosed cases of autism is changing. It used to be 65 to 70 percent. There is some suggestion that that rate may be dropping, despite the fact that there are no changes in diagnostic substitution, in some settings.

Savant skills can occur both in the setting of a normal intelligence or in the setting of profound mental retardation. What can these tell us about the functioning of the brain? Learning and memory and abstract thought, of course, are also disturbed.

There are disturbances of growth and some reports of children who are both large and small, sort of falling a little bit outside of the normal bell-shaped curve. The effects of growth and immune challenge, nutritional challenge, and also viral challenge on growth curves are very well-known. Head and brain size are reported to be abnormal, but not in all children with autism.

Seizures also occur. There seem to be a couple of spurts of seizure disorders that correspond with periods of myelination. Immune disturbances we noted. Connective tissue -- ligaments are lax. How does this occur in a disorder that is supposedly only a central-nervous-system disorder? How do we understand this complexity in terms of etiopathogenesis?

Sensory disturbances can also occur, with hearing, taste, and even synesthesia of a cross-modal analysis of sensory input.

Brain-imaging studies can also be quite informative. This is a study from Eric Courchesne's group that shows that, although at birth -- these are kids with autism. This is using the CDC norms for head circumference. These are kids with autism. There is slightly smaller head circumference at birth. By the age of 6 to 15 to 28 months, there is a significant increase in head circumference. This seems to level off after time. This phenomenon seems to be most highly associated with kids with ASD. This is in the black circles here. You see this increase in autistic disorder, classic autism. Then the children with the more atypical PDD, pervasive developmental disorder, NOS, seem to have a smaller

increase.

We need more studies that do serial and perspective analysis to understand what the nature of this brain growth is, to define it, and also to understand its correlation, perhaps, with the normal pruning mechanisms of the brain, apoptosis-related products. There is a normal process. We are born with too many neurons. We should be pruning them out over time. We need to understand the biochemical correlates that go along with these changes in head circumference.

I also draw here from Martha Herbert's work on the dissociations of volumes in cerebral cortex, subcortical region, and in white matter, in slightly older boys with autism. You can see here in the stars that there are increases in globus pallidum, putamen, diencephalon, and cerebellum, and also in the white-matter tracks.

Interestingly, she notes that there are very prominent effects in bridging white-matter structures, which are structures that continue to mature postnatally. Some of the biggest volume increases are in the bridging structures, suggesting postnatal effects.

The neuropathologic findings, as I noted, are very few and far between, and based, in large part, on

older preservation techniques and older analytic techniques, until the past couple of years. There are fewer than 50 autopsies presented in the literature. We do know that there are changes in the cerebellum that are reported. It may be an age-dependent phenomenon. Purkinje cells seem to be lost in the older population. Both hypo- and hyperplasia of cerebellum have been reported. The branching of the dendrites of the cells seems to be present in some. GluR1, which is a glutamate receptor of the AMPA variety, is also found to be abnormal in cerebellum of individuals with autism.

Similar dendritic-branching abnormalities are noted in hippocampus and amygdala, some regions of the brain that are involved in emotional processing, and there are increased cell packing and GABA abnormalities. GABA is another inhibitory glutamate neurotransmitter.

Association cortex shows migration defects at times, with malformations in the gyri.

Biochemically, we have identified a wide variety of abnormalities in the serotonin, glutamate, and a variety of other neurotransmitter systems. We know from heel stick of children with autism at birth, but also children with MR, that there are abnormalities in neuropeptides, such as

VIP, calcitonin gene-related peptide, brain-derived neurotrophic factor, and NT4/5, and also abnormalities found in other studies in reelin, which affects migration, but also has effects postnatally in neural function.

Sex steroids have been implicated. We know that there are some studies now that implicate apoptosis-related factors as being abnormal in children with autism.

Immune abnormalities are a very distinct subset that have had a fair amount of attention. Even using older techniques, there has been an interesting convergence in the literature. More recent studies seem to corroborate the findings of the older studies.

Th2 cytokines -- the so-called Th2 shift, which is an indicator of an autoimmune propensity, is found in kids with autism, but also Th1 cytokines, like interferon-gamma and IL-12, which are also found in lupus, rheumatoid arthritis, and a wide variety of other humerally mediated, antibody-mediated autoimmune diseases. We also know that interferon-gamma is a cytokine that plays a role in mercury-related immune disturbances and autoimmunity. More on that to come.

There are isotypes of immunoglobulins, IgG4 and IgE, that are of the Th2 type that are increased. In *in*

vitro mitogen responses of T cells and natural-killer cell activity, responses are dampened. There is a decrease in the C4B complement. This actually happens to be an interesting molecule that has some other implications -- we will talk more about that later -- in association with the vaccine-strain measles virus receptor.

There is also an increase in the transmission of the C4B null allele in the families.

Autoantibodies are found to a wide variety of agents. I will allow Dr. Singh and others to describe some of that very nice work in autoimmunity. There is also the strong family history.

Curiously, there have been several studies of HLA linkage, and it is only the studies that look at children that have just a single individual affected by autism that seem to show the linkage. So if you look at multiplex families, you do not see the HLA linkage. We need to make sense of those data and understand. One explanation is that you also need an environmental factor in association with HLA, since HLA is an immune response change that changes your vulnerability to a wide variety of exogenous agents.

There are some reports, case reports primarily,

of IVIg immunomodulatory responses in children with autism. The predictors are unclear. We need to understand more about the basic biology to understand how to help these children more effectively.

One interesting integrative pathway that allows for the connection amongst some of these factors is the kynurenine pathway. This is also the so-called tryptophan-degradation pathway. These enzymes that degrade tryptophan, tryptophan 2,3-dioxygenase or indolamine 2,3-dioxygenase, happen to be activated by a variety of exogenous influences. Interferon-gamma and a variety of other cytokines can influence the activation status of indolamine 2,3-dioxygenase, and glucocorticoids affect the tryptophan dioxygenase. So a variety of infections can then increase the degradation of tryptophan down this cycle.

There are a variety of apoptosis promoters and effects on NMDA receptors and other glutamate receptors, both protective and excitotoxic, depending upon the time at which they are introduced into the nervous system.

What do we know about the genetic-susceptibility factors? We know that 10 or more loci are likely. We know that twin data, although this is clearly a highly

genetically influenced disorder -- for the broad autism phenotype, one sees in monozygotic twins 90 percent concordance -- it turns out that the early reports of only zero percent dizygotic twin rate compared to the sib concordance rate of 4 percent have been incorrect. There were very small numbers of dizygotic twins upon which that initial literature was based. With much higher numbers of dizygotic twins to allow for greater security in the data, it seems that we may be up as high as 35 percent, suggesting that either the intrauterine environment or the shared early postnatal environment may be important in establishing this higher concordance of dizygotic twins relative to sibs.

A wide variety of chromosomes are suspected. We talked already about the HLA association and the linkage to the familial history of autoimmune diseases. We haven't touched very much on the possible role of epigenetic influences. We know that, developmentally, the gene product that is involved in Rett's disorder, the MECP2, is affected by a variety of transcriptional and posttranscriptional mechanisms, so no mutations are necessary. Transcriptional and posttranscriptional mechanisms may affect these in autism spectrum disorders,

Prader-Willi and Angelman syndromes. We need to understand more about the actual normal developmental pathways and the aberrations that may occur in selected subsets and with selective exposures.

Some genes relative to neural-immune interactions are stress responses, as we just saw with the kynurenine pathway, the tryptophan 2,3-dioxygenase, or may be activated by infection or stress through cytokine induction.

What can we learn from animal models? This is the bulk of our grounds-up way of looking at the issue. The importance of timing -- one cannot necessarily translate what is done in an adult system to what is relevant for a neonatal organism. In this brief story of Borna disease virus -- we don't think that children with autism have Borna disease virus, but we think it is a good model for understanding the developmental sensitivity. Adult rats have a very different scenario than newborn infected rats, even though they are given the same virus. The disease manifestations are based upon the variations in the maturity of the immune and the nervous system.

We know that in the adult rats, it is a biphasic disease. There are startle responses acutely, with

hyperactivity. During the chronic phase, they have a stereotyped motor syndrome, with dyskinesias and dystonias. Encephalitis, though, is a very large part of this, with a very large cell loss. The dopamine system seems to be largely the system affected.

Neonatal rats, in contrast, who are infected during the equivalent of the primate third trimester -- this disorder unfolds as the nervous system develops. These animals have hyperactivity, a variety of social disturbances with play behavior, many features reminiscent of the disorder that we know as autism. There is no encephalitis here, however. Yet one loses one distinct portion of the hippocampus completely, dentate gyrus, and we lose Purkinje cells, up to 75 percent, by 7 months. We believe that the lesion is in the serotonin and the glutamate system here.

It is a very complex virus. It is a negative-strand virus that replicates in the nucleus, non-cytolytic, minimally productive, and has a very, very complex biology that involves splicing of genes and unique phosphorylation patterns that may create a very special pathobiology.

This is a normal brain of a rat. If you infect the adult animal, early on, in the acute phase, you see

perivascular cuffing, encephalitis. Then, in the late phase, you see cortical atrophy, this hydrocephalus, and sort of a generalized degeneration of hippocampus.

In the neonatal animal, you see no encephalitis, and you see a distinct ablation of the dentate gyrus.

The adult animals, which I will focus on just very briefly as a way of example, have complex examples. This is an animal just dragging a petri dish back and forth. You can see, if you look at the D2 receptors, using autoradiography, a reduction in the striatum, a motor-control center, a 40 percent decrease, and you see a 20 percent increase in D1-receptor expression. This D1 overdrive seems to be related to tail-biting behavior. If you give a D1 antagonist, Schering 23390, during the period of the drug, until it clears, you are able to abrogate that behavior.

In the neonatal scenario, in contrast, we find a variety of affects that seem to be very similar to certain aspects reported in autism. This comes from Phil Teitelbaum's work in 1999, where he found that there were abnormal righting movements in children later diagnosed with autism using home movies. In contrast to the normal corkscrew effect when an infant tries to turn over,

crossing across the midline, you find that there is a turning en bloc or a ventroflexion and rocking over. They do not cross the midline.

This has a very nice parallel in rodents, and a time course for its development. It goes away after a period of time. By day 9, we don't see it. We only see falls onto the back with an inability to right in the animals that are infected with Borna virus.

We find that there are disturbances in social communication. This is showing, in a maternal separation paradigm, that there is an abnormal waveform, there is an abnormal increase in the number of calls. We believe that these calls fail to elicit the mother's normal maternal response -- certainly, evidence of social communicative disturbance.

Hyperactivity is found in these animals as well. We know that, if you look at the same time that the hyperactivity occurs and all the changes are occurring in the brain, there is an increase in a variety of proinflammatory cytokines that seems to correlate with the loss of cells by apoptosis and an increase in the proapoptotic Fas and caspase-1, and a reduction in the antiapoptotic or neuroprotective bcl-x.

This shows you the ablation by 5 weeks postinfection of the dentate gyrus in these neonatally infected animals. Just by H&E, we have corroborated by TUNEL labeling that these are cells that appear to be undergoing apoptosis. You can see here the hypereosinophilia and pyknotic nucleus of a Purkinje cell in cerebellum undergoing apoptosis, and many cells in dentate gyrus.

We know that dendritic branching is abnormal, as has been reported in the few neuropathologic studies of children with autopsy data. You can see the normal branching and nice complexity here in the control. The infected animal, in the dentate gyrus granule cells, has a reduced complexity.

Of course, I don't have the dentate gyrus granule cells. That has not yet been studied. But in another region of hippocampus, CA1, you can see a similar effect in pyramidal cells, a spindly appearance.

These are typically developmental changes that are under the influence of glutamate -- again, another intriguing set of characters that are important for us to study.

GluR1 expression -- as I noted earlier, GluR1 is

an AMPA receptor that was found through microarray technique to be abnormal in the cerebellum of children with autism. Normally, at 4 weeks, you see a nice distribution, this brown staining here of GluR1 in the molecular layer of dentate gyrus. In the infected animal, you see a complete bleaching in the molecular layer and a coalescence of protein staining at the level of the granule cells that are ultimately lost, so a redistribution to this cell soma.

This 4-week time point happens to coincide with a developmental switch in splicing. It is sort of a way of taking genetic code from one isoform to the other. This development switch from flip to flop isoforms typically occurs at the 4-week time point. We hypothesize that an arrest in the flip isoform caused by the virus or viral proteins or the secondary mediating changes may have led to continued glutamate sensitivity, since the flip isoform remains sensitive to glutamate, despite chronic exposure.

We know that, if you take an AMPA-receptor antagonist -- there is no specific GluR1 antagonist, but we administered an AMPA-receptor antagonist and found that you get this nice dose-dependent reduction in the hyperactivity. Here is the hyperactivity in the untreated but infected animals compared to the control animals. You

can see this dose-dependent reduction relating to the GluR1 and other AMPA-receptor antagonism.

We did find that there is an increase in the flip versus flop isoform, if you look 4 weeks after infection.

We also note that a kynurenine-pathway product, quinolinic acid, is increased in a variety of brain areas. This may suggest that there is an excitotoxic pathway that may be the convergence of cytokine-mediated changes, tryptophan degradation, and glutamate-receptor alterations.

So what do we learn from this model? We know that genes, environment, and timing all interact with one another. We know that there is a wide variety of infectious agents that have very similar effects, and probably xenobiotic agents that have similar effects. We want to look more and more to the sites of convergence, like the kynurenine pathway, or to understand normal programs of epigenetically influenced developmental gene expression, to understand these issues of vulnerability.

We have been trying to build more generic models. We use a synthetic mimic of viral infection, poly(I:C), polyinosine-polycytidylic acid. We know that across mouse strains the timetables for maturation vary. We believe that the genetic contributions of the maternal immune

response and the offspring immune response are important in determining this. We note that the pathogenesis, then, is not virus-specific, although a wide variety of viruses are implicated, both in clinical epidemiologic studies and animal models, but, rather, the genetically coded maternal response to infection and what that induces in the pup is what is important here.

We took two strains of mice, one with a high susceptibility to immune damage, the SJL, and the C57, with low. We went late in gestation, GD 16, and found that, if you look at the dams -- there are up to 70 that we have studied; these are just the ones that are above the no-injection control -- you can see a wide range of differences in the profiles of cytokines and chemokines. This will take a long time to sort out, but it does give us some sense that there is some biochemical basis for understanding the differences that do occur.

Postpubertally, the offspring of these dams have some hyperactivity. They also have a variety of other abnormalities. They have altered BDNF expression, a dose-dependent reduction. You have an increase in reelin expression both at week 8 and week 16.

So this gives us a sense that multiple soluble

mediators may contribute to neurodevelopmental injury. It may not be a specific process in all cases. Understanding of generic models or general models may be helpful.

But what susceptibility of receptors may be developmentally sensitive? What may create the sensitivity? Are there cross-reactive antibodies that may target specific brain components?

We have moved to a model called PANDAS. It stands for "pediatric autoimmune neuropsychriatic disorders associated with streptococcal infection." We find that there is a misdirected immune response to exogenous agents that may result in neuropsychiatric syndromes in susceptible hosts. We are looking at this syndrome, first described by Sue Swedo back in 1994, in conjunction with Sydenham's chorea. It has been long known that this St. Vitus dance is associated with an autoimmune response. But even in Sydenham's chorea, which is a very well-established autoimmune disorder, the CNS targets of these antineuronal antibodies may vary. There is a risk factor, the D817 marker, but also this occurs in 78 percent of children with autism, even in the absence of recent streptococcal infection. These children respond quite profoundly at times to plasmapheresis and intravenous immunoglobulin, and

also to antibiotics.

There is swelling in association with the syndrome in distinct areas that are involved with compulsive movements, the striatum or basal ganglia. So you have caudate putamen and globus pallidus, but not the sensory processing area or thalamus.

We injected inactivated streptococcus of the type that is associated with the PANDAS syndrome, group A beta-hemolytic strep.

I am sorry. We are having problems with translation. There was a photo here. If I had my movie, this animal would be doing back flips.

So in addition to the staining in the PANDAS mouse brain, showing the immunoglobulin deposits -- and also showing that using PANDAS mouse serum on rat striatal neurons in culture, you have a very high level of staining -- we also see that there is very profound staining in deep cerebellar nuclei that co-localizes with the synaptic marker, SNAP-25. These IgG deposits are correlated very well in the PANDAS subset with rearing behavior and also with ambulatory distance.

Had we gone just with a normal western blot, we would have missed this phenomenon, because all of our

initial efforts were not successful. We found that, using nondenaturing western blot, we got this band. It was taken to the nondenaturing condition immunoprecipitation, and found through LC/mass spec, that there was complement C4 and alpha-2 macroglobulin. These are both very interesting molecules, for a variety of reasons. The actual section where they have homology would indeed be altered by denaturing conditions. That made sense with our western blot and nondenaturing western blot data. But also C4 complement is associated with a wide variety of autoimmune syndromes. It is important in strep pathogenesis, because it binds the N protein of strep. We also know that there is the abnormality in C4B in children with autism and in their families.

Alpha-2 macroglobulin also can be activated by neurotransmitters such as monoamines and has differential effects. Again, an intriguing set of molecules.

But one of the really key issues, and the last factor in our equation here, is the timing. One of the big issues has been, is it possible that there can be really profound postnatal challenges that will affect the development of the brain? We know that in humans limbic structures continue to develop through the second year of

life.

This brings us to the mouse strain-dependent model of ethylmercury neurotoxicity. The sensitivity to this adverse event in mice predicts neurotoxic effects following postnatal thimerosal. We hypothesize that it would not be just any individual that would have these effects, but rather those that had a specific genetic vulnerability that created a mercury-related sensitivity.

Eighteen percent of the population has skin sensitivity to thimerosal. Population differences also occur in glutathionase transferase and metallothioneins. We know that there are a wide variety of effects, apoptosis, et cetera. I will leave some of those, I am sure, to Dr. Baskin for later today.

But of note here is that methylmercury and ethylmercury, or thimerosal, have very different effects, and that you need to look at these very carefully. Th1-predominant responses occur with methylmercury exposures, Th1-related isotypes, and also reduction in T-cell and natural-killer cell activity. But the metabolism to inorganic mercury must be considered. Ethylmercury has a pattern which looks much more similar to inorganic mercury, but is somewhat mixed, because it also has the organic

forms.

Here you see a Th2 shift. You see isoforms, IgE, that are relevant to a Th2 autoimmune scenario, and polyclonal B-cell activation -- this is recent work by Helpman's(?) group in Sweden -- and also increased autoantibodies.

We basically replicated the childhood immunization schedule in a mouse. We took 2, 4, 6, and 12 months of early thimerosal exposure in the U.S.-required childhood immunization schedule, as reflected by these vaccines at each of those time points. This was the ethylmercury load at those time points. This was the 10^{th} percentile weight for U.S. boys. We basically did our division and came up with a microgram-per-kilogram dose at day 7, day 9, day 11, and day 15. Day 7 was chosen to attempt to come close to the end of most migrational events in brain.

We looked at mouse strains that were known to have differential sensitivity on the basis of their major histocompatibility complex. The H2S is a sensitive strain to mercury. C57 and BALB are not. H2B and H2D have less sensitivity.

We found here that there is a profound difference

only in the SJL mice, as we predict. The autoimmune sensitive mice have a significant delay in weight gain -- very profound and early, just through day 15, as we are finishing the injection. There is a behavioral impoverishment that occurs across a wide variety, highly significant effects across the board -- again, only in the autoimmune sensitive strain of mouse.

Again, my movies are not playing in their entirety. But what you would see here, if you had both movies, is an animal that is frantically self-grooming. Its partner here -- we have tail biters. About 40 percent of the animals after 6 months become self-mutilatory. He bites his tail. He grooms excessively. But he also grooms his partner. Unfortunately, our other movie did not import appropriately to the PC. What you would see here is that this animal, who wildly self-grooms, not only takes care of his partner -- and they have wonderful protective dyadic interactions -- but he also grooms. He has groomed through the skull, and eventually destroys his partner.

This is what the brains look like. We have very profound disturbances in SJL brains. I will just orient you for a moment. These are the normal, the controls, in SJL and BALB. What you see here in the thimerosal

animals -- we don't see differences, by the way, between thimerosal-only and thimerosal-vacs animals in mercury equivalents. If you look at the hippocampus here, in the normal animal you should see sort of a nice increasing stream here, going from CA3 to CA2 to CA1. What you see here is this widening at CA3, at the elbow, a huge enlargement in stratum radiatum at this area. You see this notch at what is putatively the CA2 start point, and you see increased cell density, hyperchromic neurons, and also in dentate gyrus. The brain is, overall, enlarged. There is some enlargement in the BALB thimerosal animals, particularly in dentate gyrus, but not to the degree that we see in the SJL animals.

Curiously, this hyperchromia has been reported by several investigators in Rett syndrome, in various regions of hippocampus -- Margaret Bauman and a variety of other investigators.

We know that host-response genes are really critical in determining sensitivity to mercury-induced autoimmune disturbances in this animal model, and that postnatal xenobiotic challenges are at least possible in these types of settings. Of course, we need to determine the relevance of such animal models for human neural

development.

That brings me to the last set of slides, which very briefly describe for you our autism birth cohort. The Norwegian government has funded a mother-and-child study, the MOBA study, upon which we have set up the autism birth cohort, 100,000 pregnancies, where sampling begins during the 17th week of gestation. This is funded by NINDS, just recently. We follow the mothers and babies through with both questionnaire data and ongoing biologic sampling, and also trios -- mother, father, and child -- as well, with cord bloods at birth, as well as assessments for autism and other neurodevelopmental disorders, beginning at 30 months.

Organic mercury exposure in this study does not have great relevance for thimerosal. Methylmercury in fish is their major exposure, and that is large in that setting. We will have the opportunity to understand more, at least, about methylmercury metabolism.

These data in the U.S., if one is considering just methylmercury exposure through fish, up to 16 percent -- this was 8 percent before last week, and now this has been doubled, because our data were insecure. We were basing the risk of children on maternal blood levels only, and it turned out that there was a great disparity

between maternal and umbilical cord blood levels of mercury. So this went from 3000 children to 600,000 children at risk, simply due to the levels of organic mercury present in their blood through dietary means.

We also are pursuing a study of the MMR vaccine in autism. There are many more qualified individuals to discuss, certainly, the epidemiologic data on that issue. But I just wanted to return to what the original findings were. Besides there being this finding of ileal nodule or hyperplasia, there was measles virus. So, in conjunction with the original reporting lab, John O'Leary, as well as our group at Columbia and the CDC, will, in blinded analysis, all get split samples from the same specimens. We will be looking, again, just to answer that question as to whether measles virus is actually present in the gut.

Of course, there are a number of outcomes in this scenario. There may not be any difference. The prevalence may differ in cases and controls. Then the work becomes trying to figure out what the nature of the meaning of those data might be.

I just want to close on this issue with respect to paradigm shift, just as a reminder that 10 years prior to this report of *Helicobacter pylori* and its role in

ulcer, we were talking about stress for duodenal ulcers and which type of stress created whatever type of outcome. Then we knew about *Helicobacter pylori*.

So it brings us to this quote: In the period that Einstein was active as a professor, one of his students came to him and said, "The questions on this year's exams are the same as last year's." "True," Einstein said, "but this year all the answers are different."

So it behooves us to keep our eyes open to the possibility of new answers.

DR. MCCORMICK: Thank you.

I forgot to remind you, but your first question is to talk about your funding.

DR. HORNIG: I don't have any specific vaccine-related funding from manufacturers of pharmaceuticals. I have funding from the Coalition for Safe Minds and from NIMH, et cetera, to look at neurodevelopmental aspects and to consider the possibility of xenobiotic exposures.

DR. MCCORMICK: The mike was off. Can you repeat that?

DR. HORNIG: I don't have any specific funding for looking at safety and efficacy of vaccines. I have

done work for a variety of manufacturers of vaccines, but for psychopharmacologic drugs, not for vaccines. I have funding from the Coalition for Safe Minds -- I think that was on my first slide -- as well as from a variety of NIH institutes.

DR. MCCORMICK: Questions from the committee?

DR. CASEY: My name is Rosemary Casey. I am a pediatrician.

May I ask you, in the children with macrocephaly, the autistic children with macrocephaly, do you know if they have communicating hydrocephalus and increased extra-axial fluid? I saw your data about the cortex and so on, but I was wondering about any degrees of atrophy, because we see that.

DR. HORNIG: I don't know that answer. I do know that over time -- head circumference, of course, is a cumulative measure -- eventually, this starts to level out, and there can be changes in the volumetric patterns as the children age. That is seen somewhat with autopsy data, as well. But I don't know the specific answer to your question.

DR. CASEY: Because that is what happens if they eventually reach an equilibrium, and they do have

communicating hydrocephalus. You can just do it with an ultrasound.

DR. BAYER: Ron Bayer, on the committee.

I wonder if you would stretch a bit to the answers for next year rather than this year and talk some about the impact of changing dietary patterns in industrialized societies and the level of mercury in fish as the overwhelming determinant of whatever pathological consequences there might be as compared to vaccination, which, after all, had been constant for many decades.

DR. HORNIG: One of the important concerns, of course, is to try to understand some of the continued increase in the methylmercury exposures. There is some work that is ongoing now to look from the Hudson River to waters and lakes in Norway, to understand the factors that are increasing the exposures. There are some nutritional factors that can act as sort of a counteractive force. Selenium, for instance, and selenium status, is an important determinant of the response to mercury-related damage. The better your selenium, the less mercury-related damage one would have.

But I think this is a continued concern. I know that we have a lot of work to do to understand how to

chelate, if we are not going to remove fish from our diets or not going to move to a program that tells us how much each can of tuna has on the nutritional warning label. Chelation, I think, is something where we have not had enough controlled trials to know about its safety, to better it.

DR. GOODMAN: Steve Goodman, from the committee.

It is often said that there is not a good animal model for autism. You have presented this compelling little movie, even though we didn't see the rest of your movies. I am wondering if you could talk about the degree to which the behaviors we just saw in these particular mice and the pathophysiology are indeed a good model for autism, and whether there are any other toxins or insults that produce the same sort of behavior in either these mice or other strains of mice.

DR. HORNIG: My guess is, starting with the end of your question, that there are going to be a number of toxins that, if studied in this fashion, may indeed show very similar types of effects. Much of the work that has been done for toxic insults in developmental neurobiology has been done with prenatal exposures. The ability to extrapolate from prenatal to postnatal, even in a mouse, is

difficult. It is extraordinarily difficult when you try to think about the immune and nervous system differences in these animals.

I think, at best, we can think of these animal models as being reminiscent of certain aspects of autism, being able to model certain types of neurocircuitry. I think, as we gain more knowledge about the heterogeneity of autism and the endo-phenotypes, if you will, we may be able to utilize a variety of animal models to answer specific questions that will enable us to tailor diagnostic schemas, as well as treatment and intervention schemas, for those select subsets.

DR. KABACK: Mike Kaback, from the committee.

Again, staying with the thimerosal mouse susceptibility story, you indicated that the dates at which these doses were administered were somehow calculated to be comparable to the exposures that children would get, and the doses per kilo would be comparable as well. But did you do pharmacokinetic studies to indicate what the peak and saturation levels and excretion levels and rates of excretion were? It seems to me that a two-day interval in a mouse, a growing mouse, is perhaps quite different than a one- or two-month interval in a growing child.

DR. HORNIG: We are trying to obtain those data now. I agree that they are very important. Nonetheless, the data also do tell us that there are differences across the strains, no matter the peak level that is achieved in these animals. We feel very strongly that there are, perhaps, a variety of explanations. It may be nothing relating to the autoimmune genes of these animals, for instance. Just because they happen to have differences in H2 linkage and just because that happens to have been previously linked to mercury sensitivity doesn't mean that that is why it is working in this scenario.

We have a lot of work to do, back-crosses and so forth, knockouts, to understand the genetic influence, number one, and also to determine, as you say, what blood level is achieved? All we have now are the strain differences, and one animal strain in which the phenotype is quite striking.

DR. FOXMAN: Betsy Foxman, from the committee.

It sounds as if your impression is that there is a wide possible number of etiologies for autism. Is that correct? So how would you anticipate, then, in terms of these measures of host response in immune function to vary across these subtypes? How well characterized are any of

these subtypes today?

DR. HORNIG: We need a lot of work in the characterization. My impression, I think, is at best a guess, based upon my reading of the immune literature, where a subset of children with autism is taken. These vary somewhat. About a third of individuals, on average, I think, if you were to go across the wide range of studies that have been done, both positive and negative, looking at children with autism and looking for a range of immune abnormalities -- the incidence is quite high. It is about 40 percent in the autoimmune familial link, again mostly down the maternal lineage, in terms of transmission -- hypothyroidism, for instance. Hashimoto's thyroiditis is a strong one.

But even there, we need better studies that are not survey-based, to corroborate and to strengthen these types of findings.

DR. JOHNSTON: Dick Johnston.

Could you correlate for us the neurodevelopmental relationships in the mouse model you used compared to the human? What was the basis of your selection of the timing? I know that the immune system is very immature in a newborn mouse compared to the human. How does the central nervous

system relate in that regard? What was the basis for your selection of those dates?

DR. HORNIG: A day-7 mouse actually is far more advanced relative to a rat, even. It is a little bit further along. But we know that also there are infants that have immune immaturity as well, and that, perhaps because of genetics, may have differences. But in terms of the selection, all of these are, in the end, arbitrary choices based upon a reading of the literature and making the best choice available.

The day-7 time point is one where organogenesis -- the structures of the brain are largely in place. There is still migration that is just ending for certain late-developing structures. Maturation and synaptogenesis are continuing. But maturation and synaptogenesis are continuing well through the second year of life, even up to the third year of life, in humans.

So we feel that this was not a bad guess. But, of course, yes, these animal models can give us information that we can then bring to human birth cohorts and other types of human studies, and test the hypotheses out.

DR. MCCORMICK: Thank you very much.

We will now hear from Dr. Kumanan Wilson, from

the Toronto General Research Institute in Canada, who is invited to discuss his review of the current epidemiologic evidence regarding the association of autistic spectrum disorder and MMR vaccine.

This will be a 20-minute presentation, followed by 10 minutes for questions.

Association of Autistic Spectrum Disorder and MMR Vaccine: A Systematic Review of Current Epidemiological Evidence - Kumanan Wilson

DR. WILSON: Thank you very much.

I have been asked by the committee to review the current epidemiologic evidence surrounding the hypothesized link between the MMR vaccine and autistic spectrum disorders.

This study has been funded by the Canadian Institutes for Health Research and is part of a larger study we are conducting, looking at the overall debate that is occurring over the safety of vaccinations.

At the first stage in our project, we wanted to look at the information that was informing this debate. We first began by examining the safety of the MMR vaccine, as put forth by the hypothesis by Wakefield that it may be linked to autistic spectrum disorders. This emerged out of

the 1998 study, which you are all, probably, familiar with, in which Wakefield found that the MMR vaccine in several children was soon associated with the onset of autistic spectrum disorder, often characterized with developmental regression and bowel symptoms.

This study led to the following hypotheses: That a new variant of autism had developed and that this variant was associated with the MMR vaccination.

Following Wakefield's study, several epidemiologic studies have been conducted to investigate whether this association between the MMR vaccine and autistic spectrum disorders does exist. The objective of our study was to identify and summarize the results of these studies, examining for an association between MMR vaccine and autistic spectrum disorders. We were specifically examining the studies that were addressing the hypotheses put forth by the Wakefield paper. We focused on the issue of the heterogeneity of the questions being examined. These studies are actually examining several different questions of association.

We used standard systematic review techniques to conduct our search, using a variety of search terms that we hoped would be inclusive of the majority of studies that

were conducted on this topic. Our search was conducted up until January of 2003. We also contacted authors and searched the references of reviews to see if there were further studies that we may have missed.

Our broad inclusion criterion for studies was any epidemiologic study examining the association of MMR vaccine and autistic spectrum disorders. In total, we reviewed 379 abstracts and identified 12 relevant studies.

As I mentioned before, these studies were actually examining several different questions. I think it is important to look at it from that perspective. The hypotheses that the studies addressed were the following: Are rates of autistic spectrum disorder higher in vaccinated children compared to nonvaccinated children? Is there an increase in autistic spectrum disorder as a consequence of an increase in the MMR vaccine? Is the development of autistic spectrum disorder temporally associated with receiving the MMR vaccine? Most specific to the Wakefield paper, is there a new variant form of autism associated with the MMR vaccine?

It is also important to identify some of the questions that we did not attempt to answer from this review: Is there an association of GI symptoms and the MMR

vaccine? We weren't trying to identify studies that were comparing the monovalent vaccine to the trivalent vaccine. We did not attempt to determine an association of thimerosal vaccines and autistic spectrum disorders. We did not try to identify whether there was an existence of a variant form of autism. We also didn't include biological studies or animal studies.

The results of our review: The studies we examined were conducted in five different countries, the U.S., U.K., Finland, Denmark, and Sweden. A total of nine data sources were examined. One data source was examined three times and another was examined twice. In the studies that provided age of vaccination, vaccines were received at ages 13 through 17 months.

This was quite a challenging review to do, because of the variety of study designs employed. This is primarily necessary because, in general, there are very small numbers of unvaccinated children. So the researchers had to come up with innovative methodologies and make use of ecological study designs to help answer these questions.

The study varied in how ASD was diagnosed, although most relied on the records of services for autistic children or children with disabilities, or from

ICD codes.

I was asked, in particular, to focus on the evidence that has emerged since the last Institute of Medicine review. We identified four studies that were not included in the previous review.

The first was a study by DeWilde, which actually may have been available at the time of the previous review, but not considered to be of sufficient quality. This was a case-control study conducted in the U.K. It examined 71 children with autistic spectrum disorder and had four-for-one matching to identify 284 matched controls. These are children who didn't have ASD. They identified these children from the U.K. general practice database.

It specifically was looking for evidence of a temporal association between the vaccine and signs of autism. In particular, between the two groups of autistic children and normal controls, they compared the changing number of consultations from six months before the vaccine to six months after the vaccine. They found no significant difference in the changing number of consultations between the autistic patients and the controls, and furthermore, only identified one case of autistic spectrum disorder diagnosed within six months of vaccination.

However, it is important to note in this study that this was any consultation, and not consultations specific for autism. Based on that fairly significant limitation of the study, we would have to state that this was comparatively weak evidence not favoring causation.

The next study, which was conducted by Taylor et al., in 2002, as a follow-up of a previous study they had reported in 1999, employed both time-series methodology and case-series methodology. It was conducted in the United Kingdom. This study was based on a sample of approximately 470 children born from 1979 to mid-1998 with autistic spectrum disorders, in eight health districts. In this sample, approximately 120 children had, along with autism, evidence of late developmental regression, and another 80 children had signs of GI-tract symptoms. This study was specifically looking at the question of whether there was a variant form of autism associated with the vaccine.

The way it sought to answer this question was to determine whether the vaccine was received in these children before the development of parental concern regarding regression or GI-tract symptoms, or after the parents developed these concerns, or in some children, perhaps the child didn't receive the vaccine at all.

The study also conducted a time-series analysis to see if there was an increasing percentage of children with autistic spectrum disorder and either GI-tract symptoms or regression between 1979 and 1998 -- actually, that should be 1988.

When they conducted their analysis, they found that roughly equivalent percentages of children received the MMR vaccine before the parents developed concern about GI-tract symptoms, after they developed concern, or did not receive the vaccine at all. This was similar for developmental regression.

They also found that there was no increase in the percentage of children with autistic spectrum disorder who had either bowel symptoms or who had developmental regression over the time of the analysis.

Overall, of the several studies that we reviewed, this would likely have been the one to provide the strongest evidence not favoring causation with respect to, in particular, the fourth hypothesis of a variant form of autism and the MMR vaccine.

The next study is probably the one with the strongest study design of the 12 studies we looked at. This was a large cohort study conducted in Denmark by

Madsen and colleagues and reported in 2002. This study looked at all children born between 1991 and December 1998 registered in the Danish civil registration system. It obtained vaccination data based on GPs' reports to the National Board of Health. They have a national health surveillance system that was able to identify all kids with hospitalizations for autism or outpatient consultations for autism, based on ICD codes.

This study specifically examined the rates of autistic spectrum disorder or autism in children who were vaccinated compared to those who weren't. It conducted unadjusted analyses and analyses that were adjusted for important confounding variables. In both of these analyses, the relative risk of developing autism or autistic spectrum disorder was less than 1, suggesting that there was no association.

However, it is important to note here -- this signifies the difficulty in ruling out risk -- even in these studies that were fairly well powered, the confidence intervals do cross 1. So we cannot say with 95 percent certainty that there is no association. In particular, the confidence interval in the first was up to 24 percent relative risk increase.

Nevertheless, this was probably the strongest study design of the 12 we examined, and it didn't find evidence of an association.

The next study was a study by Makela and colleagues, which looked at just over a half-million vaccinees who were enrolled in a surveillance study between November 1982 and June 1986 in Finland. Again, the diagnosis of autistic spectrum disorder was based on ICD codes. This study determined if there was a clustering of hospitalizations for autism after the time of MMR vaccine. The study was actually looking at the link between the vaccine and encephalitis, meningitis, and autism. We were just extracting the data on autism for this review.

This study also determined if any recipients of the MMR vaccine hospitalized with autism were also hospitalized with inflammatory bowel disease.

The results of this study: There were 309 children, vaccinees, who were hospitalized for autism after the MMR vaccine. They didn't find any clustering of cases. There wasn't an increased likelihood of being hospitalized in certain time intervals after receiving the vaccine. Similarly, there were no hospital visits for inflammatory bowel disease among the 309 children hospitalized with

autism.

A major limitation of this study is that it was relying purely on hospitalization data, and many children with autism may not have been hospitalized. With respect to the issue of variant autism, it was only specifically looking for evidence of inflammatory bowel disease, as demonstrated by hospitalization records.

So how did these four studies fit into the overall literature, up until the present?

The first hypothesis is, is there an increased rate of autistic spectrum disorder in vaccinated individuals compared to non-vaccinated individuals? Again, there is only one study that was able to examine this question in concurrent vaccination and non-vaccinated individuals. That was the Madsen study, which I described, which did not demonstrate evidence of a statistically significant risk in rates of autism or ASD between the two populations.

The second hypothesis is, are there increasing rates of ASD associated with the introduction of the MMR vaccine? This is a very difficult question to examine. Studies have relied upon ecological analyses in particular, and these are susceptible to several forms of bias. One,

there are secular changes in diagnoses, the introduction of additional confounding variables, as well as erroneously inferring individual cause-and-effect data from aggregate data.

Four studies conducted time-series analyses to examine this question. They specifically were looking at whether there was an association between an increase in MMR coverage and the increasing rates of autism. Each of these studies in its reported analysis did not find an equivalent increase in the MMR coverage to explain what was viewed as an increase in rates of autism.

The other two studies were primarily before-and-after designs, looking at rates of autism prior to and after the MMR vaccine, and didn't find evidence of an increase in the rates of autism.

The third hypothesis was the evidence of a temporal association of autistic spectrum disorder and the MMR vaccine. Again, this emerged from the Wakefield study, that children presented with their symptoms soon after receiving the vaccination. Eight studies examined this question, in total. Three of these studies compared the age at which autistic spectrum disorder was diagnosed or parental concern developed, in individuals who were

vaccinated and those who were not vaccinated. The hypothesis in these studies was that if the MMR vaccine caused autistic spectrum disorder, populations exposed to vaccine should develop autistic spectrum disorder at a different age than populations who weren't exposed. They should cluster around the time of vaccination.

These studies found no differences in the mean age at the time of diagnosis of autistic spectrum disorder, suggesting that whether or not children were vaccinated, they would develop the symptoms of autistic spectrum disorder or create parental concern at approximately the same time.

The only point to note here -- the only analysis that was significant was in the Taylor study. At six months after vaccination, they did find a statistically significant increased risk of parental concern. That was the only time interval of the several that they looked at in which that was identified.

The other studies examining for evidence of a temporal association looked for evidence of clustering of diagnosis of ASD after receiving the MMR vaccine. These studies did not find evidence of clustering of cases of autistic spectrum disorder following the MMR vaccination.

Probably the most important hypothesis to examine based on the Wakefield study is, is there a specific association of a variant form of autism and the MMR vaccine? Even if there is no association between all forms of autism and MMR, a specific association may exist which could be washed out by these aggregate analyses. Again, in these studies, the variant autism is identified by the presence of developmental regression or GI symptoms.

Four studies examined this question. In one study, none of 31 children who were identified in a vaccine adverse-event reporting-system database in Finland who developed GI symptoms subsequently went on to develop autistic spectrum disorder, based on up to 14 years of follow-up. This study is obviously limited by the small sample size and potential for reporting bias in the database.

The other study, by Fombonne and colleagues, found no difference in rates of developmental regression in children with autistic spectrum disorder, sampled children after the introduction of the MMR vaccine, when compared to a historical sample prior to the introduction of the vaccine. The study also did not find a higher-than-expected prevalence of childhood disintegrative disorder in

the post-MMR sample.

This again provides evidence against an association. However, it is important to note that the historical sample began in 1954, and there was a large time gap between the pre-MMR sample and the post-MMR sample. In addition, the sampling was primarily convenience sampling, based on the authors' own clinical cases.

I have already reported the results of the Taylor study, which likely was the strongest study, as far as study design goes, examining this specific question. It didn't find an increase in the percentage of autistic children with GI symptoms or regression after the introduction of the MMR vaccine to that population.

I have also reported the results of the Makela study, which found no evidence of children who were hospitalized with autism after the MMR vaccine developing inflammatory bowel disease. But again, this study was limited by the fact that it was only looking at hospitalizations, and only specifically looking at the association of autism with inflammatory bowel disease.

So the overall conclusions: There is no evidence of an association of autistic spectrum disorder and MMR from 12 epidemiologic studies. Based on the time-series

data, it would be difficult to argue that all of the causes of autism that are being seen are due to the MMR vaccine. Furthermore, it appears that in the study that looked at the specific questions, vaccinated and non-vaccinated children were developing autistic spectrum disorder at approximately the same age, and there was no clustering of cases of autistic spectrum disorder after vaccination.

There is also no evidence of a variant form of autism related to the MMR vaccine. However, I think it is important to note the limitations of current study designs. Only one study was really of sufficient quality to examine this question.

I will just sum up by saying that future studies that examine this question should focus on examining the risk of obtaining variant autism from the MMR vaccine. As the number of non-vaccinated children is potentially increasing, prospective cohort studies may be able to be conducted, as well as case-control studies.

Thank you.

DR. MCCORMICK: Thank you.

Just to be certain, the only funding you have received for your research is from the research council?

DR. WILSON: That is right.

DR. MCCORMICK: Dr. Wilson's paper is open for questions.

DR. BERG: Al Berg, with the committee.

Speaking as an epidemiologist, could you clarify your comment about not being able to rule out the null hypothesis? These studies, a couple of them, had extremely large numbers. Have you done any modeling to figure out how large a population it would take to have a 95 percent confidence in saying the association is no bigger than -- fill in the blank?

DR. WILSON: The challenge in answering that question is that the only way you could say with 95 percent certainty that an association didn't exist is if you found a statistically significant association in the opposite direction, if you found in these analyses that the MMR vaccine protected against autism, which then would make people wonder about the potential for confounding or systematic error in those analyses.

Based on epidemiologic studies, it is very hard to definitively rule out evidence of risk.

The other limitation, even in that Madsen study, is that, while the sample size was very large, the number of unvaccinated children was reasonably small compared to

the number of vaccinated children, which is also limiting these analyses.

I am surprised we didn't see any case-control studies. They are usually the types of studies that are used to examine the specific question. But I understand a few are under way.

DR. MCCORMICK: Any further questions from the committee?

(No response)

DR. MCCORMICK: From the audience?

DR. DETH: Dr. Richard Deth, from Northeastern University in Boston.

The MMR is typically given after other vaccines which might or might not contain thimerosal. Therefore, the possibility of an association that is not causative -- that is, the MMR not causing the autistic spectrum disorders -- but the possibility that an MMR, especially a measles infection, might be associated with a vulnerability induced by, let's say, the prior vaccinations in which the thimerosal content might have weakened the resistance to, let's say, measles infection, is a separate hypothesis, not something that was tested, I gather, in any of the studies that you examined.

Furthermore, the possibility that individuals who are susceptible to autism might have poor methylation capacity is a theme that has been, I guess, presented already today and may be developed further. My understanding is that methylation is an important mechanism for suppressing foreign DNA and for suppressing infection, and therefore leads to the possibility that there is an association, but not one in which MMR causes autism, but that the autistic population might be simultaneously vulnerable to, in particular, a measles virus infection as a result of a biochemical defect.

I guess the question is, is it possible to test whether or not, in equivalent populations that have, let's say, a vaccine history, with thimerosal or not, the liability of developing measles infection is equal?

DR. WILSON: We didn't look for that in these studies. I can only base the analysis on the studies we examined. However, my sense is, given that the parental concern in autistic children develops around the time the vaccine is given, and if there are these potential interactions being hypothesized, the only way to definitively answer the question would be to do trials in which the vaccine is potentially delayed, so that it is not

coinciding with these other vaccines. Again, that introduces concerns about being in uncovered periods.

MR. ANIATY(?): My name is Albert Aniaty. I am a father of a child with autism. I am also a member of Safe Minds.

My question is, as you heard this morning from Congressman Weldon, the majority of these studies, except Wakefield, either had a conflict of interest with the CDC or vaccine manufacturers. When you were reviewing these articles, did you look at the author to see if he had any conflict of interest with CDC or vaccine manufacturers, especially the Denmark study? If they did, did you ask them to give you the raw data, how they arrived at the statistical analysis? If you have not done that, would you be able to go back and ask them if they had any conflict of interest or not?

That is a really major problem -- me, as a father of a child with autism, and the concern that Congressman Weldon had this morning.

DR. WILSON: I appreciate those concerns. There is no way in our methodology that we could rule out any systematic error in publication or non-publication of these data. We weren't able to elucidate clearly if there was

any conflict of interest or not. All I can report are the results of the studies that we did identify from the literature.

MR. ANIATY: (Not at microphone)

DR. WILSON: As part of this overall study, we are hoping to do interviews with all of the opinion leaders, both those supporting vaccinations and those concerned about them. We would hope to be able to gain further information from those interviews.

MS. BIRD(?): My name is Elizabeth Bird. I am with Safe Minds and Medical Interventions for Autism. I have a child who has documented bowel disease that has gotten worse since he was scoped at the Royal Free.

I have a question about the Taylor study. Was this based on clinical records that were reviewed, as far as the GI symptoms present in the autistic children?

DR. WILSON: I have it here. It was based on computerized special-needs/disability registered at child-development centers and records in special schools and child psychiatric records. These were checked by pediatric registrars using ICD-10 classification codes.

MS. BIRD: Did they look at the medical histories of the children in the clinic notes?

DR. WILSON: It suggested they did and compared --

MS. BIRD: And if they did, there is a basic flaw in that, because many children's GI symptoms are not documented in their medical records. Sometimes those GI symptoms, it takes years to get care for. I personally know, from my experience, for the time that I got care for my son, it took four years. There were no notes in his pediatric chart about his GI issues. But now he has severe inflammatory bowel disease. So I think a lot of that information is missing from those records.

DR. MCCORMICK: Thank you.

We will move on to our next speaker. We will now hear from Dr. Frank DeStefano, from the Centers for Disease Control and Prevention, who is invited to discuss a recently published study examining the age at first MMR vaccination in children with autism and in a control population.

This will be a 20-minute presentation, followed by 10 minutes for questions.

Age at First Measles-Mumps-Rubella Vaccination in Children with Autism and School-Matched Control Subjects - Frank DeStefano

DR. STEFANO: Thank you. I am from the National Immunization Program, the Centers for Disease Control and Prevention, which provided all the support for this study.

I would like to acknowledge my co-authors on this study: Bill Thompson, Tanya Karapurkar, Marshalyn Yeargin-Allsopp, and Coleen Boyle.

You have heard much of the background on this issue, so I think I don't need to go into that -- in particular, the critical role played by the case series reported by Wakefield in 1998. When this committee reviewed the evidence in 2001, the conclusion was that the evidence favors rejection of the causal association between MMR vaccine and autistic spectrum disorders at the population level. But the committee also recommended additional studies to examine possible associations between the vaccine and ASD subgroups.

When we started this study -- actually, it was going on at the time that your first report came out -- our primary objective was to evaluate the association between ASD and age of receipt of first MMR vaccine. The secondary objective we emphasized subsequent to the release of your report. We tried to compare MMR vaccination histories among ASD subgroups and matched controls. Much of our data

collection had been completed by that time, so the subgroups that we could evaluate were restricted by the information that we had abstracted.

The study was conducted within the Metropolitan Atlanta Developmental Disabilities Surveillance Program, or MADDSP. It is a population-based surveillance program, which began in 1991. It covers an area that includes about 300,000 children 3 to 10 years of age, in the five-county metropolitan Atlanta area.

Initially, the surveillance system was for specific developmental disabilities, which included mental retardation, cerebral palsy, hearing loss, and visual impairment. ASD was added to the list of conditions in 1996.

Our study used a case-control design and included 624 children with ASD and 1824 children from regular-education school programs.

First of all, I will describe the cases. These 624 children were born between 1986 and 1993. Again, they were identified through the MADDSP system in the 1996 surveillance year. School and clinical and other source records were abstracted by trained abstractors using standardized forms and methods. ASD classifications were

determined by clinical psychologists, developmental pediatricians with expertise in diagnosis and classification of ASD. They used the DSM-IV criteria to classify the case children.

Some of the inclusion criteria -- this applies also to the controls, which I will be mentioning later -- these were children who were in school. Georgia has vaccination requirements to attend school. So most of the children were vaccinated. Be sure that we had good records on these children. We required that there had been at least a valid MMR vaccination date from a school immunization or at least one DTP vaccination recorded on the form by age 2, or else an immunization-exemption form.

As I mentioned, we tried to look at overall ASD, as well as different subgroups. Some of the specific clinical subgroups that we were able to look at I will describe now. One subgroup would be children who had preexisting conditions under 1 year of age. That would include children who had any known birth defect, other co-occurring developmental disabilities, such as cerebral palsy, or children who had a major perinatal or postnatal insult that could have contributed to developmental delays, such as central-nervous-system infections or traumatic

brain injuries.

We also looked for evidence of developmental delay before 1 year of age, including evidence that speech was not appropriately developing or that the child exhibited some signs of social unresponsiveness, under 1 year of age.

We also tried to look at the critical question here of regression. We included the concept of plateau in with regression. Regression would be children with an indication of loss of age-appropriate developmental skills. What we called plateau would be children who were developing appropriate skills, who then did not lose those skills, but did not progress either, as would be expected. In the analysis, the plateau was combined with the regression.

Here are the clinical characteristics. This shows some of these features among our case group. As you see, 61 percent had mental retardation, 38 percent had evidence of a preexisting condition, and 80 of the cases, or 13 percent, had regression or plateau. These groups are not mutually exclusive.

As for the controls, these were identified from regular-education public-school programs in the MADDSP

surveillance counties. We selected a 3-to-1 ratio, controls to cases. They were matched based on age within 1 year, sex, and school of attendance at the time of abstraction. If the case child was attending a special-education school, the control would have been selected from the local home school that the child would have attended. Then we had the same vaccination-form requirements as for the cases.

For a subsample of the cases and the controls, we were able to obtain additional information on potentially confounding factors by matching with a Georgia state birth certificate sample. The information we were able to get from the birth certificate included maternal age, maternal education, child birth weight, multiplicity, and parity, as well as others. These are some of the factors that were important or had some association with case status.

In addition to the clinical subgroups, we also tried to look at different groups within the population, by demographic or socioeconomic factors or birth characteristics, to see if there were groups of children who may have had different susceptibilities to the vaccine. We were able to look at factors stratified by age or gender, and also, for those individuals where we were able

to match the birth certificates, we also looked at whether there were different associations according to race, birth weight, maternal age, or maternal education.

The specific hypotheses that we evaluated: First of all, we assessed whether variation at age of first MMR vaccination was different for cases and controls. Then we went on to look at those distributions at three different age cut points, the first one being at 18 months of age. We looked at this because it would have been the age according to the recommended vaccination schedule, which calls for first MMR vaccination at 12 to 15 months. So by 18 months of age, this would have represented children vaccinated at or shortly after the recommended schedule.

The 24-month age cutoff was to look at the age when, according to other studies, most children with autism or with regression would have manifested symptoms or parental concern. The 36-month cutoff was a definitional cutoff. DSM-IV criteria require that autistic symptoms have been manifested by this age, to receive an autistic-disorder diagnosis.

The analytic methods: We used conditional logistic regression analysis, stratified by the matched case-control sets. The analyses were a bit different for

the total sample and the birth-certificate sample. In the total sample, we were not able to adjust for other factors, although they were stratified, and thus controlled for age and sex and, actually, local school. But we did not require a Georgia state birth certificate for these. In the Georgia state birth-certificate sample, we did unadjusted analyses, similar to the total sample, and then we also did adjustments for factors that we were able to obtain from the birth certificate and were related to case status. Actually, the unadjusted and the adjusted analyses were fairly similar, so we won't be presenting the unadjusted results for the birth-certificate sample, just the adjusted analyses.

As for the descriptive data, in terms of the age distribution, about a third were, in both cases and controls, since they were matched, about 3 to 5 years old; roughly two-thirds, 6 to 10 years of age. As is known, autism affects, predominantly, boys. Eighty percent of the cases were boys. Since there was matching on sex, 80 percent of controls were also boys. Maternal age of the cases tended to be a bit older than the controls. Case mothers also tended to have higher levels of education than control mothers. Birth-weight distribution was not

terribly different, although there was a slight increase of low-birth-weight infants among the cases and a little bit of an increase in twin or higher births among the cases.

The main results of the associations with vaccination: This graph shows the distribution of age of first MMR vaccination between the cases, in green, and the controls, in purple. As you can see, the ages when the cases and controls were first vaccinated were fairly similar. When you compare these two distributions, there is no statistically significant difference. About 60 or 70 percent here were vaccinated between 12 and 17 months.

I just draw your attention to this tail here, the 36+-month cutoff here. About 9 percent of controls and about 7 percent of cases were vaccinated after 36 months. Any differences that we did find when we looked at different age cutoff analyses were at this tail end.

This is the main result of the logistic regression analyses for all cases, for the total sample. When we looked at the 18-month cutoff or the 24-month cutoff, there was no association. With vaccination under 36 months, the odds ratio was 1.49, and the confidence interval did not overlap 1.

Results in boys were fairly similar in both the

18- and 24-, and even the 36-month. The girls, again no association at 18 or 24 months. Again, a lower risk here. The much smaller number of girl cases made it difficult to say whether these are actually different results. In formal tests of interaction, this was not a statistically significant difference.

Looking at differences in age, again in the 3-to-5-year-old cases, no association with the 18-month or 24-month cutoff -- at least not a significant association. The association with the 36-month -- the odds ratio is 2.34 and just barely overlaps 1. For 6 to 10 years of age, there is no association at any of the age cutoffs.

We performed similar analyses to the cases that could be matched to the Georgia state birth certificates. The number of cases was half of what we had before. In all groups, there is really no association with the 18-month or 24-month cutoff. The 36-month cutoff was fairly similar to what we had seen for the total sample, although, with a smaller sample size, all the confidence intervals were wider and overlapped 1.

Looking at some of the clinical subgroups that we were able to analyze, these would be children who had no preexisting conditions or evidence of delay before 1 year

of age. These would be children who you might say were at risk for developing autism or developmental delays after MMR vaccination. At the 18-month and 24-month cutoffs, there is no association. In this case, the 36-month cutoff, there was this odds ratio, and it had a confidence interval that overlapped 1.

When we looked at regression, 80 cases of regression, no association with the 18-month or 24-month, or even, in this case, the 36-month cutoff. Among those cases with mental retardation, no association with any of the cutoffs. Without mental retardation, no association with the 18- or 24-month cutoff, but here we do have a 2.45 odds ratio with the 36-month cutoff, with a lower bound of the confidence interval that excludes 1.

Similar analyses restricted to the birth-certificate sample are not much different than what we saw before, except that the confidence intervals are wider because of the smaller sample size. This cell here, the 3.55, takes really wide confidence intervals. There were only three exposed cases in this cell.

We tried to look at associations within different demographic subgroups or subgroups determined by birth or maternal characteristics. We didn't find any statistically

significant associations for any subgroups in this table. The 18-month cutoff, the odds ratios are all under 1; the 24-month cutoff, all the odds ratios are under 1.

This may be noteworthy here. The 36-month cutoff, there was an odds ratio over 2 for children whose mothers were 35 years of age or older. But again, this was not significant. There is a fairly wide confidence interval around this. Also, there is a tendency for mothers who have had 16 or more years of education to have a higher odds ratio. But again, this was not significant, with a fairly wide confidence interval.

A summary of the findings: First, the variation in age of first MMR vaccination between children with autism and matched controls was similar. There were no significant associations found between vaccination at under 18 or under 24 months and risk for autism or for any autism subgroups, including regression.

Cases were more likely than controls to be vaccinated before 36 months of age. Again, that was that tail of 93.4 percent versus 90.6 percent. I list here the profile of the factors that were associated with an elevated and/or significant odds ratio at that 36-month cutoff. These tended to be children aged 3 to 5 years,

boys, children without mental retardation, older moms, or better educated moms.

The 36-month cutoff reflects, we think -- one of the factors that has to do with it is that in 1991 the Individuals with Disabilities Education Act mandated the provision of special-education programs for children with autism beginning at 36 months. Georgia required the MMR vaccine for attendance in these school-based special-education programs. In our sample, 98 percent of the ASD children aged 3 to 5 years were enrolled in preschool education programs.

In summary, there were similar patterns of age at first MMR vaccination among cases and controls. We found similar proportions of cases and controls vaccinated according to the ACIP schedule, that is, under 18 months. We find a similar proportion of cases and controls vaccinated by typical age of onset for autism, that is, under 24 months. Children with autism were more likely to be vaccinated before 36 months of age, compared to matched controls.

Thank you.

DR. MCCORMICK: Do we have any questions from the committee?

DR. FOXMAN: I just had a number of questions about your main results. We just got your paper this morning, and I didn't have a chance to really look at it closely. I am assuming that in all these comparisons they were all compared to the 36+-month as the referent group. Is that correct? So when you say less than 36-month cutoff, everything is compared to children that were -- the vaccination status of those were 36 months and older. Is that correct?

DR. DESTEFANO: Yes.

DR. FOXMAN: Actually, in that group, as you noted at the beginning, there was a higher rate of -- the controls were more likely to be vaccinated later.

So I am confused as to why, when you did the analysis, you did these cutoffs instead of looking at the less than 18 months, then between 18 and less than 24 months, and then 24 to less than 36 months, because of how it collapses across categories, and it doesn't tell us any biological differences across those age groups in terms of the potential impact. Can you help me?

DR. DESTEFANO: Our reasoning for the various cutoffs -- let me just reiterate what I said. The 18-month cutoff was primarily to look at associations with

vaccination according to the recommended schedule. Secondarily, that would be the median time when regression is usually noted.

The 24-month cutoff is sort of the outer limits of when autistic symptomatology or regression or parental concern has been reported in other studies. We would have expected any etiological associations to have been found with either the 18-month or the 24-month cutoff.

The 36-month cutoff was definitional, because the DSM-IV criteria impose that 36-month cutoff.

DR. FOXMAN: So I don't have the reference group right, then. In fact, if you use the 18-month cutoff, you are comparing the less than 18 months to the 18-month and greater.

DR. DESTEFANO: Right.

DR. FOXMAN: So you have a roving reference group for each of these.

DR. DESTEFANO: Yes. I guess I misunderstood. I thought we were just talking about the 36-month group.

DR. FOXMAN: So these odds ratios really can't be compared across, because the reference group is different for each of these.

DR. DESTEFANO: Right.

DR. FOXMAN: Did you do any comparisons where you looked for a test for trend across these different age groups, in terms that there might be -- so you considered each of these separately?

DR. DESTEFANO: No, we didn't do that. I am not sure I understand your question, but we didn't.

DR. FOXMAN: What I was trying to get at was if you looked at the effect, looking at zero to 11, 12 to 17, 18 to 23, all compared to one reference group, and if you saw any trends and differences that would give us an idea of a biological effect?

DR. DESTEFANO: No, we didn't do that. Our first analysis, when we compared that whole figure -- those five categories were actually put into a conditional logistic regression. We did a likelihood ratio test just to see if the age-of-vaccination variable was a significant predictor. It was not.

DR. FOXMAN: One last question. If you want to speculate as to -- since you found this association with older mothers and more educated mothers, if that might have some influence -- what your interpretation is relative to vaccination, and use of services, how those might go together. Any speculations in that regard?

DR. DESTEFANO: Not right now.

DR. CASEY: The group that is this tail that you were talking about, greater than 36 months, those children are really, by most of our definitions, delayed in their immunization, cases and controls. We are trying to think of reasons why that may have happened, whether they had less access to care, as a total group, cases and controls. Am I hearing you say that one thought is that the group of children with autism basically was immunized as a requirement for -- okay, so that is what you are thinking.

DR. DESTEFANO: Yes. There may be other sort of design effects. If you read the papers, there was some effect from being born out of state or not having a birth certificate that seemed to contribute to it.

DR. CASEY: Then, as Betsy was just talking about, better educated or older moms may, in fact, be bringing their children to the attention of the medical-care and educational system. Is that what else is probably going on?

DR. DESTEFANO: That is a possibility.

DR. MCCORMICK: I just have a quick one. When you were talking about developmental delay, you mentioned only social and verbal skills. Did you also consider gross

motor delay as part of that spectrum?

DR. DESTEFANO: I would have to ask our developmental pediatrician who did those reviews. I will be glad to check.

DR. GOODMAN: Could you elaborate a little bit, again, on the exact way you established the diagnosis? Was it from a developmental pediatrician who was examining the records?

DR. DESTEFANO: Yes, reviewing records. This was all records-based. A lot of the records came from the special-education assessments that were done in the schools.

DR. GOODMAN: And who was doing those assessments? Was that a structured assessment?

DR. DESTEFANO: We abstracted any and all assessments that we could find on a child, and then trained abstractors went through, using as standardized as possible a format, and abstracted all relevant information from those abstractions. Then, from these abstracted forms, either developmental pediatricians or psychologists reviewed the abstracted forms for the specific DSM-IV criteria.

DR. GOODMAN: Did you do any sort of validation

subsample, where anybody went in and looked to see if these diagnoses were --

DR. DESTEFANO: That is being done in MADDSP, or at least is being planned. As of this study, it hasn't been done.

MR. ZEHORICK(?): Peter Zehorick, with Autism Canada.org.

Can you comment on Congressman Weldon's observations about the conflicts of interest and growing relationship between CDC and pharmaceutical companies? Specifically, can you address that, and not being made available to independent researchers, along with the notion that any data presented by the CDC is inherently biased because they serve as a promoter of vaccinations and as their own watchdog group?

DR. DESTEFANO: I can just tell you my own case. I have 25 years of epidemiologic research experience. We approach these studies in as objective and scientific a manner as possible. We have had no affiliation, no support. I personally get no funding, and none of my co-authors have any funding, from the pharmaceutical industry.

In terms of access to data, the data for this study, we are making a public-access database available.

DR. MCCORMICK: And I would note that this study is under a different auspice than the immunization group. This was done under a different group at CDC.

We will now move on to the break. We will come back here at 11:10.

(A brief recess was taken.)

DR. MCCORMICK: Our next speaker is Dr. Elizabeth Miller, who is head of the Immunization Division at the Communicable Disease Surveillance Center in London. Dr. Elizabeth was invited to discuss her study of exposure to thimerosal-containing vaccines in the U.K. and autism. We have allotted 20 minutes for Dr. Miller's presentation and 10 minutes for questions.

Exposure to Thimerosal-Containing Vaccines in U.K. Children and Autism - Elizabeth Miller

DR. MILLER: I would like to thank the committee for inviting me here to give this presentation.

The two studies that I will present have been funded, respectively, by the World Health Organization and by the U.K. Department of Health.

I will take a little bit of exception to Congressman Weldon's assumption that if you are doing a study that is funded by government, you are there to prove

that there isn't an association. As an epidemiologist, very much like Dr. DeStefano, I am there to test hypotheses and to form opinions based on the evidence, not to confirm a preexisting opinion. I would be grateful if the audience would bear that in mind when they hear this talk. I certainly have done studies that have resulted in the global withdrawal of certain strains of MMR vaccine.

My department does, on occasion, do collaborative work which has commercial sponsorship, and we have a very clear policy about the circumstances under which such commercial sponsorship is obtained. I would draw your attention to this Web page, where the policy that we follow is made absolutely explicit. A key element of that is that no personal funds are obtained from any such sources.

I am currently an expert witness in the MMR litigation that is going on in the U.K. I have done this without accepting any funding, either for myself or for my division.

That said, let's go on to the science.

The background to the U.K. being involved in studies of thiomersal and developmental conditions came as a result of the background work that was done in the U.S., where, as you know, there was an exceeding of a stringent

safety limit for ethylmercury based on the number of vaccines given in the U.S. program. The preliminary analysis of the U.S. VSD study suggested an increased risk of certain developmental problems in those exposed to high levels of thiomersal at a younger age. Those levels were within the range of exposures that we have in the U.K.

More importantly, the WHO, through its expanded program on immunization, were concerned that some of the exposures that were coming out positive were consistent with exposures that were given in the EPI program -- hence, the WHO funding this study. Although there is a commitment to move to thiomersal-free vaccines, this is not going to be possible, at least in the short term, for developing countries, where the importance of having antibacterial agents in multidose vials is important to continue.

These are the kinds of data that we saw. I think that the final results that were published from the U.S. study, having controlled for various potential confounders, did not show increased risks for the developmental disorders that were shown in the preliminary analysis. As you can see, levels around here were suggesting increased risks.

If we look at the thiomersal exposure in the

U.K., the only routine vaccines that contain thiomersal -- we call it thiomersal; I think you call it thimerosal, but it is the same product -- are DTP or DT-containing vaccines. We have some in hepatitis B and flu, but these are not routinely given. They are only given to children in high-risk groups. We have excluded them from the analysis, as, inevitably, they will be an atypical subset.

We used to have the vaccine scheduled at 3, 5, and 10 months, but in 1990-91, we changed to an accelerated schedule of 2, 3, 4 months. That is similar to the EPI schedule. As you can see, the maximum exposure to ethylmercury is lower in the U.K. than in the U.S., and certainly lower with the former extended schedule compared to the current accelerated schedule. So the 75 µg of mercury, which is the constituent that thiomersal is broken down to, will have been given by 4 months of age in the U.K. Clearly, the total exposure in the U.S. is greater, but understand that that is for a child who has all the vaccines on time. In the U.K., we do tend to give the vaccines on time.

That was not meant to be a pejorative comment at all. Sorry.

You can see there is a big difference between the

former extended and the current accelerated schedules.

The first thing to look at -- and this is not a strong arm of the analyses -- is the kind of ecological relationship between autism prevalence in the U.K. and use of thiomersal-containing vaccines. I was a co-author of the studies that were done by Professor Taylor et al. These data come from those studies.

This is the picture. Clearly, we do see an increasing prevalence. Our view is that this is largely due to changes in diagnosis and awareness of the condition. That was when MMR was introduced, and that was when we changed to the 2-, 3-, 4-month schedule.

We have some updated data from a paper that was published last year in *Archives of Diseases of Childhood*, where, in five of the eight districts, we have tried to update the prevalence estimates. Those are the actual cases by year of birth. Quite clearly, because of the delay in diagnosis of autism, there is an apparent decline in the most recent cohorts. But you can estimate, based on delay in diagnosis and trends in reducing the age of diagnosis over time, what the expected prevalence is in those birth cohorts. We have seen a leveling out. That is when the change to DTP at 2, 3, 4 months occurred. So you

could say there was an increasing incidence prior to that, as shown on the previous slide.

Ecological analyses are essentially weaker than the type of study that we have done, looking specifically at the individual-level thiomersal exposure. For this, we have used a database called the General Practice Research Database. It is similar to the U.S. VSD data, in that there is the whole experience of a birth cohort that is in the general-practice research practices. We could examine outcomes significant in the preliminary analyses in the VSD study.

About 500 general practices take part, which is about 5.7 percent of the population in the U.K. We don't have a large private practice in the U.K., so this will be a nonselected population accessing the free health care that we have for the entire population. It has data on patient consultations, referrals, and prescribed medicines, including vaccines.

For the selection of the GPRD cohort, we used data available for the period 1988 to 1999. The system was only set up in 1988, so there is not a lot of experience with birth cohorts who would have been exposed to the extended schedule. We looked at children who had been born

into a GPR practice, so that we had their experience from birth, and had been enrolled in a GPRD practice for at least two years.

We also only used a cohort for which the exact date of birth was available. Usually, there isn't an exact date of birth available for GPRD data sets, but by looking at clinical events that could only have been recorded on the day of birth, such as the Apgar score, it was possible to identify an exact date of birth for the majority of the cohort.

There is also a data-quality criterion for release of the data from the practice, that it should be up to standard. That was another selection criterion.

The full cohort was some 107,000 children. We excluded where there were data anomalies, such as dates of vaccine being given before dates of birth. We also excluded various congenital, prenatal, perinatal conditions, which, themselves, may be predictors for the developmental outcomes and may, themselves, be associated with delayed vaccination, or, indeed, early vaccination. These are potentially true confounding factors.

We also excluded some of the postnatal conditions that were, again, likely to be predictors of outcome, and

outcome events, developmental-delay events, recorded in the first six months of life -- again, to avoid the confounding of clinical event with timing of vaccination.

This left a cohort of some 103,000 children, of whom 2471 were preterm. We identified the preterm cohort, again, because there were different results in the original VSD study between preterm cohorts and term cohorts. I will not present any of the preterm data here, as none of those children had a diagnosis of autism. But, essentially, the results of the preterm cohort were very similar to those for the term cohort.

We also analyzed the data restricting it to those who had a record of three doses of vaccine by 1 year of age, as well as children who had incomplete records of vaccination. It may be that the dose was not recorded or they may not have actually had the vaccine.

Clearly, there are a number of variables that could cause potential confounding in such an analysis -- year of birth, month of birth, sex, region, GP. There are limited data available on potential confounding variables in the data set, other than the ones that I have mentioned about preexisting clinical conditions. The analysis was only able to adjust for sex and year of birth.

The exposure to mercury was determined by the number of doses of DTP. I say it is DTP or DT-containing vaccines. That includes Hib-DTP combinations, each of which contains 25 µg of mercury. So the exposure variable was defined either as the number of doses given by 3 months, by 4 months, or we had a variable that attempted to encapsulate the exposure to thiomersal-containing vaccines in the first 6 months of life. So it is looking at the number of doses and the age at which they are given.

It is a complex variable, but rather than just looking at cutoff at a particular age, which might divide the data arbitrarily at a point in the distribution, we felt this variable was another way of looking at the data that was not subject to those potential criticisms of cutoffs.

The outcome codes that we looked at were those that had been studied in the original VSD study in the U.S., defined by ICD codes. In order to identify these conditions, the coding on the GPRD is done in something called Read and OXMIS codes. A very detailed set of mapping was done in order to convert these clinical codes, Read and OXMIS, to ICD codes.

As I say, these stars are the ones that in the

original, preliminary analysis of the VSD study had come out as significant, although in the paper subsequently published, after better controlling for confounding, many of these disappeared as significant.

The method of analysis: The Cox proportional hazards survival from 183 days of age to the age of the event, censoring at age of event or last date of follow-up, and the results were reported as a hazard ratio per DTP or DT dose.

This is showing, actually, how compliant the U.K. population is in receiving vaccines according to the schedule. This is by 4 months of age. You can see that about a third of the population do actually receive their third dose by 4 months of age. Relatively few have no doses by that age. So we do have a population which, although overall may not have as high a cumulative exposure as the U.S., do receive their vaccines on time and at an early age.

The outcome data: To get a feeling for the veracity of the data, we looked at the median age at which the various conditions were picked up in the GRPD data set. For this, we restricted it to children who had at least 8 years with a follow-up on the GPRD, because children,

clearly, who have only been followed up for 2 years of age cannot contribute to events which are diagnosed later than 2 years of age.

It looks very much as you would expect in relation to the median age at onset and the percentage male. The autistic cases comprise some 104 in this data set, with a median age at first recorded diagnosis of autism -- clearly, that is not the same as first onset of symptoms or parental concern. It was not possible to extract that from this data set. Other studies have addressed that question. This is purely looking at the final outcome of autism in relation to the timing of exposure to thiomersal-containing vaccines in the first year of life, and specifically in the first 6 months of life.

The Cox regression results: This shows the autism cases, according to these three methods of defining exposure, doses by 3 months, doses by 4 months, or this Hg-all variable, encapsulating the experience in the first 6 months of life. As you can see, there is no suggestion that any of these exposure variables is pointing to an increased risk of an autism outcome.

If you look at it compared with the other

developmental outcomes -- as I say, the purpose of this study was very much the general developmental delay categories that had come up positive -- you can see that in some cases there are apparent protective effects, which almost certainly -- I don't think it is reasonable to have a hypothesis that the vaccine protects against these outcomes, unless, specifically, it is protecting against a disease that itself could cause that outcome. So there probably is some residual confounding.

We have tics coming out as marginally positive in one of the analyses when we exclude children who hadn't received three doses of vaccine by 366 days of age.

These show the reverse Kaplan-Meier plots -- basically, they are survival curves, if you like -- according to number of doses received by 4 months of age. I have chosen this as an illustrative example for autism. Here is the three-dose group, slightly lower than the two-dose group. So there is a suggestion of protection, although, as I say, I am not postulating that that is a real protective effect. But certainly there is no suggestion of an increased risk with increasing number of doses received by 4 months of age.

The pattern for general developmental delay was

very similar to that we saw for autism. There we have the one-dose group, the no-dose group, two-dose, and three-dose.

The autism diagnosis in the GPRD: As I have said, the diagnosis was based on first mention of the ICD code 2990. We didn't validate the autism diagnosis cases in these studies. First of all, that wasn't the primary hypothesis in this study. It had not come out as significant in the VSD study. Also there have been a number of studies using the autism cases in the GPRD data set, specifically in relation to the MMR hypothesis, which have validated these cases and found a high degree of reliability in the records.

We did validate the other diagnoses, or at least subsets of the other diagnoses, and found 80 percent of them could be confirmed, that the child did present with the condition. In a further 7 percent, there was a record of a parental concern, but no independent medical verification that that condition was either existing at the time or continuing. Some 7 percent did have an incorrect coding. In 5 percent, there was no record of the diagnosis found in the GP notes, although it was on the computer.

Importantly for this analysis, all dates of

vaccination were correct, and the dates of the events were correct or close to the date in the GPRD.

So the comments: No evidence that increased exposure to thiomersal at a young age increases the risk of autism from this study. Confounding may be an issue explaining the apparent protective effects for some conditions. Except for tics, there was no evidence of an increased risk. Validation of tics showed them to be minor and transient, and, interestingly, some of them were of the parasitical variety -- t-i-c-k-s.

The conclusion to this study, as accepted when it was presented to the WHO Global Advisory Committee on Vaccine Safety -- remember, it is WHO that has sponsored this study -- was that they were confident that this study did not show any evidence of increased risk, as in the preliminary analyses of the VSD study, and no reason for WHO to accelerate changes in EPI countries to thiomersal-free vaccines, particularly as withdrawal of thiomersal-containing vaccines may have very substantial health risks for those populations.

Just briefly, I want to talk about a parallel study. This was funded by the Department of Health, done by researchers at Bristol University, Professor Jean

Golding and John Heron(?), her statistician, done in parallel. It wasn't looking at autism as an outcome, because the study didn't have sufficient power. But it was able to look at the effect of confounding in much more detail than was possible with the GPRD study.

Avon is a district in the U.K. between Wales and England. In that population, all women resident in the southwest region of England with an expected date of delivery between the 1st of April 1991 and the 31st of December 1992, were enrolled into this prospective study. They were enrolled from the point at which the woman presented for her antenatal booking. There is very detailed information on antenatal exposures, as well as postnatal exposures to various things, including vaccines. The purpose of the study was not a vaccine study, but, clearly, it was possible, opportunistically, to use the data from this study to test hypotheses relating to vaccines.

There were some 13,000 children in the study. As you can see, it wouldn't have been enough to address autism questions.

It is a bit like the proposed study that I think an earlier speaker spoke of, the one in Norway, with

100,000 children. Very ambitious, these studies are. But they are the best way of answering questions where you need information about exposures prior to events.

The information here was on childhood behavior. It was collected by questionnaires throughout the child's development. Information on potential confounders, as it comes from questionnaires given to the mother both during pregnancy and the period that followed, and information on immunization was taken from the child health surveillance database. In the U.K., all children are put on a computer network at birth, which invites the parents to bring the child for vaccination and also records the vaccinations given. That is part of the reason why adherence to the vaccination schedule is so good in the U.K.

There are a large number of outcome variables in this study, some relating to behavior, fine-motor development, speech development, tics, special educational needs. This LEA statement -- it is local education authority-"statemented." If you have a child that is "statemented," that means it has been assessed as having special needs. So the autism cases will be within that category. Professor Golding wasn't able to give me, at the present time, the number of autism cases within that

category for the analysis, however. Various questionnaires were done at these different time periods, and outcomes, sometimes just a yes/no, and sometimes a quantitative variable or cutting off of a particular part of the distribution.

I am not going to go into the results in detail, but the important thing here is that there is a lot of information on potential confounding variables, including things like maternal education, gender, parity, housing tenure, smoking history, ethnicity, breastfeeding, all collected prospectively and completely in this cohort. There were many more, but these were the ones that were associated with vaccination, and therefore potential confounding variables.

The exposure analysis: We agreed to have the same exposure analysis as in the GPRD study. We are only showing the results here for 4 months of age. The other results are similar, including the Hg-all variable.

I will show you, just for an example, the unadjusted and adjusted odds ratio, adjusted for the potential confounders that I showed you on the previous slide. You can see here that I have taken one set of results, at 47 months. This was the questionnaire on

behavior. You can see that there is some apparent protective effect in the unadjusted odds ratios, but when you adjust for the confounders that I showed before, then the shift up towards 1 -- surprisingly little effect, I would say. In some cases, the apparent protective effect disappears, and it shifts towards 1, but there are none that become significantly greater than 1.

So that is just an example of the magnitude of the effect of confounders.

This group were able to look at tics. There was no association between tics, at all, and exposure. In fact, there was a protective effect. Clearly, there is some confounding here, but certainly nothing that is in the same direction as in the GPRD study.

In this group of special needs and LEA-statemented, again there is a protective effect, which is interesting, but no suggestion in the adjusted odds ratios of a significantly increased risk.

My comments here are that the unadjusted odds ratios sometimes show significantly protective effects, and the adjusted odds ratios tend to be slightly higher, although some effects remain protective. The confounding effect is usually very small. Parity was found to have the

largest effect. This suggests that, while the GPRD study could not adjust for many potential confounders, this should not have led to large biases.

Returning to the tics, no evidence of a higher risk for tics in the ALSPAC study.

So the conclusion of the U.K. expert group who looked at these data, as indeed the WHO group who looked at both studies in detail, was that, overall, there is no evidence of an increased risk of developmental problems, including autism, from thiomersal exposure in vaccines given in the U.K. in infancy.

Thank you.

DR. BAYER: Given your findings, do you think there is any grounds for WHO to be moving, even eventually, towards thimerosal-free vaccines? Do you think that the move away from thimerosal-bearing vaccines in developed countries has any basis in science?

DR. MILLER: That is quite a direct question, isn't it?

Personally, I don't. This question arose because of the situation in the U.S., when one particular criterion, safety level, was exceeded. There are questions about methylmercury and ethylmercury and so forth. It came

from that. It certainly didn't exceed the WHO. I think, had the U.S. not gone down this road, it would have been a non-question.

There has, however, been a stance taken by regulatory agencies in Europe and other developed countries that, in principle, we would move towards thiomersal-containing[sic] vaccines. Here WHO has a problem, in that, while there is no evidence at all of any damage from these products, different standards of care cannot be seen to be acceptable for developed countries and developing countries. So I think we have a political dilemma, but not a scientific dilemma.

DR. GOODMAN: As I understand it -- and correct me if I am wrong -- you have shown that there is no dose effect of thimerosal. That is, if one posited that the risk is incurred by the very lowest levels of exposure, you really haven't ruled that out. Is that right?

DR. MILLER: Ours was not a hypothesis-generating study. It was a hypothesis-testing study, based specifically on the sort of analyses, outcomes and exposure, that were done in the VSD study.

SPEAKER: In your study, you have only chosen three vaccines that you give out, but you have excluded

many other vaccines, such as hepatitis B and so on. You simply said you excluded them because they are given to high-risk -- therefore, the concentration comparison is actually inaccurate in your investigation. That would be my understanding.

DR. MILLER: Very, very few children receive hepatitis B vaccines or flu vaccines. We are talking about --

SPEAKER: (Not at microphone)

DR. MILLER: Less than 1 percent. In fact, none of the children with any of the outcome conditions in our study had received flu or hepatitis B vaccine.

What is your question? There are more children that receive hepatitis B or flu vaccine?

SPEAKER: (Not at microphone)

DR. MILLER: The concentration measurements. There was only one source. With the exception of children -- the less than 1 percent -- who receive flu or hepatitis B vaccine, the only source of exposure to thiomersal-containing vaccines in U.K. children in the first year of life is DTP- or DT-containing vaccines. So there were no groups who were excluded who had thiomersal exposure, other than this very atypical and very small

subset of children who, by and large, have risk factors for other conditions -- as I say, less than 1 percent. None of the children with any of the outcome conditions had received those vaccines.

SPEAKER: (Not at microphone)

DR. MCCORMICK: Sir, there are other people requesting --

DR. MILLER: We don't have a routine hepatitis B immunization program in the U.K. It is not given routinely. It is only given to children whose mothers are hepatitis B carriers, and even then, it is very poorly implemented. Those are the facts. I am sorry that you don’t accept them, but those are the data.

DR. MCCORMICK: Mike?

DR. KABACK: In the Thames study and the inverted survival curves that you showed, if you compared the zero exposure with the three-dose exposure group, it looked like that comparison would be a significant difference in terms of developmental disabilities as a group. Was that analysis conducted?

DR. MILLER: It is looked at as the variable of how many doses received by 4 months of age or 3 months of age, not specifically naught doses.

DR. KABACK: There was a zero curve in there. The zero curve looked like it was significantly different than the full-dose regimen.

DR. MILLER: This slide here.

DR. KABACK: Yes. Between the zero-dose and the three-dose, if the analysis is done between those two groups, although the groups may be very small --

DR. MILLER: They are very small. They are very small. This is based on a very small percentage of children. That is increased risk rather than decreased risk.

DR. KABACK: That is exactly what I am asking. Is that a significant difference?

DR. MILLER: We didn't test specifically. We try not to do statistical tests after the event, when you see things that look as though they are different. The Cox proportional hazards model said that there is no significant dose-related effect across there.

DR. MCCORMICK: I think we are going to have to move on, because we are so far behind schedule.

Our next speaker will be Dr. Robert Davis, from the University of Washington Group Health Cooperative. Dr. Davis was invited to provide an update on the Vaccine

Safety Datalink study. Dr. Davis has been allotted 20 minutes for his presentation and 10 minutes for questions.

Vaccine Safety Datalink Study: Autism Outcome - Robert Davis

DR. DAVIS: Thank you very much.

I have a fairly full agenda today, over 20 minutes. I am just going to be reviewing the Verstraeten findings, talk about some questions about the effects of various criteria upon our findings, sub-analyses that we performed, and a chronology of our findings, a discussion of some other concerns, and then update the committee on the planning stages for the current study.

I am a professor of epidemiology and pediatrics at the University of Washington, and I am a scientific investigator at Group Health Cooperative, which is an HMO in Seattle. My current funding comes from AHRQ, for the Centers for Education and Research on Therapeutics, from the CDC for the Vaccine Safety Datalink project and the clinical immunization safety assessment project, and I do teaching as well. My past funding has come from the NIH, AHRQ, and the CDC. I have done some funded work for Merck, GlaxoSmithKline, Wyeth, Pfizer, Amgen, and I have done some work for the Packard and Culpepper Foundations.

I think it is appropriate at this point to say that all the work that I have ever done, both for federal and for pharmaceutical companies, comes with very strict criteria, in the sense that I have absolute freedom to publish whatever results I find. In fact, some of my published work led to the removal of the rotavirus vaccine from the U.S. vaccine-administration schedule. I also published some work that Merck was very disappointed in. Be that as it may, I have complete independence. I am an independent investigator.

The VSD is a partnership between the CDC and seven health-maintenance organizations in the United States. The large, linked database includes vaccination, clinic, hospital discharge, and demographic data. It was initiated in 1991, and covers between 2 and 4 percent of the U.S. population, depending on the specific age groups that you evaluate.

We specifically looked at the ethylmercury content of vaccines in the VSD study population. Particularly, as has been reviewed in depth previously today, we looked at DTP, Hib vaccine, hepatitis B vaccine. Polio, MMR, varicella, and pneumococcal vaccines contributed no thimerosal exposure to the children in our

study.

As you can see, at various ages of exposure, there were various opportunities for different levels of exposure. People could get 12.5 µg of total mercury exposure in the first month of life, and at 6 to 7 months of life, could get between 25 and 62.5 µg of mercury, leading to variations in the cumulative mercury dose at the end of the period.

As you can see, specifically in HMO A, in the study that I will be presenting today, there were a substantial and appreciable number of children who exceeded the EPA mercury exposure limit. Hence, our interest in actually doing the study.

We performed a two-phase study. Phase I was to screen a range of neurodevelopmental and renal disorders for assessment for association with thimerosal; in phase II, to reassess whatever associations we encountered in phase I in an independent data source.

Our study methods were a retrospective cohort study of automated vaccine and outcomes data. We used the exact exposure of mercury from thimerosal-containing childhood vaccines at different ages, since we actually had the information on the manufacturer of the vaccine that the

child received.

We looked at a range of plausible neurologic and renal disorders, including autism, that could possibly be associated with thimerosal exposure.

Our study population included children born between 1992 and 1998, who were born into one of two HMOs of the VSD. They had to be continuously enrolled through the first year of life, so we could make sure that we had complete thimerosal-exposure data on them. We wanted to make sure that they were actually users of the health-care system, as some parents have dual coverage. So we insisted that subjects receive at least two polio vaccines by the first year of life. We followed all children until December 2000 or the onset of disease, whichever came earlier.

We excluded -- although subsequently did a sub-analysis -- children with low birth weight, and we excluded children with congenital or severe perinatal disorders or mothers with serious medical problems with pregnancy, as we felt that the possibility was that they could act as confounders for our study results.

Our exposure assessment was the cumulative mercury exposure calculated from individual automated

vaccine records, which included the type, manufacturer, and lot number. We looked at total ethylmercury exposure modeled both as a continuous and as a categorical variable, measured on a metric of risk per 12.5 µg. We assessed their exposure periods at 1, 3, and 7 months of life.

We used a proportional hazards Cox survival model, and modeled it separately for each HMO. We stratified on gender, year and month of birth at one HMO, and gender, year and month of birth, and clinic at the other HMO, since we found that clinic acted, actually, as a confounder in our data. We adjusted for health care-seeking behavior. For our final model, we restricted the comparison groups to children with at least one visit to the clinic or emergency department at the time of the autism diagnosis.

Person-time began with the first birthday at HMO A and with the first birthday or January 1st, 1995, whichever came later, at HMO B, since that is when the outpatient data began to be collected at that HMO. Person-time was censored on the diagnosis date or last date of follow-up. Temporary disenrollment was allowed, but person-time and diagnoses were only used while the child was enrolled.

In addition, we did a medical-record validation, where we reviewed 120 medical records of children with an ICD-9 code indicative of autism and found that 81 to 92 percent showed that the diagnosis had been made by either a clinician or a behavioral specialist who took care of children with autism. There were no individual examinations, however.

Results: We had a total of 223 children with autism, 21 in one HMO and 202 at the larger HMO. The mean age at diagnosis was 44 to 49 months of age, and 80 to 90 percent were male.

I apologize for the small print on this slide. I just thought it would be informative to go through how the cohorts were, in fact, formed.

At the very top, we started with children, the entire birth cohort. Then 150,000 were left after we excluded children who were not continuously enrolled in the first year of life; 143,000 after excluding low-birth-weight children; 114,000 after excluding children with severe perinatal or congenital disorders; and then 110,000 in this HMO, which was the final cohort, after excluding children with fewer than two polio vaccines in the first year of life.

Here are our results. This is the relative risk for autism, categorized on a continuous basis by 12.5 µg increased mercury exposure, at HMO B, which was the one with the larger number. You can see that there really is no evidence for an increased risk of autism. Looking at cumulative 1-month, there is a 16 percent increased risk of autism, with wide confidence intervals which include 1. There is a 6 percent increase at 3-month cumulative exposure, and no evidence of an increase at all at 7-month cumulative exposure.

When we look at the data in the categorical fashion, we see that there is no evidence of a dose response among children who, by 3 months of age, got the mid-level of dosage. There was a 1.61 relative risk. Again, the confidence intervals included 1 in all dose categories.

At 7-month cumulative exposure, the relative risk actually tended to suggest a protective effect. But in any case, there was no evidence of an increased risk for autism based on the cumulative exposure by 7 months of age.

We have been asked repeatedly about the appropriateness of the inclusion and exclusion criteria upon our autism findings. These are actually data from a

prior presentation to the IOM of 150 cases, which is three-quarters of the size of our final cohort. Regardless of including or excluding the congenital and perinatal conditions, there was no appreciable change in the relative risk. Then again, cumulative exposure by 7 months of age, there is, in essence, no change in the relative risk, regardless of including or excluding children with severe congenital or perinatal conditions.

How about if we simply don't exclude any children whatsoever? Here is our study cohort, and here are all the children with autism. If you include all the children with autism, you find that, looking at cumulative exposure at 1 month of age, the relative risk goes from 0.99 to 0.94. If you look at cumulative exposure by 7 months of age, the relative risk goes from 0.98 to 1.03. So, in essence, there were no substantial differences, no appreciable differences, due to our exclusion criteria.

Other sub-analyses of autism and thimerosal: If we just look at the chart-verified diagnoses -- this is a much smaller data set -- we find that, for children whose charts actually verify that they had autism, there was no evidence of an increased risk for autism related to cumulative exposure by 7 months or cumulative exposure by 1

month.

If we actually look at an implicit adjustment for care-seeking behavior and we look at children whose thimerosal exposure differed solely because they had received different types of DTP-containing vaccine, we find that, in terms of a 25-µg difference of exposure to thimerosal, there was no evidence of an increased risk for development of autism.

At Mrs. Redwood's request -- this was shown previously -- we categorized as finely as possible the cumulative ethylmercury exposure among children in this cohort. Again, this is the highest exposure; this is the lowest exposure. There is simply no evidence of a dose response or, in fact, any particular category that appears to be at increased risk for thimerosal exposure.

A brief summary of the autism chronology. We began this study as a thimerosal working group. It was identified as a priority study, because of its obvious public-health implications, in September of 1999. Over the next month, we developed a protocol, in collaboration with the thimerosal working group and VSD principal investigators. We did the initial data analyses and then had discussions of the interim results, and involved the

FDA at that time.

Our initial findings were somewhat worrisome. We found that exposure at 3 months, based on 62 cases today, showed almost a 2.5-fold increased risk of over 62.5 µg versus 37.5 µg of exposure. The P value here was not significant. Confidence intervals were not given at that time. But it certainly was cause for concern on my part and on other's parts, and internally.

So we wanted to continue to accrue data, assuming that, if this were a real finding, we wanted to know about it, and that the finding, if anything, should obtain statistical significance if we were able to add more cases as they accrued in the data set. When we presented our data to the Simpsonwood and the ACIP, we had, at this point, more than twice as many cases. Now we see that at the highest exposure level, at 3 months of age, the relative risk is 1.69, again with confidence intervals that include 1.

I believe you have seen this before. If we look at the relative risk with exposure at 3 months of age, the first presentation to this panel showed that, based on 150 cases, there was no evidence of a dose response, and the highest group had a relative risk of 1.52, with confidence

intervals that included 1.

The differences have been that the updated data sets have extended the follow-up, allowing additional cases to be identified in the HMOs. The exclusion criteria have been modified at times based on scientific input from yourselves, from the CDC, and from VSD investigators and others. We have improved our adjustments for health care-seeking behavior, and we have corrected some initial problems with the data, just in terms of correcting some mistakes in terms of exposure and timing of vaccines.

The data have been made available to outside investigators at the Research Data Center, the RDC. There have been some recent outside analyses of VSD data. In fact, there was a recent investigator who went to Atlanta. This is a direct quote from an interview that they gave. They were asking whether, among children that got a minimum of either three consecutive thimerosal-containing DTaPs or three consecutive thimerosal-free DTaPs, there was a difference in the number of autism cases in the two groups. That is a very nice hypothesis, and it certainly is one that can be addressed. They claim to have found large differences, more than 20 times higher.

This, of course, was very concerning to us. Had

we missed a signal that was present in our data that we, in fact, should have seen? So we attempted to replicate this analysis using the exact same data, the RDC, that were available to the investigator. We limited this analysis to children born after 1997, since only children born after this date had the chance of being given thimerosal-free DTaP vaccine. We looked at all children receiving DTaP vaccine between 1/1/97 and 12/31/00. The exposure was the total thimerosal dose from DTaP, and the outcome was the first inpatient or outpatient autism diagnosis -- in fact, a very similar approach to the analysis that we had used in the VSD.

Children were, in fact, able to have five categories of exposure, 0 µg, 25 µg, 50, 75, and 100, depending on which combination of thimerosal-free or thimerosal-containing vaccines they had received in the first two years of life.

There were 76 cases of autism. This analysis that I am about to show you is controlled for gender. It is done using logistic regression, since this is a case-control analysis.

In fact, we find what the investigator found, which is that there are, in fact, very large differences.

Over 100 µg of thimerosal, mercury exposure -- children with that have an 18.4-fold increased risk for developing autism compared to children who do not receive any. If you do the math, it is about a fourfold increase, a 100-µg category exposure versus a 25-µg category exposure. All the odds ratios are statistically significant, at P less than .05.

There is, however, a substantial issue, problem, other shoe that has to drop here, in the sense that if you look at children with 0 µg of exposure, the median age at the last follow-up was 1.03 years. If you remember our study of autism and Liz Miller's studies of autism, the median diagnosis date of autism is 4.4 years. Children with 100 µg of exposure had 2.2 years of available follow-up. You can see that, in fact, there are substantial differences in the amount of follow-up that is available from category to category. Children with the highest exposure had up to three times more opportunity to be diagnosed with autism. In other words, age at last follow-up has the opportunity in these data to act as a confounder.

So we reanalyzed these data, matching cases to controls on month and year of birth, which will equalize

the group according to the length of follow-up and the ability to be diagnosed with autism. We also deleted DTaP vaccines with unknown thimerosal content.

This is what we found. If you look at children with 0 µg of exposure to thimerosal, mercury, the reference group -- in fact, we see odds ratios that show no statistical association. They vary now, instead of between 1 and 18, between 0.75 and 1.21. In all situations, it looks as if they are simply random snapshots of the data taken from the same bell-shaped curve that centers around 1.0. In other words, there is no evidence here of an increased risk for autism, in this analysis.

The IOM said that evidence was insufficient to accept or reject a causal association, in July of 2001, but felt, as we originally did, that the hypothesis was biologically plausible, and a portfolio of additional studies was recommended, including a case-control study of thimerosal and autism.

This is a large case-control study, in the final preparation stages, in collaboration with the VSD project and Abt Associates. We do have an external advisory board that is reviewing the protocol at many stages. This will include an in-depth examination of children with autism,

along with extensive collection of data on prenatal mercury exposures and other environmental exposures in infancy.

The primary research question is, is there an association between cumulative exposure to thimerosal vaccines or RhoGAM from the prenatal period up to 7 months of age and autistic disorder? Is the timing of exposure from thimerosal related to autistic disorder?

This is my last slide. It just summarizes where we have been and where we are going. The screening analysis was a retrospective cohort. We are now embarking on a case-control study. The screening analysis incorporated two to three HMOs. The case-control study will derive its subject population from three HMOs. The birth years were 1992 to 1998 in the first study, and 1994 to 1999 in the final planned study. The age range in years had one-quarter of the child population under 3 years of age. The case-control study includes children between 4 and 10 years of age.

The screening analysis was automated and postnatal-exposure history only. The case-control study will include automated data, we will interview the parents, and we will collect information on prenatal and postnatal exposures, both to thimerosal and other environmental

insults. The outcomes will include autistic spectrum disorder. We will confirm this by doing in-person ADOS and ADI-Rs. In essence, we will be giving ourselves a detailed assessment for potential confounding.

Thank you very much.

DR. MCCORMICK: Questions from the committee?

DR. FOXMAN: I have two questions. What was the loss to follow-up in the first VSD study? They were born between 1992 and 1998, and followed until 2000. Presumably, there were some people whom you couldn't follow for that whole time period.

DR. DAVIS: Yes. I don't have that information at my fingertips. I am sorry. I don't know.

I will say that, compared to the general population, it tends to be a population where loss to follow-up tends to be less, because they are a covered population with comprehensive health-care delivery. But I don't know of the exact amount who were lost to follow-up.

DR. FOXMAN: Especially given that the median age at diagnosis is about 4 years, there is a potential loss of cases.

DR. DAVIS: Yes. And for them to impart bias to the study, of course, the loss to follow-up would have to

be biased with respect to thimerosal exposure.

DR. FOXMAN: Yes, exactly. Just apropos of your reanalysis that you did, in this first analysis for the VSD data, how did you adjust for varying lengths of follow-up?

DR. DAVIS: It was a Cox survival analysis, which, in essence, follows people during the entire duration of follow-up, censoring upon the date of diagnosis or last date of known follow-up.

DR. FOXMAN: So it takes into account --

DR. DAVIS: The varying lengths of follow-up that each person, individually, contributes.

DR. FOXMAN: So, in fact, even though the ones that were enrolled in the last year, in 1998, were only two at the end of follow-up, they were essentially, in a way, sort of non-contributory in terms of risk of autism, because they wouldn't have even reached the --

DR. DAVIS: If they are diagnosed with autism, they do contribute, and they are compared, of course, to children who are not diagnosed with autism.

DR. MCCORMICK: Other questions from the committee?

DR. BAYER: Given your most recent analysis and that of Professor Miller, going into your case-control

study, do you expect to find any association between thimerosal and autism?

DR. DAVIS: I don't. I think we are going to learn a lot about autism, and I think we are going to learn a lot about environmental influences. I actually have no honest-to-God feeling about it one way or another. I haven't even attempted to formulate an expectation prior to launching the study, one way or another.

DR. MCCORMICK: Any other questions?

DR. DAVIS: Obviously, I am thinking about this for the first time, but one thing I think we will be able to address is this sort of newer question of whether -- I hope, and we will be limited by statistical power, of course -- whether or not there is any synergistic effect, just to put it in epidemiologic terms, between thimerosal exposure early in life and later receipt of MMR. Given the variation we have seen to date, that is a study-able question.

DR. GEIER: I am Dr. Geier. I have a question.

In the Verstraeten study that you have recently published in *Pediatrics*, did you correct for the fact that one-third to one-half of your children received thimerosal-free vaccine? Did you count those as having thimerosal?

If you did not, which you should not have, why does your first table not show intermediate values that would have to be allowed -- your first table in that study shows the values that are possible outcomes. You have left out of that table the possible intermediates that would be generated for the three in one, two in two, one in three, and zero in four.

DR. DAVIS: Thank you. A good question. In fact, we categorized people's thimerosal exposure according to the exact thimerosal-containing or thimerosal-non-containing vaccine that they were administered. So we actually accurately categorized them according to their known exposure, whether that meant they were exposed to thimerosal at the time the vaccine was delivered or they were not exposed to thimerosal.

The question about why our table appeared not to show intermediate values -- actually, our table does show intermediate values. Because this is PowerPoint and because I am a real klutz with PowerPoint, I ran out of space on the right side, and so it says from the lower to the upper category. So it basically is inclusive in terms of the exposure categories that they could receive.

DR. APOSHIAN: I am not an epidemiologist. I am

a toxicologist. I am a little disturbed about your talking about the mercury exposure of your children, when we know that the mothers have a substantial mercury load. There are good data. The question I am asking you is, do you expect you would have the same results if were able to get data on what the mother's body burden is, especially since we now know that cord blood is quite different, as far as the body burden of the child *in utero* and when the child is born?

I am disturbed about only looking at thimerosal while ignoring the body burden of organic mercury.

DR. DAVIS: That is a great question. I would just have to agree that we are disturbed by that, too. As a matter of fact, in the paper, that is one of the reasons, explicitly, that we call for detailed interviews of the parents, to try to assess body burden. I will be the first to say that we will be limited. Ask me what I ate yesterday. I actually am not sure I could tell you, honestly. Ask me what my wife ate four-and-a-half years ago, when our daughter was born. I don't think I could tell you that either.

So we are going to be limited. But that is the limitation of all retrospective studies. I wish it weren't

so. Short of actually doing a randomized clinical trial, where you bring people in and forcibly vaccinate them -- which is anathema to all of us, with thimerosal-containing vaccines -- it is the best we can do. We are including nutritional epidemiologists, who have been able to find, using these kinds of retrospectively collected data, the effect of various additives on food, in terms of either increasing or decreasing the risk for certain congenital conditions that you would expect to be subject to the same types of recall bias.

I am not trying to say that all is lost. I think we have 35 pages of prenatal interviews going on in terms of getting the exposure that the parents have. We will do the best we can.

DR. MCCORMICK: One more question.

DR. BASKIN: David Baskin, from Baylor College of Medicine in Houston.

I have a different question about study design. I am not the first person to bring this up. If you look at the example of neural-tube defects and folic acid, this type of study would have never found that. In other words, the populations at risk are randomly distributed between the two groups. There is no controlling for whether you

are actually studying populations at risk.

What do you think about the idea of actually studying families with one child with autism and looking at the incidence in the second child? In other words, you might still miss a real effect with this study, but you don't have equal -- you don't have any control for what populations are at risk in each group.

If you look back at the history of neural-tube defects, folic acid was missed until they studied populations at risk. What do you think about that?

DR. DAVIS: It is an interesting idea. As a matter of fact, I proposed to Bob Byrd -- I am not sure if Dr. Byrd is in the audience today, from UC-Davis -- that we study this exact question, based on your exact reasoning. The hypothesis was this: Among families that had one autistic child, we thought that there would a large variation in the number who would accept a second MMR vaccine. He thought it was a great idea, I thought it was a great idea, and we wanted to do that study.

For various reasons, including the fact that our initial anecdotal reports -- the major reason we didn't do the study was that our understanding was based on original anecdotal reports of the proportion of families who had a

second child born following a first autistic child that did not receive MMR vaccine. In fact, it turned out to be much lower than we expected, when we actually started collecting the data. So we were not statistically powered to actually answer that very good question with a high degree of accuracy.

DR. BASKIN: But it is a good question, I think. Actually, from some other databases, like the AGRE databases, there are enough children --

DR. DAVIS: I am sorry, from the what?

DR. BASKIN: From the Autism Genetic Resource Exchange, which is the blood bank we have with autistic kids and nonaffected sibs, there are enough children who have subsequently been -- the second child has been vaccinated, often because the diagnosis wasn't clear until the second child was already born and went through the vaccination schedule.

So I am just suggesting that if you really want to knock this out of the ballpark one way or the other, it is a much better study to do. There is some analogy in other serious issues in medicine, like neural-tube defects, where the whole thing wasn't discovered until this type of epidemiologic study was done.

DR. DAVIS: Right. But it is also worth saying that there are lots of associations that actually have been found using this. What you are saying is that this kind of study would actually miss a mild-strength association or even a moderate-strength association, if it occurred in a very small subgroup of the population. I don't think there is a person in this room who would disagree with that.

DR. BASKIN: If it occurred in 30 percent, it would miss it. I think it is fair to say that if it occurred in 30 percent, you would miss it.

DR. MCCORMICK: I think we are going to have to move on to the next --

SPEAKER: May I just ask one real quick question? It will take Dr. Davis three seconds to answer it. What is the average age of children in your cohort?

DR. DAVIS: The median age at autism is 4.4 --

SPEAKER: No, no, no --

DR. DAVIS: There is no average age. It is an open cohort, where people age through the cohort. There is no right answer. If I actually give you a number, it is a wrong number.

SPEAKER: My understanding was that the average age was around 30 months. Fifty percent of the children --

DR. DAVIS: I am not sure who gave you that information, but I would actually disagree with that interpretation. It is an open cohort. I was 20 years younger 20 years ago. I am now what I am now, and I am older. I guess you could say my average age is that over 27, but it is an inappropriate way to characterize the data set.

I am not trying to be difficult; I am just trying to be exact. The median age at the time of autism --

SPEAKER: The median age of the whole cohort. What is the median age of your whole cohort?

DR. DAVIS: I will be happy to look through our data and give that to you. I will be here today.

DR. MCCORMICK: Our next presentation is by Mr. Anders Peter Hviid, from the State Serum Institute in Copenhagen, Denmark. Mr. Hviid was invited to present a study of the association between thimerosal-containing vaccine and autism in Denmark. Mr. Hviid has been allotted 20 minutes for his presentation and 10 minutes for questions.

Study of the Association Between Thimerosal-Containing Vaccine and Autism in Denmark - Anders Peter Hviid

MR. HVIID: I would like to thank the committee for the invitation and the opportunity to present a study from Denmark here today, a study of the association between thimerosal-containing vaccine and autism in Denmark. The study was published late last year.

With respect to disclosure, the study was funded by the Danish National Research Foundation and the Danish Medical Research Council. During the conduct of the study, I was employed at the Department of Epidemiology Research at the State Serum Institute, and I still am today. The institute is state-owned. Its mission is the control and prevention of infectious diseases, through research, surveillance, diagnostics, and vaccine manufacturing.

I should say that the institute is by law obliged to supply the vaccines for the Danish childhood vaccination program. This is done either through producing the vaccines themselves or by buying them.

Other than that, I don't have any further disclosure.

We wanted to study the association between thimerosal-containing vaccine and autism. Our specific research question was, are there more cases of autism among children vaccinated with thimerosal-containing vaccine than

we would expect?

To study this association, we designed a population-based cohort study. The term "population-based" refers to the fact that we included all children born in Denmark from 1990 through 1996. The term "cohort study" refers to the fact that we obtained for each child in the cohort date-specific information on vaccinations and date-specific information on possible autism diagnosis. Thus, we could perform a study where we prospectively followed these children according to relevant vaccination exposure, relevant thimerosal exposure, and we could follow them prospectively for an autism diagnosis from 1990 through the year 2000.

This is an overview of the range of different registries which we have used in the construction of this cohort. In Denmark, we have very complete registries of our citizens. For example, all citizens living in Denmark are registered in the Danish Civil Registration System, where they are given a unique identification number. I call it the "P" number here. In this registry, there is a range of information -- date of birth, information on who your parents are, addresses, and so on -- for the entire Danish population, with complete follow-up. It is updated

continuously.

From this registry system, we obtained the information of person numbers for all children born from 1990 through 1996. That was the study cohort. That was approximately a half-million children.

Exposure information with respect to thimerosal was obtained from a vaccination database which contains information on all childhood vaccinations administered since 1990 in Denmark, also nationwide and complete.

With respect to outcome, we obtained information on the autistic spectrum disorder diagnosis from the Danish Psychiatric Central Registry, also nationwide.

Both the vaccination database and the Danish psychiatric central registry I will give more details on in a minute.

Furthermore, we obtained information from the Danish medical birth registry and some further information from the Danish civil registration system on factors which could possibly confound an association between vaccination and autism -- birth weight, gestational age, 5-minute Apgar score, sex, place of birth, mother's age at birth, mother's country of birth. We obtained information on conditions strongly related to autism, and children with these

conditions were excluded.

I am going to say a little bit more about our vaccination database, but first I need to make some key points about the Danish childhood vaccination program. The Danish childhood vaccination program is voluntary, and it is free of charge to the vaccinees. It is administered by general practitioners, and they are reimbursed for their expenses when they report these vaccinations to the National Board of Health.

To construct a nationwide vaccination database, we obtained these vaccination reports, which cover childhood vaccinations since 1990. We obtained for each child a complete individual history of the vaccinations they received. The type of information is, again, person number, which uniquely identifies the child, the type of vaccine administered, the dose number, and the exact date of vaccination.

You need to know something about how thimerosal was used in the Danish schedule. It is quite simple. In the study period, which for this study was from 1990 up to 2000, only the whole-cell pertussis vaccine contained thimerosal. It did contain thimerosal until the middle of 1992.

I should say that there are some minor misprints here. One dose of the whole-cell pertussis did actually contain 100 µg of thimerosal. So that is a minor error here. It was administered in half-a-dose at 5 weeks, one dose at 9 weeks, and one dose at 10 months of age.

In the figure, you can see a comparison between the Danish schedule and the American schedule, just before thimerosal was withdrawn in 1999. It shows the total exposure to ethylmercury through vaccination, according to the age of the infant. In the first 4 months of life, the exposure levels are actually quite similar in the Danish and the U.S. programs, except that in the U.S. program there is the 12.5 µg mercury-containing hepatitis B.

From 4 months of age up to 9 months of age, the discrepancy widens between the Danish and the U.S. schedule. Then, when the last whole-cell pertussis dose is given, the total amount in the Danish schedule was then 125 µg compared to the U.S. schedule of 187.5 µg.

How did we determine the actual thimerosal exposure in our cohort? There was one problem, and that was that we did not have exact information on which of the whole-cell vaccines actually contained thimerosal. We knew which children were vaccinated with whole-cell pertussis

and when they were vaccinated, but we didn't know whether the vaccine contained thimerosal.

To determine a cutoff date where we could say that vaccines administered before this date contained thimerosal and vice versa, we went through the production lots. Because the State Serum Institute is the only supplier for the Danish childhood vaccination program, and because it is a constant, closed population, we could extrapolate a good cutoff date based on when the last batch of thimerosal-containing vaccine was produced.

Then we classified the children in three categories: unvaccinated, vaccinated with thimerosal-containing whole-cell pertussis, and a group vaccinated with thimerosal-free whole-cell pertussis. If the children did not receive throughout the study period any whole-cell pertussis, we classified them as unvaccinated. That was about 4.4 percent of the cohort. Children vaccinated before June 1, 1992 were classified as having received thimerosal-containing whole-cell pertussis. That was about 30 percent of the cohort. Children vaccinated with whole-cell pertussis between June 1, 1992 and December 31, 1996 were classified as vaccinated with thimerosal-free whole-cell pertussis.

The autism diagnosis, as I mentioned, was obtained from the Danish Psychiatric Central Registry, which is a nationwide passive administrative registry that records contacts to psychiatric departments. Throughout our study period, there have been some changes in how autism has been recorded, or how any psychiatric diagnosis has been recorded, in the system. ICD-8 was used from 1990 through 1993. In 1994 and forward, ICD-10 was used. Up until 1995 -- I apologize; there is an error there -- only inpatients were included. From 1995 throughout the study period, both inpatients and outpatients were used.

To the right you see the actual codes used. We only included cases which were ascertained using ICD-10. Cases identified by these ICD-8 codes with these two autistic spectrum disorder outcomes were only included if they were later ascertained in ICD-10 coding.

Here I show how we have analyzed these data. We have used simple survival analysis. This is an example of how we would treat a child from the cohort analytically. In this case, it is a child who has received two thimerosal-containing vaccines and a thimerosal-free vaccine. It is a child which is diagnosed with an autism diagnosis at about 4½ years of age.

As I have said earlier, our study period was from 1990 through the year 2000. We followed the children from 1 year of age until the end of the study period, if possible.

In this case, the child is followed from 1 year of age to 4½ years of age, when he was diagnosed with autism. In this case, that gives us one case of autism in 3½ person-years at risk. That is how this table on the left side is completed when you have gone through all the children in the cohort.

These data are then analyzed using Poisson regression to produce ratios. The natural referent was, of course, children who received thimerosal-free vaccine.

These are the main results from our study. In the first analysis, we compared children who had received at least one thimerosal-containing vaccine with children who had only received thimerosal-free vaccine. In the first column you can see the length of follow-up. There was approximately 2.9 million years of follow-up and approximately 400 cases. You can see that, if you calculate the crude incidence rates for these two categories, the thimerosal-containing vaccine is protective. That is simply a result of the cohort design

and the fact that the age and calendar distribution is unequal in the two groups.

If you then adjust for confounders, which in our case were age and calendar period, you get a rate ratio of 0.85, in comparison between thimerosal-containing and thimerosal-free.

In a second analysis, we evaluated the association with respect to number of doses. As you can see, none of these were associated with autism -- 0.99, 0.71, 0.96. Finally, we conducted an analysis where we evaluated the dose response between thimerosal and autism. We didn't find any association there either.

The final column is the rate ratios fully adjusted for all the variables which I showed in one of the first slides -- birth weight, gender, and so on. As you can see, there is absolutely no confounding of this association. That is simply a result of the fact that we compared vaccinated children to vaccinated children. So the factors which would usually confound with respect to whether you would be vaccinated or not are simply not present here.

We also conducted further analysis to evaluate the robustness of our results. First, we evaluated how any

misclassification of the cutoff date for thimerosal exposure could affect our results. We excluded children who were vaccinated from the cutoff date, June 1, 1992, through 1992. The result was almost identical with what we had on the previous slide, 0.87.

In the second robustness analysis, we evaluated whether the skewness of the cohort design -- the fact that if you were born early in the cohort you have longer follow-up and you can reach a higher age, and if you are born late in the cohort, you are not followed up as long -- whether it had any influence on our results, and also the homogeneity. If there were some external factors which were associated with calendar period, this analysis would also show that. So we restricted our cohort to contain only children born in 1991 through 1993, and it didn't change our results either.

These results were not published in the article. They show, for our main result, the 0.85 rate ratio for comparing thimerosal-vaccinated children to children vaccinated with thimerosal-free vaccine, how this varies according to subgroups of children, according to birth weight or gestational age or sex. We see in the first column that birth weight and sex are risk factors for

autism. There wasn't any interaction between the thimerosal effect and these risk factors for autism.

The strength of this study was that it was a large study, with 440 cases of autism and 787 cases of autistic spectrum disorder. It was nationwide and population-based, which eliminates selection bias. All the data we used came from different registries and were prospective data. This clearly also eliminates selection bias. Maybe the main strength of the study was that it was a comparison between vaccinated children.

Some of the weaknesses of the study -- I don't know if you could call them weaknesses -- we had only the date of diagnosis for autism instead of the date of onset of symptoms, and we didn't have any clinical information on the cases, so we didn't have an opportunity to define different autism outcomes.

In conclusion, the results we obtained are not compatible with the hypothesis of causal association between thimerosal-containing vaccines and autism or other autistic spectrum disorder. I didn't show the results for all the autistic spectrum disorder, but they are presented in the article.

Last but not least, I would like to thank my co-

authors, Michael Stellfeld, Jan Wohlfahrt, and Mads Melbye, all from the State Serum Institute.

Thank you.

DR. MCCORMICK: Questions from the committee? Ron?

DR. BAYER: As I recall from the news stories that followed the publication of your piece in *JAMA*, there was some report of the incidence of autism in Denmark in the period subsequent to the elimination of thimerosal from vaccines.

MR. HVIID: Yes. The data that were obtained were not prevalence data.

DR. BAYER: They were incidence data, yes?

MR. HVIID: No, they weren't incidence data. They were just year-by-year data obtained from the Danish Psychiatric Central Registry. As I said, they register contacts. If you do not have the person number, you can't get incidence or prevalence. It depends on how many contacts you have.

DR. BAYER: But can you say something about the diagnoses of autism in Denmark in the period since the -- one of the questions confronting both this committee and people in this room more generally is, what accounts for

the rising number of autism cases? One hypothesis is thimerosal. The question is, in Denmark, subsequent to the elimination of thimerosal, what has happened to cases of autism? Can you say anything about that?

MR. HVIID: Not from the data we have, because of the changes in diagnostics and recordings. I can't really speculate on that.

DR. GOODMAN: I just want to second that. You could have calculated -- you have incidence rates right here. Because this was a time-series study, you basically have the incidence rates before that cutoff date and the incidence rates after that cutoff date. So, if I am interpreting this right, you are effectively saying that the incidence dropped after that cutoff date. No? Even with the change in diagnostic --

MR. HVIID: No. The incidence increased throughout the period. This is a study of the association at the individual level.

DR. GOODMAN: Right, but you have person-years, you have a period of risk before the cutoff date, and you have a period of risk after the cutoff date.

MR. HVIID: Yes, and the incidence continues to rise.

DR. GOODMAN: Okay. How did you handle the addition of the inpatient diagnoses to the data?

MR. HVIID: We adjusted for calendar year and age.

DR. GOODMAN: Did you exclude them at any point? You said, before 1995, the databases only include outpatient contacts, and after 1995, you added in inpatient. Is it the other way around?

MR. HVIID: Yes.

DR. GOODMAN: How did you adjust for that?

MR. HVIID: By adjusting for calendar period. Adjusting for calendar period takes the increase into account.

DR. GOODMAN: I see. Thank you.

DR. FOXMAN: I have a follow-up question about that. You said all cases were ascertained using ICD-10.

MR. HVIID: Yes.

DR. FOXMAN: So does that mean that if someone did not have an inpatient diagnosis, say, in 1992 and then they had an outpatient diagnosis as an ICD-10 code later, that is when they were added in? I am a little confused.

MR. HVIID: If you had an ICD-8 code in 1992 and then, later, an ICD-10 code, the incidence date would be in

1992.

DR. FOXMAN: So you would refer back -- but let's suppose we had someone who -- if they were in the earlier cohort when the ICD-8 was in force and you only had it for inpatients, if they were only an outpatient visit in that earlier time period, and then they subsequently had an outpatient visit that was coded as ICD-10 -- so their date of diagnosis would have corresponded to when they first had the ICD-10 code?

MR. HVIID: No, the date of the ICD-8 code.

DR. FOXMAN: But what if they didn't have an ICD-8 code? The ICD-8 is a narrower definition, right, and it was only for people who had inpatient visits? So if someone would have been diagnosed only under ICD-10 and they didn't have an inpatient visit that corresponded to ICD-8, then they would have been diagnosed later?

MR. HVIID: Yes.

DR. FOXMAN: Okay. But if they had both an ICD-8 and an ICD-10, then you would refer back the ICD-10 to the first ICD-8 code?

MR. HVIID: Yes.

DR. FOXMAN: But they had to have both to be included in your cohort?

MR. HVIID: Yes, or they had to have an ICD-8 or an ICD-10, or an ICD-10.

DR. FOXMAN: So when you said all cases ascertained using ICD-10, if they --

MR. HVIID: All cases had at least one ICD-10 diagnosis.

DR. FOXMAN: At subsequent visits?

MR. HVIID: Yes.

DR. FOXMAN: Is it typical, when a child is diagnosed with autism in Denmark, for them to be hospitalized at the first visit?

MR. HVIID: Come again?

DR. FOXMAN: Is it typical to be hospitalized for a diagnosis?

MR. HVIID: No. I think we have some numbers, what the relation is between inpatients and outpatients, in our autism publication, which covers approximately the same cohort.

DR. MCCORMICK: Any other questions from the committee?

(No response)

MS. BERNARD: I am Sallie Bernard, with Safe Minds.

I have a question. Your analysis is dependent upon knowing when the thimerosal-containing vaccines actually stopped being administered to children. You are assuming in your main analysis that it is three months, from the end of March to the beginning of June, and then for your sub-analysis, you carried it out to nine months, to the end of the year. My question is, how do you know that that is actually when these vaccines stopped being administered? Here in the United States, it took more than two years for thimerosal vaccines to be cleared, actually, from the providers' offices and administered to the children.

MR. HVIID: In Denmark, again, it is the State Serum Institute that supplies vaccines and controls the Danish childhood vaccination program. You know how many children are born each year and how many are vaccinated. It is very, very implausible that there would be any thimerosal-containing whole-cell on the shelf of the general practitioner. There is a fast turnover of the batches.

MS. BERNARD: Have you ever done any studies to look at that? I haven't found any. I don't know if you all have ever looked at how long it goes through the supply

chain into actual use.

MR. HVIID: As I said, we looked through the production lots.

MS. BERNARD: But you have an equivalent cohort every year. It has to be pretty flat.

MR. HVIID: But the institute wouldn't continue to produce an excess amount of whole-cell if it wasn't used in the program.

DR. SINGH: Vijendra Singh, from Utah State University.

I just have an informational question. Are vaccines that are given in Denmark manufactured in Denmark or are they imported from the U.S.?

MR. HVIID: The whole-cell vaccine was manufactured at the institute.

DR. SINGH: I mean all vaccines.

MR. HVIID: All whole-cell --

DR. SINGH: MMR, for example.

MR. HVIID: That is bought.

DR. SINGH: It is imported from the United States?

MR. HVIID: I think (inaudible).

DR. HALEY: My name is Boyd Haley, from the

University of Kentucky.

What I am wondering is if we are really comparing two similar incidences. The rate of autism per 10,000 in Denmark compared to the United States and Britain, could you tell me what those are?

MR. HVIID: Come again?

DR. HALEY: The rate of autism per 10,000 population in Denmark versus the United States and Britain, I would like to know what they are, because you are showing two different vaccine schedules, one getting mercury on the day they are born and a much larger, faster rate. So I would like to know, what is the rate per 10,000 of autistic children born in Denmark versus the United States and Britain?

MR. HVIID: I don't have the numbers from the U.S. or Britain. In our cohort, you can calculate the crude incidence rates from this table here and from --

DR. HALEY: I know I can, but I would expect you to know that. I mean, if you are coming here saying this -- people know. That is what is printed in all the papers. I read your paper. You had something --

DR. MCCORMICK: I think he has answered your question.

I think we need to move on. The final talk this morning will be from Dr. Mark Geier, who is president of the Genetic Centers of America, and David Geier, who is president of MedCon. They were invited to discuss their analyses of the Vaccine Adverse Events Reporting System, or VAERS, and autism. They have been allocated 20 minutes for their presentation and 10 minutes for questions.

Autism and Thimerosal-Containing Vaccines: Analysis of the Vaccine Adverse Events Reporting System (VAERS) - Mark R. Geier, David Geier

DR. M. GEIER: On conflict of interest, we don't receive any funding from any vaccine companies or any other companies. We are consultants at times to petitioners before the Vaccine Compensation Act in civil litigation. I am an expert witness in some civil litigation before the Vaccine Compensation Act.

MR. D. GEIER: What we have done here, because we are very, very strapped for time -- we are people that have pretty much been convinced at this point that there is a causal relationship between thimerosal and autism. We haven't just analyzed the VAERS. We have seven different publications on this subject. We have attempted, in very, very brief fashion, to review some of that evidence that we

have.

To add one more point, when we were initially presented with this idea by parents, we were among the most highly skeptical people. The last time the Institute of Medicine held this hearing, we didn't have any evidence, we didn't believe in what was being said here at all, and we were, and still are, strongly pro-vaccine.

First, starting with the autism epidemic, what we have done here -- and this is consistent with the rest of our talk -- since we have so little time, we are simply presenting published papers. We have submitted to the Institute of Medicine a complete review and a summary of all this evidence and how it intertwines. We are simply going forward in this talk.

DR. M. GEIER: But it is very important to understand the magnitude of the problem facing this country. There is just overwhelming evidence that autism has risen from about 1 in 25,000 in the seventies to 1 in 2500 in the eighties, to 1 in 250 in the nineties, to 1 in 150 currently. Neurodevelopmental disorders and speech disorders have risen 30-fold. One in eight children are now in special education. When that figure is updated, it will probably come out to be one in six. In fact, it is

one in six in the Verstraeten article itself.

MR. D. GEIER: This is the actual substance, thimerosal, that we have purchased. There is extensive evidence on the biological plausibility of this issue, both from theoretical sources, who have compared the similarities of thimerosal exposure and autism, and found them to be very similar, and we have also evaluated, in comparison to the federal safety guidelines, the instantaneous exposure children were exposed to from thimerosal-containing vaccines.

DR. M. GEIER: And it is more than 100-fold, slightly over. Additionally, in your slides, you will find -- I know our talk is hard to follow, but we have very little time -- you will find the references. We have given the IOM committee all the references. You guys, unfortunately, can't have the 1200 pages given out to each of you, but you can look up each one of them that we have referenced in these slides.

MR. D. GEIER: Here are some more samples from there. We have the National Toxicology Program, part of the National Institutes of Health, describing the signs and symptoms of thimerosal exposure: mental retardation in children, loss of coordination in speech, writing, and

gait, stupor, irritability and bad temper progressing to mania-- very similar symptoms to what is seen in autism.

The CDC actually published a study back in 1984 warning that giving more than the 25 µg from a single DPT dose could be extremely hazardous to vaccine recipients.

Of course, in this country, we have approximately tripled the amount of mercury children receive.

Biological plausibility has also been demonstrated. This evidence dates over many decades. People back in the 1950s recognized that mercury in vaccines was harmful, people like Warkany and Hubbard. There have been animal models that have been developed by various authors.

Of course, another interesting point here is about methyl- and ethylmercury. Much controversy has raged about that. We have assembled from the literature 17 different references showing that they are at least similar. Authors from the FDA published in 2001 that, because high-dose exposure to ethylmercury from thimerosal results in toxicity comparable to that observed after high-dose exposure to methylmercury, because of the chemical similarity of the two compounds, it appears reasonable to consider the toxicity of both low-dose methylmercury and

ethylmercury to be similar.

In addition, despite what has been widely circulated, that there are no exposure limits to ethylmercury, back in 1969, there was an international committee meeting that had people like Clarkson and Magos, who specifically set an exposure blood-level ceiling value. The authors concluded that, for methyl- and ethylmercury salts, the ceiling value for mercury in whole blood should not exceed 10 µg of mercury per 100 ml total mercury. So that is simply a lie.

DR. M. GEIER: Additionally, if you take the 17 articles as establishing they are similar, the National Academy of Sciences has already ruled on this issue. They have ruled on it on methylmercury and have determined that it causes neurodevelopmental disorders.

MR. D. GEIER: Chronic low-dose exposure in children can cause neurodevelopmental disorders in children, and they also have affirmed the .1 µg/kg body weight per day from the EPA's valid safety limit.

DR. M. GEIER: It is over 100-fold, and it is not hard to calculate.

MR. D. GEIER: Of course, as we already heard, there has been an animal model for thimerosal-induced

autism. I won't go into detail about that.

Then we have the epidemiological evidence. We have four studies analyzing VAERS in the U.S. Department of Education, in various different ways, finding the effect, as well as Mark Blaxill's study that has been published now in the peer-reviewed literature, his ecological study.

This is from our first VAERS analysis, published in *Experimental Biology and Medicine*. This is comparing thimerosal-containing versus thimerosal-free DTaP vaccines. Incidentally, this thimerosal-free DTaP vaccine has never contained thimerosal, contained 2-phenoxyethanol. Unfortunately, we are not allowed to tell you which company it is, because when the CDC provided us with the number of doses of vaccines broken down by each type of company, they said we were not allowed to release that to the public. We have since been criticized for that, but we are not allowed to release it.

What you see here is a very large, statistically significant difference for autism, a sixfold difference in reported autism, following thimerosal-containing versus thimerosal-free DTaP.

DR. M. GEIER: And we did all the controls. We looked at all sorts of other things in the VAERS database.

Only the neurodevelopmental ones were elevated. Fever, sore arm, even seizures -- none of those were different when you compared the thimerosal-free to the thimerosal-containing. Only neurodevelopmental disorders pop out.

MR. D. GEIER: As Mark Blaxill presented -- this is now from the peer-reviewed literature -- here is his dose-response ecological study for thimerosal and the prevalence of autism in California.

This comes from one of our recent publications. It is in press right now. This is from the United States Department of Education. What we took into account here are birth cohorts. When you have a report -- take the 2000 report, the U.S. Department of Education. It tells how many autistic children there are, 6 years old, 7 years old, 8 years old, and so on. This is a plot of the average mercury dose, on one axis here, versus the prevalence of autism. You can see that there is a very dramatic apparent correlation here between the two factors. I would like to point out -- we heard from Denmark here -- if you look in the Denmark study, what you find is that they were giving this kind of mercury exposure to children, and they had a prevalence of autism very similar to that.

The reason we are here, and probably the IOM is

holding these hearings, is that we went from here in the childhood schedule out to here, for a total exposure in the childhood vaccines.

This is looking at further analysis we did from VAERS, from our article in *Pediatric Rehabilitation*. We have done dose-response curves from VAERS. VAERS provides which dose number the adverse reaction was reported after. What you see here are averaged-together exposure values. The first and second shots, you get an average exposure level. We looked to see how many outcomes there were comparing thimerosal-containing versus thimerosal-free.

This is looking at autism. You see the increasing dose-response curve.

Most recently, we have gone to the Vaccine Safety Datalink. Issues have been raised about that, as presented here. We present this in direct response to what was said.

This is looking at children. They got four doses of DTaP vaccine. DTaP vaccine, in the VSD database, was given in various combinations. Some got completely thimerosal-free, some got three and one, two and two. So there are many intermediate values. With those intermediate values, you can ask the question, what is the risk of autism for additional mercury doses?

Lo and behold, when you do this -- and by making it four doses, we are talking about children that are 18 months to 2 years old, so these children have had time to have a diagnosis of autism here -- you find these similar stark results that we found from VAERS and the U.S. Department of Education.

DR. M. GEIER: In addition, it doesn't take epidemiology. You simply go to the VSD database and you parse those that have thimerosal-free DTaP with those that have thimerosal-containing DTaP. Every case but one in the database -- we don't have the exact base they had, because we have been denied, and our database has been scrambled, and they have, in every way, tried to not cooperate with us -- the bottom line is, there is no way to look at it, when all of them are in one group and only one of them is in the other group -- and the one that is in the other group is a clerical error -- there is no way to turn it around. Any which way you want, that database supports all the other databases that thimerosal is a major contributor to the current autism epidemic.

MR. D. GEIER: And, of course, the CDC, inappropriately -- one of the agreements in getting into this database was that they agreed not to look into our own

data sets and reanalyze the data that we were looking at. That was a specific condition that was agreed upon by us and them when we went into this database, and they simply went in and rated what we were looking at.

Of course, it is not just this kind of epidemiology we are just presenting. There are mercury-retention studies. This has been extensively looked at in the literature. I won't go into a whole lot of detail, because Dr. Bradstreet will present this. But we worked with him. You find there are very, very high levels of mercury in children that have autistic spectrum disorders, in comparison to normal children. This is chelation with DMSA. Very similar levels of cadmium and lead.

Independently of us, from Holmes et al., with Boyd Haley on the paper, again finding that autistic children have a very, very significant decreased ability to excrete mercury in comparison to normal children.

It goes beyond just retention of mercury in children. It has been identified that there are very, very specific biochemical defects in these children that allow for the accumulation of mercury and damage from mercury accumulation, by various authors. We have submitted this to the committee.

This is from Jill James. We have done similar work by ourselves, with Dr. Bradstreet. One of the molecules of specific interest here is the glutathione molecule, which is a sulfhydryl-containing molecule that has the ability to bind the mercury and allow children to be able to excrete it. Some of these other molecules are accessory ones. They will be gone into more detail by some of the later presenters here. But you notice how much its presence is in autistic children in comparison to normal children.

There are specific genotypes. Not only do we have the biochemistry, we have DNA, specific kinds of DNA found in these children. It has been found that they have DNA that codes reduced levels of sulfhydryl groups. It has been found that the distribution of thimerosal and ethylmercury in the body -- there are many people looking at that, published over many decades. What you find here is that -- I will quote here from Slikker, from the FDA, who has published: "Thimerosal crosses the blood-brain and placental barriers, and results in appreciable mercury content in tissues, including the brain."

The buildup of considerable doses of thimerosal in the brain is a very serious cause for concern.

Thimerosal has been implicated to cause neuron degeneration. People like Baskin have looked at apoptosis, and others have seen that it causes neuron degeneration, at extremely low levels.

Most recently, Waley et al., from the Johns Hopkins University, U.S. Department of Agriculture, and Tufts, and a bunch of other places, have -- and I will just quote here: "A recent analysis of the VAERS found a significant correlation between use of thimerosal-containing DTaP vaccines in comparison to thimerosal-free DTaP vaccines." They discovered a specific pathway and its potent inhibition by the vaccine component of -- thimerosal provides a potential explanation for how the increased use of vaccines could promote an increase in the incidence of autism.

It has also been shown by various authors that thimerosal interacts with other substances present in the vaccines and can cause more damage that way. Of specific interest, it has been shown that testosterone tends to potentiate the effects of thimerosal -- again, Boyd Haley will elaborate more on this -- whereas estrogen seems to protect.

It has also been identified that there are

specific places in the brain that are producing these glutathione molecules I mentioned before that tend to protect certain regions, and other regions are damaged in the brain.

When you do metabolic perfusion scans -- specs, PETs -- on these children, what you find is that there is very specific damage in the areas that you would expect, where there is decreased protection from these glutathione kind of molecules.

I will shift to a slightly different topic here. Many authors, including IOM here, have recommended removing thimerosal from vaccines.

DR. M. GEIER: All the way back to the 1950s, 1960s, 1970s. There are just hundreds of papers recommending this.

MR. D. GEIER: What has happened -- and this is something we can demonstrate as we speak -- we have been assembling vaccines, and what has happened is, they haven't removed it from all vaccines. Thimerosal is present in quite a number. We are talking about here the full amount, 25 µg, 12.5 µg, pediatric vaccines included.

DR. M. GEIER: And they haven't taken the recommendation of the IOM or the American Academy of

Pediatrics to remove it, nor do they have plans to remove it in the immediate future.

MR. D. GEIER: This is DT vaccine. You see from the box here, it is still present in the vaccine, with its expiration date here in 2004.

DR. M. GEIER: But what they did do is, they removed it from the bottle. We told parents, and others have told parents, to look at the bottle and make sure you get thimerosal-free. I have a collection of vaccines all the way back to the seventies, and it always is on the bottle. But it has accidentally been removed from every single vaccine that has thimerosal -- no longer has it on the bottle. These vaccines, incidentally, are no longer in the PDR. If you look it up in the current PDR, there are no vaccines listed that have thimerosal. They have been accidentally left out.

MR. D. GEIER: These are our children. It just goes on and on -- influenza vaccine --

(Applause)

DR. M. GEIER: And influenza vaccine is available with and without thimerosal. It is very hard -- I have tried really hard for my collection to get one without. Ninety-nine percent of them -- not 100 percent -- contain

it.

MR. D. GEIER: Then we have the additional list here, meningococcal, Td, tetanus toxoid. It is very interesting, too, to look at the expiration dates on these things. Take Td vaccine here, recommended for all children 10 to 12 years old. We are talking about an expiration date, 2 September 2005. We bought this in the last couple of weeks. It means right here, as we are convened, they are making thimerosal-containing vaccines for our children, with the full amount, 25 µg per dose.

This is the conclusion of what it is we are saying. It sort of explains everything that has happened, as we briefly scan through it here. If a certain segment of the population has a decreased ability to excrete mercury, as has been demonstrated for several different genotypes, there can be little doubt that mercury concentrations once administered as part of the childhood routine vaccination schedule resulted in a significant number of children developing neurodevelopmental disorders. This is especially true when a sudden shift in the amount of mercury administered, as occurred in the United States when the amount of mercury virtually tripled from thimerosal-containing vaccines -- since the gene pool

contained many susceptible individuals that under the previous environmental conditions would have been normal, but under the new environmental conditions are unable to thrive.

DR. M. GEIER: It is not a genetic epidemic. There are no genetic epidemics. I am a geneticist, as my clinical practice. Genetic changes occur very slowly. An epidemic is a rapid change in human disease. The fastest known genetic shift is 1 percent per 100 years.

There are, however, some genetic differences among us, called polymorphisms. If you happen to have the wrong one and you are hit with enough mercury, yes, you may have problems. If you have a different one, you may have problems with something else.

And I must say that I am a little bit embarrassed to stand here and listen to Verstraeten's work being presented, after what they said at Simpsonwood, and to listen to that the table doesn't contain the error that we said. In my view, this is not a scientific issue. This is about as proven an issue as you are ever going to see. What is occurring here is a cover-up, under the guise of protecting the vaccine program. And I am for the vaccine program. If you keep covering it up, you are not going to

have a vaccine program.

MR. D. GEIER: That is it.

DR. M. GEIER: Just one more comment. If the committee determines, despite what I think is the overwhelming evidence that thimerosal is strongly contributing to this vaccine[sic] -- if they determine that either they are not sure or there isn't an effect, I would suggest that, as your next recommendation, you recommend spending $10 billion or $20 billion to go find out what you think is causing this epidemic. Nobody is doing that. The reason, I purport to you, is that it is well known to the authorities, and they are slowly correcting it.

But there is no way that we could allow this to continue to happen. If it is not thimerosal, this society is going to be in big trouble. We cannot have a whole generation of people damaged the way this is happening. Our answer is that we are going to build homes for these people? We had better prevent it from happening anymore in the future.

(Applause)

DR. MCCORMICK: Questions?

DR. BERG: If I may ask a question about the article you recently published -- you didn't have time to

present the results -- we have seen many analyses of VAERS data in this committee, and it usually is accompanied by a litany of its strengths and weaknesses. That is a part that is missing in your paper. I wonder if you could comment, from your point of view, on the strengths and weaknesses of using VAERS data for this kind of analysis.

MR. D. GEIER: I would like to comment that the CDC itself has developed the methodology that we employed to analyze VAERS. Dr. Robert Chen published specifically that comparing vaccines administered to similar-age populations provides accurate qualitative and quantitative risk-assessment analyses. What we are not reporting is that VAERS gives you a true incidence rate of the reactions.

But why should there be a biased reporting in the way the reactions are? As a matter of fact, we have actually addressed that in many cases, like with the thimerosal. Why is it only the neurodevelopmental disorders that are so elevated following the thimerosal-containing vaccine, whereas things like fever, injection-site pain, swelling -- those are reported similarly. It is only for the neurodevelopmental disorders.

In the material that we have submitted to the

committee, we have recently been invited by *Expert Opinion on Pharmacotherapy* to write a review specifically addressing the issues that you mentioned. What we have found is that VAERS consistently, across the board, has a very good causative predictive value, using this method developed by the CDC to analyze it, for vaccine adverse-reaction problems.

DR. M. GEIER: It predicts whole-cell versus acellular, which has been changed. It predicts rubella. It predicts the rotavirus problem. It predicts the Lyme vaccine problem. In fact, that is how you test a method, to see how well it predicts. There is not one thing that we have found in VAERS that has not been confirmed -- except, perhaps, if you want to say this one. But everything else that we have ever studied by this method has been confirmed in spades elsewhere. So it is a very good method, looking at it, and it is the CDC's own method. They have described and defended it and shown how to do it.

MR. D. GEIER: And as recently as 2003, CDC and FDA came out with a study out of VAERS calculating the incidence rate of tetanus toxoid vaccine reactions and Td vaccine reactions, which we have done as well, and there you find that they are similar. They published this in

Vaccine, and they are using that to say that Td vaccine is a safe vaccine, which it clearly is.

DR. BAYER: Excuse me. Ron Bayer, the committee.

Given your conclusions about the toxicity of thimerosal, how do you account for the data from Denmark and from Great Britain?

DR. M. GEIER: We showed you Denmark. We didn't have much time. They did their study -- and he refused to answer the question. But the answer to the question is, their level is at the level that we were at when we did not have an epidemic. So withdrawing it did not make the epidemic go away.

DR. BAYER: I asked a different question. You made a statement about the toxicity of thimerosal going back to the 1950s. I am asking you how you explain the data from Denmark and Great Britain, which use thimerosal at levels that should be showing toxic effects, given your analysis.

MR. D. GEIER: What we are quoting here are studies not necessarily specifically on vaccines. They are demonstrated in tissue-culture systems. They are demonstrated following gamma-globulins given to people, RhoGAM. They are not all specifically for vaccines --

although some of them are.

DR. M. GEIER: Again, the answer is that, at low levels, it is much less, and maybe it isn't even real. But at the high levels, it is undeniable, and we have gone to the high levels. Their study, therefore, is not relevant -- I am not saying it is wrong, although there are many criticisms of it. It is just not relative to the U.S. situation.

DR. KABACK: Dr. Geier, you mentioned expert witness activity in this area. I wonder if you could tell the committee how frequently that occurs, and is that done pro bono?

DR. M. GEIER: No. We are paid to appear before the Vaccine Compensation Act, as are experts on -- everybody that appears there. As far as it influencing what we find, people in the Vaccine Compensation Act are interested in whatever we find, in either direction. It does not in any way influence what we earn. We probably could be paid a lot more if we had data that it didn't cause than if it did cause.

Over the years -- and I was the very first expert. In fact, I was the one that helped set it up. The judges asked me to be a theoretical witness, to train the

lawyers. Over the years, I believe I have appeared in the Vaccine Compensation Act 90 times as an expert witness.

DR. GOODMAN: Could you comment on two things? First, on the value of the use of VAERS for acute reactions versus long-term and delayed reactions. All of the other reactions that you referred to as not being elevated were all acute reactions versus this.

Second, did you say that you had analyzed the VSD data as well?

DR. M. GEIER: Yes.

DR. GOODMAN: And how did you account for the differing lengths of follow-up? Those are two questions.

MR. D. GEIER: For the VAERS analysis, what is going on is, the CDC follows up patients in the VAERS database. Despite the fact that the CDC and FDA often maintain that there is no follow-up and VAERS is just completely passive junk, they take a very involved interest in following patients that report. FDA follows up on all deaths. CDC contacts patients at, I think, 60 days and then one year, to inquire about the condition of the people in the VAERS.

DR. GOODMAN: Those are the ones that report early. They don't follow up people who don’t report.

DR. M. GEIER: Therefore, it is an underestimate. So there is no question that it is an underestimate. Also, as we said, if you wanted weaknesses, it is not a very good estimate of what the rate is, but it is a darned good estimate of relative risk, as long as you have two groups that you can compare, because it is equally likely to be under-reported in each group. Doctors today don't even know whether they are giving thimerosal-free or thimerosal-containing vaccine. So there is no way that that biased the data.

We have done various studies to look at bias, using control, things that we know are not affected, and we have not found significant bias. Where we have found small biases, we correct them.

DR. M. GEIER: I would like to point out, too, in our VAERS analysis, we have looked at the thimerosal-containing versus thimerosal-free DTaP. We looked at thimerosal-containing whole-cell DTaP versus thimerosal-free DTaP. You see these same dose-response effects that we briefly showed you here. We have looked now at DPT and Hib vaccine versus DPTH. When DPT and Hib were given, that is 50 µg, in most cases from the same company, and when it was combined, DPTH, you got 25 µg. We saw the exact same

kinds of effects. So this effect is repeatedly being shown in this database.

Regarding the VSD, what we did to control for length of follow-up is the fact that we required children to have four doses of vaccine. By requiring four doses of vaccine -- DPT is given 2, 4, 6, 15 to 18 months -- we are extending the period of immunization out far enough that we are into the time of diagnosis.

DR. M. GEIER: Also, we only looked at 1997 -- that vaccine was only made in 1997. So the chart that was shown behind us to show that we were wrong showed one dose of DTaP when we excluded those children. In order to get into our study -- and we are trying to do more studies, but we are being interviewed with -- you had to have taken four doses of DTaP to be in the study, so you had to be more than 18 months old. To show up there that they are under a year -- I don't know how they did it, but that is not how we did it.

DR. MCCORMICK: We do have the papers, and we will be reading them.

In order to try to make up some time, we will be reconvening here at 2:05.

(Thereupon, at 1:20 p.m., the meeting was

recessed, to reconvene at 2:05 p.m.)

AFTERNOON SESSION (2:10 p.m.)

DR. MCCORMICK: As a reminder to our listening audience, the Power Point slides of most of the presentations that we are hearing today are available on the project website at www.iom.edu/imsafety. Additionally, a signup sheet is available at the registration table for people interested in commenting during the public comment session at the end of this series of talks. Comments must be kept brief, approximately two minutes.

We will now hear from Dr. Vasken Aposhian, professor in the Molecular and Cellular Biology Department at the University of Arizona. Dr. Aposhian will present a toxicologist's view of thimerosal and autism. We have allotted 20 minutes for his presentation, followed by ten minutes of questions.

Agenda Item: A Toxicologist's View of Thimerosal and Autism, by Dr. Vasken Aposhian

DR. APOSHIAN: I would first like to read a statement, if you'll bear with me. I learned during the week prior to this meeting that Merck is a vaccine manufacturer. I am a benchtop investigator, I did not know this. Since I do not wish there to be any conflict of interest to arise with my presentation at this meeting, I

have issued written instructions via certified mail that my family's holdings in Merck stock be sold. That was as of last Thursday.

At the present time, none of the research in my laboratory is being supported by funds from any autism related research foundation or from any people in any way related to autism, although a week ago we did start a preliminary autism research program. In the past, I have been a consultant to a manufacturer and distribution of DMSA, which is used to treat autism. I have been a consultant to Johnson & Johnson Corporation, and to the Ohio company, the makers of DMPS.

In 2003, I have also been a consultant to a number of multinational consumer product companies. As of January 1, 2004, I no longer have a consulting relationship with any commercial organization. As we say in academia, consulting is feast or famine.

I am an educator. Therefore, when anyone comes to me for advice or for an education on mercury, no matter what side of any problem they are on, it is my duty as an educator to educate them. I sometimes charge for this duty, but sometimes I don't. Sometimes I do pro bono work.

So I wanted everyone to know that as far as I

know, I have no conflict of interest. Thank you for your time.

A toxicologist's view of thimerosal in autism. Let me say to begin with that three years ago, I didn't even know what autism was. I had heard the word, and because of a number of reasons, primarily political reasons, I was asked to learn about autism, and have become fairly familiar with the subject.

I want to give you an outline. It has been changed to some extent. I am first going to very quickly review mercury toxicology, talk about the significance of recent papers, ask my pharmacokinetics of thimerosal in most infants are not applicable to pre-autistic or autistic children, point out the need for new data. I may or may not say something about epidemiology. Then I'll give you a summary. I want to be absolutely clear that I believe in the use of vaccines.

Elemental mercury. You are all familiar with the liquid silver, as it is called. In that form, elemental mercury in its liquid form is not toxic. People have injected it directly IV, they have swallowed it in medical stomach balloons. Those balloons have broken, some of these people have had intestinal obstructions before the

Balloon broke. We don't think elemental mercury in its liquid form is toxic.

Mercury vapor on the other hand is. The elemental mercury liquid gives off at room temperature mercury vapor that has severe toxicity.

Organic mercury. What I want to point out to you at this point is, of all the mercury forms that we are going to be talking about, organic mercury has a delayed response, sometimes called a latent response. In the case of Karen Winterhan, who died of dimethyl mercury exposure, she was exposed in August. Her first neurological signs occurred the following February, and she died in August. That was dimethyl mercury exposure.

Mercuric mercury we need not say very much about. It is a form of mercury that is found in the brain. We'll talk about that in a moment.

Mercurous mercury was used in children's teething powder at one time, and it was also used to rinse diapers, as an antiseptic. Mercurous mercury is the basis of what used to be called pink disease. Not all the children exposed to mercurous mercury in this way got the rash, got the symptoms of pink disease. It was a susceptible number of children in the population.

Target organs. Mercury vapor, methyl mercury, thimerosal. The target organ in this case both for concentration as well as toxic effects is the brain. In particular, the brain of the developing child, brain of the developing fetus, young children, their brain is very sensitive to mercury vapor, methyl mercury and thimerosal.

What I want to remind you of is that children are not small adults. Children have developing organs. Most of the people in this room, their biochemistry is maintaining the state of their organs, their tissues. Children are continuously developing, and each day is another event for the development.

Mercuric mercury goes primarily to kidney, where it can exert some toxic effects there.

Let's just briefly review the forms of mercury in the brain. This is supposed to be the blood-brain barrier. Mercury vapor from the amalgams in your mouth very quickly get into the lungs, about ten micrograms of elemental mercury vapor is absorbed by the average person from the average number of amalgams. That mercury vapor very quickly gets in the blood, and is very quickly transported in the blood, comes across the blood-brain barrier because it is fat soluble. The mercury vapor is quickly oxidized

to mercuric mercury.

Methyl mercury from fish. It is believed that the methyl mercury forms a compound with the amino acid cystine, and that cystine methyl mercury compound is analogous in chemical structure to another amino acid called methionine. The methyl mercury cystine compound is transported across the blood-brain barrier via the methionine amino acid transport system.

Thimerosal. This is an old slide. I didn't know very much about thimerosal at the time. There is no question that thimerosal gets into the brain. Tom Clarkson, one of the world's best experts in mercury toxicology, clearly showed in 1977 that thimerosal is taken up in a human and is in the brain.

In all these cases, we believe some of these compounds are converted to mercuric mercury, which has a very high affinity for the sub-hydro groups of proteins. In the case of methyl mercury, at one time we thought the toxicity of methyl mercury was due solely to the methyl mercury in the brain. We now know from studies at the University of Washington, at the Karolinska Institut in Sweden, that methyl mercury is slowly converted to mercuric mercury. Whether the toxicity of methyl mercury is due to

methyl mercury per se or to the mercuric mercury formed or a combination of both, we don't know at the present time.

For those of you who would like to continue your education, if I may say that, as far as various forms of mercury are concerned, I recommend very highly to you this article by Lynn Goldman, by Michael Chen and committee on environmental health. It is a very, very, very good article on all aspects of mercury.

Now I want to address the question, is autism an efflux disorder. What I would like to do is give you an example of an efflux disorder before we go on to talk about autism.

Wilson's disease. As most of you remember from your medical school days, Wilson's disease is a movement disorder, inherited metabolic disease. It has very definite central nervous system signs and symptoms. It is due to a mutation -- we now know this -- it is due to a mutation in the ATP7B gene, which I will explain in a moment.

Wilson's disease was first described in the late 1800s. I want to say 1875, but the exact date has slipped my mind. It wasn't until 1953 or 1954 that the first therapy, useful therapy, for Wilson's disease was

discovered. Now it has just been almost within the last three years that the genetic error, the mutation, has been found.

We now know that it is due to mutation in the ATP7B gene. The mutation is a single mutation, but that one single mutation can occur in various places, in different people in this one gene.

The ATP7B is a copper transport protein. Wilson's disease is -- the signs and symptoms of Wilson's disease is the accumulation of copper in the tissues. The ATP7B copper transport protein is expressed primarily in the liver, and it is deficient and lacking in Wilson's disease. There is hepatic and central nervous system copper accumulation toxicity, and there is hepatic and central nervous system signs and symptoms.

Wilson's disease is one of the rare treatable genetic disorders. Not very many genetic disorders can be treated. In this case, there is a group of chelating agents that have been used very successfully, they have kept people alive, and now they use zinc for a different reason, but it still is very effective.

So now let's talk about a paper that you will hear more about, reduced levels of mercury in the first

baby haircuts of autistic children. Amy Holmes is a practicing physician. She treats people. She is not a research physician, but she had the bright idea, remembering that most people keep some of the first haircuts of their children.

What she did was collect this hair and sent it to a laboratory where the mercury content was determined. This shows you exposure differences in the autistic group as compared to controls. The autistic group had a mercury level in their hair of 0.47 parts per million, the control group had 3.63.

Now, one can always criticize an experiment. This experiment could be criticized that it wasn't done in a research environment, the hair was sent off to a commercial lab. There are many things one could say. But I want to point out that this initial observation was very, very important, and has now been confirmed.

I was very fortunate on Friday night to run back to my computer to check something, and I found on my computer a note from someone pointing out, in the transactions of the Society of American Radiology, there is a paper that appeared in November of last year. This paper is from MIT, from three nuclear scientists who went out and

got the hair, did the analysis at MIT, using neutron activation analysis.

What is very good about this study, the two studies put together, was, the first study by Amy used ICP mass spec, as I remember; this study used even the more sensitive neutron activation analysis. So this now is a study that has been confirmed. I have with me the reference, if anybody wants it. There is no question the results were the same, remarkably close.

Another study that you will hear more about -- I'm not going into the details of all these studies -- a case controlled study of mercury burden in children with autistic spectrum disorder. Jeff Bradstreet is the lead author. He found that if he gave -- this was a retrospective study as I remember, and he found that if we went back and looked at the data, children who were autistic who received DMSA put out in certain cases six times more mercury in their urine than did control children who were non-autistic.

This is a very good study. Among other reasons, there is a tremendous amount of anecdotal evidence for this. This is the first solid piece of work with autistic children and the effects of DMSA on them.

So it occurs then that from this study in particular that autistic children have a greater burden of mercury. How does that fit? Autistic children have a greater burden of mercury, yet their hair has less mercury in it.

The significance of these two papers. It appears that autistic children lack an effective mercury efflux system. Let me just describe this by a diagram. Here we have ethyl mercury efflux from autistic tissue. It appears to be inhibited. Here we have the tissue. Normally there is an efflux system which involves combination with glutathione molecules, an efflux system that sends it out of the tissue into the blood, and then gets over to the hair follicles. As the hair grows, there is a chronological record of how much mercury has been laid down, or the person has been exposed to.

In the autistic child, you are going to see a block. I think we should have had this arrow right here, rather than in between. The mercury supposedly cannot leave the tissue to get into the blood. Some of it does, there is always a little bit, and some of it is in the hair.

When you have this, all you need to show that

autism is a disease concerned with a mercury efflux disorder is to analyze the tissue. Obviously I got on the phone. I called on Wednesday people I know that are experts in this area, in a variety of cases in England, Japan, this country and Sweden, and was shocked to learn that no one has such data. It is really amazing to me as a scientist that no one has taken the tissue from autistic children to see whether mercury is accumulating there, especially since the idea of mercury being high in children with autism has been around for some time.

There is an autism bank that some people I know have tried to get brain tissue from, but I am told it is just as difficult to get brain tissue of autistic children from them as it is to get money from a Swiss bank account if you don't know the number.

So that is one thing that must really be done. I know the legal implications of all this, and if I were a CEO of a company that made thimerosal, I would want to know, and if I were the parent of an autistic child, I would want to know whether there is a lot of mercury in the tissue of autistic children. If there is, then there is no question that they have a mercury efflux disorder. However, the evidence so far is indicating that.

I haven't said they do. I said that it appears that autistic children lack an effective efflux system.

Let me now point out to you the estimated daily intake and retention of micrograms per day. Let's just deal with these figures down here. The pregnant woman, a woman of childbearing age, has methyl mercury. The numbers in parentheses equal the retention amounts. So a pregnant woman is retaining each day one to six micrograms of methyl mercury.

This is a concern of our government as far as the amount of seafood that women of childbearing age and young children do eat. But I want to point out that any epidemiological -- I say, I'm not an epidemiologist, but any epidemiologic study of thimerosal and autism certainly should take into consideration the fact that the mother of the children born had a mercury load, and that mercury load was transferred to some extent, much more now than we knew in the past, that mercury load is transferred to the child. We will come back to that in a moment.

As a toxicologist, it is my opinion -- and remember, science is the search for the truth, and the truth depends on what evidence you have. The evidence can change, therefore the truth can change. But at the present

time, I think there are different responses to thimerosal that is injected intramuscularly into a child.

The first is that the child has an efflux mechanism. He is not pre-autistic, so you inject it and the thimerosal is excreted, no autism. The second possibility is that efflux is impaired. You inject the thimerosal, and it is the final insult. It causes the cup to overflow, you might say. It is the trigger, and you get autism. That is one possibility.

A third possibility is that the efflux is impaired, you give thimerosal, it adds to the mercury burden of the child. The mercury burden of the child was obtained from his parents or her parents. So you eventually get a toxic level of mercury that is exceeded. The thimerosal doesn't cause the toxic level to be exceeded, but it contributes to it.

I made a calculation early this morning, and my calculation based on a ten kilogram child or a four kilogram child getting a bolus or one vaccine shot, where there is 25 micrograms of mercury in it, that the ten kilogram child has 25 times the dose that our government considers safe. The four kilogram child has 60 times the dose of mercury that our government has set up standards

for. The standard is .1 microgram of mercury per kilogram per day, the reference dose, the dose that is not considered to be toxic.

Let me quickly talk about mercury concentration metabolism in infants receiving vaccines containing thimerosal in a descriptive study. This study appeared in Lancet. It is a very good study, but has no implications to autism. This study was done with normal children. If all those children had a normal efflux system, a normal mercury efflux system, then there is no way that those pharmacokinetics are applicable to an autistic child.

Let me say that I know these people. They are all very, very good scientists. Elsa is one of the best mercury analysts in the world. But the pharmacokinetics are not applicable to an autistic child. Thimerosal pharmacokinetics obtained using non-autistic children are not the same as those expected for autistic children. The latter appear to have different efflux genetics.

I spoke on Friday, I spent two hours with someone who we consider to be one of the country's leading pharmacokinetists, and he agrees with this statement, because I certainly did not want to make a statement that was unreasonable.

I'd just like to remind you that epidemiological studyie cannot prove cause and effect. Rather, they reveal statistical correlations. I think we should keep that in mind.

I am almost done. Results of research using thimerosal in non-human primates may not be applicable to autistic children, and should be viewed and used with caution. There are very good studies going on at the University of Washington and the University of Rochester, dealing with thimerosal in monkeys. They are going to be very important studies. But one must realize that these are normal studies, and depending on what data they are getting from these monkeys, again they may not be applicable to children with a mercury efflux disorder.

Summary. Organic mercury compounds have a delayed response. The Holmes paper, the MIT paper and the Bradstreet paper together indicate that autistic children have a mercury efflux disorder. Pharmacokinetics in non-autistic children may not be applicable to autistic children. The results of some research using non-human primates may not be meaningful when extrapolated to autistic children because of a possible efflux disorder, and it is this toxicologist's view that the link between

thimerosal and neurodevelopment disorders in children has become more plausible.

I was asked to come to this meeting. I didn't want to come. I was asked to come to this meeting to address that question. I think after studying it very, very carefully, I believe it has become more plausible than when your committee first discussed the subject. Thimerosal appears to add organic mercury to the mercury burden of children with mercury efflux disorder.

Thank you for your attention.

DR. MCCORMICK: Questions?

DR. BAYER: You have proposed an interesting theory or hypothesis that requires study. But to your knowledge, is there any clinical or epidemiological evidence that lends credence to the theory you have put forward?

DR. APOSHIAN: You must forgive me, because my training is not as an epidemiologist, and neither am I a physician. I am used to looking at hard experimental data published in peer reviewed journals, and making an opinion as to whether it is correct or not, believable or not.

Based on the MIT study, based on the Amy Holmes study, based on the Bradstreet study, I think there is

going to be a mercury efflux disorder here. We cannot prove it at this time, because we need the tissues. Autism is not my research area, but I have gotten involved in this during the last week. When I go back, I am going to call some people and get some mercury tissue. I think I know where I can get it now, from autistic children, and analyze it.

I think that will be the key question. It has nothing to do with epidemiology. Epidemiology, as I'm sure you know much more about than I do, has been wrong many times. Epidemiology just shows a correlation or not. It does not show cause and effect.

DR. BAYER: So-called bench science has been wrong, too.

DR. APOSHIAN: Pardon?

DR. BAYER: So-called bench science has been wrong, too.

DR. APOSHIAN: Of course. We are the first to admit it. I want to make it clear that this is a hypothesis. We have gone with as much hard experimental evidence as I think we have to come to the conclusion.

What really tipped me, I must say, was the MIT study. That tipped me enough to have much more confidence

in the single experiments that were done before.

DR. KABACH: Do you have some hypothetical basis for explaining the four to one sex ratio in autism that would involve some X chromosome or other mediated mechanism? This mercury efflux mechanism, whatever it is, would have to be very strongly influenced by the sex of the child.

DR. APOSHIAN: There is evidence I'm sure Boyd Haley will bring up later, to answer that question. Let me say, one of the problems with this whole field has been, we don't know the enzymes or the carriers involved in mercury. It has not been a subject that has had intensive investigation in the past.

My own research deals with arsenic. We went down to Mexico, we have shown polymorphisms in the gene that controls arsenic metabolism in humans, because we knew what enzyme we had to deal with and what gene. We don't know the proteins involved in even demethylation of mercury.

Does that answer your question?

DR. KABACH: (Comments off mike.)

DR. APOSHIAN: That question I would much rather have Boyd Haley answer.

DR. FOXMAN: Can I follow up on this hypothesis?

I think it is a very interesting hypothesis. What I am wondering about is, on the basis of this prediction, would one predict that then autism would look like mercury poisoning? If you have efflux disorder, would you anticipate that autism would then have symptoms similar to mercury poisoning?

DR. APOSHIAN: Actually, I think I coined the term a number of years ago, micro mercurism. Micro mercurism is very similar to what Needleman found with lead. If Needleman had not done the fairy godmother teeth studies along with the correct neurodevelopment studies, we would think that the toxic level of lead in children was 50 micrograms per deciliter. But because of those -- micro mercurism, the signs and symptoms of a low level but toxic level of mercury in the body, the signs and symptoms are so non-specific, that you can't differentiate it from anything else.

Most of the time you diagnose mercury toxicity, it is based on urinary mercury or blood mercury.

DR. FOXMAN: But if you have people who have efflux disorder, then they are excreting hardly any. So you would expect over time as the child aged, that you would get more and more mercury, and then you would get

classic mercury poisoning, unless I am misunderstanding your hypothesis.

DR. APOSHIAN: If I may go back to Wilson's disease, Wilson's disease lacked the copper efflux protein. That is known. But they don't show signs and symptoms of Wilson's disease until around 15 to 20 years of age or beyond. We have a neurosurgeon here, I think he will tell you that.

DR. FOXMAN: But that doesn't answer my question with respect to --

DR. APOSHIAN: We don't know.

DR. FOXMAN: But does autism in any way mimic symptoms of mercury poisoning? I don't know anything about what mercury poisoning looks like, so I am asking you if those two are similar in any way, which based on your hypothesis I would predict.

DR. APOSHIAN: And what I am saying is, the signs and symptoms of mercury poisoning are so indefinite and so non-specific, that you can come to any conclusion you want.

I don't mean to evade the issue, but I can give you certain signs and symptoms that are similar and certain signs and symptoms that are not. But it is because we are now talking in children about a low level of mercury. The

Bradstreet studies don't show a huge amount of mercury, it is six times the amount of mercury. As we say in toxicology, all you need is one molecule to react with one essential protein, one essential enzyme. If there is not enough regeneration of that enzyme, you are going to see some adverse effects eventually. We always put in the word eventually.

I'm afraid I'm not doing a very good job of answering your question.

DR. MCCORMICK: One more question.

DR. JOHNSTON: I think this is an easy one. Could you review for us, please, the placental transmission of mercury, and the transmission in milk, and how do those levels that are transmitted within that range of one to six milligrams, whatever, compare to the exposure to thimerosal?

DR. APOSHIAN: I don't have that data. If I told it to you, it would not be exact. The data is available, especially now since Katie McHefty has just shown that maternal blood is not a good indication of the mercury burden of the fetus. In fact, the fetal blood is almost 1.7 times greater than the maternal blood.

DR. JOHNSTON: So the fetus tends to concentrate

it?

DR. APOSHIAN: Yes. What this said is, we believe the fetus cannot get rid of mercury as rapidly as the mother.

DR. JOHNSTON: How long would you expect that mercury would stay, hang around in the brain?

DR. APOSHIAN: Again, I hope this will answer your question. If not, push me a little further. About 25, 30 years ago in New Mexico, a family had a pig, and that pig got into a batch of fungicide. My indication was that it was methyl mercury fungicide, but just on the plane coming yesterday, I was reading a paper where they claim it was ethyl mercury fungicide, so I don't know which one it is, but it certainly is an organic material.

Two days later, not knowing the pig had done this, the family slaughtered the pig for their food, as their major protein source. Two of the children died within a few years, but one child lived until she was 21 years of age. This is in the neurological literature, a first-class journal it appeared in. In that 21 years later, the brain mercury of the woman who had ingested it as a child was 100 times greater than what is normally seen for the brain mercury level.

So we believe that once mercury gets into a cell and into the brain especially, it is tied up very, very strongly. I really have very little confidence in a lot of the T1 half data that has been given our for mercury for that very reason. We know it stays there.

I have spent the last ten years of my life trying to find an agent that will mobilize mercury from the brain. It is very difficult to find. We haven't been successful.

DR. MCCORMICK: Thank you. Our next presentation will be given by Dr. David Baskin, professor of neurology and anesthesiology at the Baylor College of Medicine. Dr. Baskin was invited to discuss the relation of neurotoxic effects of thimerosal to autism.

Agenda Item: Relation of Neurotoxic Effects of Thimerosal to Autism, by Dr. David Baskin

DR. BASKIN: My neurology friends would be horrified I was called a neurologist. I am a neurosurgeon.

It is a privilege to be able to speak to you today. I wanted to talk to you a little bit about some of the research that I am doing, and also a little bit about how some of this might all come together.

My funding has been from NIH, from Veterans Affairs hospital, from merit review system. I have had

fundings from the advanced technology program at Texas, in collaboration with Richard Smolling and Nobel Laureate Rice, where we have had funding to look at neuromaging of cellular damage. I have had funding in the past from pharmaceutical companies. I have had funding from Upjohn to study a drug that can reverse spinal cord injury, namely, methyl prednisolone, which is now standard of care, another drug called terilozad we were going to hopefully treat brain hemorrhage with; that didn't work out. I have also had funding from Intermedics and Fusaid to look at a way to deliver a cholinergic agonist into the brain for treatment of Alzheimer's disease. That didn't really work very well, had a very weak effect.

I also received some private funding from foundations in Houston that are basically to promote the research and treatment in neurological disease, including brain tumors, stroke and other neurological disorders. I do not have any other funding for this research or any other.

What I'll talk a little bit about -- what I talked to this committee about several years ago was the striking discrepancy between the classic neuropathology findings that are described by the master of all this to

begin with, Margaret Baumann, who felt that autism was a disorder that occurred before 30 weeks of gestation, based on the fact that there was a lack of gliosis in the infraoliveri nucleus, and there was no retrograde cell loss. She described this relationship between the oliveri climbing fibers in the Purkinje cell dendrites that occurs in this area called the laminate desicans. This disappears after 30 weeks of gestation. So since there was no gliosis and since there was no retrograde cell loss, she concluded that the injury must have occurred before those two cells came together. That seemed very sensible at the time.

However, that was all described before we knew a whole lot about apoptosis. Apoptosis could explain a lack of gliosis and no retrograde cell loss, because apoptotic cell death is designed to produce a seamless elimination of individual cells, not damage the synapse. This is essential for normal development. You can't prune cells if you prune everything about them. So if it was the case that mercury and/or thimerosal did induce apoptosis, then this could explain Margaret Baumann's finding. It wouldn't controvert what she found, but would controvert the hypothesis that this has to be a disorder before 30 weeks of life.

Indeed, there was some supportive evidence at the time, which is that people who had done MRI imaging didn't see any found loss of cerebellar gray matter in the region where we see this profound Purkinje cell loss, but that was all sort of supportive and indirect.

My laboratory has looked at apoptosis and ways to detect it for a number of years. We undertook a study looking at various methods for detection of apoptosis, including the gold standard, which is activation of caspace 3, which is the enzyme activated in apoptosis, a test looking at membrane permeability with the vital dye DAPI, a more subtle and newer test looking at mitochondrial potential changes, which both detect apoptosis and necrosis, and also just looking at light in electron microscopy.

So we did a very simple set of experiments. We took human frontal cortex from adults who had undergone a hemispherectomy for seizures. This was available through the ATCC, a standard source for NIH work in cell culture. We took fibroblasts and we took Jurkat cells, T-cell lymphoblasts.

We grew them in culture and stabilized them. We incubated them with thimerosal, and then we looked at all

these assays to see what we found. The results are that we found a dose and time dependent relationship. At six hours, you could see DNA damage and membrane damage, but you could also see activation of the active component of caspace 3, which is a gold standard and specific for apoptosis. We could actually see the apoptotic bodies in condensation in light electron microscopy, and we found that the lowest toxic concentration in six hours was two micromolar. However, if you go 24 hours, you find that for neurons, the lowest toxic dose is about one micromolar, as I will explain to you in a minute, showing of course that there was increased sensitivity with longer exposure.

We were able to document DNA damage, nuclear membrane damage, again that caspase 3 activation, and apoptotic morphology. The interesting thing, and what led me onto the path that I am taking now is, we said, let's look at some other cells. So we looked at Jurkat cells, since they are tough immortalized lymphocytes, and we found that lo and behold, they were much more sensitive than either fibroblasts or neurons, after 24 hours of exposure at 250 nanomolar, which by the way is at or below the vaccine dose, depending how you calculate how much uptake you think there is into the brain. We saw active caspace 3

detection, we saw change in mitochondrial potential, so there really seemed to be a differential sensitivity with the Jurkat cells being the most sensitive.

This is a graph that illustrates what I have just told you. We don't see anything. We have a basal rate of apoptosis, which all cell cultures have. We go up to 250 nanomolar and 500 nanomolar and 1000, we have statistically significant increases. All of these are done in wells, with a minimum of ten separate wells at the same dose.

This is just a little diagram of the kinds of things we are looking at. This is our DAPI test, where as cell membranes are damaged, the vital dye gets inside. At six hours we begin to see results at two micromolar, and as you go up on dose, you see greater toxicity.

Here is an example of the apoptotic bodies. You can see the neurons, these little budding pieces of cell that are extruded in the process of cellular apoptosis, and we see them here as well. Of course, you see more of them at higher doses, but you see quite a bit at lower doses. We have an electron microscopy that confirms the morphological changes of apoptosis.

Here is a double stained slide, where we looked at a low dose of thimerosal, one micromolar in neurons.

You can see the neurons staining green for expression of the active caspace 3, indicating that they are undergoing apoptosis. And of course they are dying. Their membranes are disrupted, so the vital dye is getting into each and every one of these cells as the membrane is being torn up by the apoptotic process.

So we asked ourselves, what was the basis for this differential sensitivity? Why are Jurkat cells, why are lymphocytes so much more sensitive than neurons? It is because they are dividing? It doesn't seem to be the case. We looked at senescent lymphocytes, lymphocytes that were old and no longer dividing, and then we looked at young lymphocytes, and there seemed to be no difference in the rate of cell damage between one or the other. If we look at a mitochondrial potential disruption though, we see again a very, very tight dose response relationship.

Indeed, in neurons, looking at this more subtle assay, we see some effects even at 500 nanomolar. So in this part of our work, we concluded that thimerosal toxicity was dose, time and cell type dependent. T cells were more sensitive; 250 nanomolar was toxic to Jurkat cells, and it didn't seem to be due just solely to the fact that the cell was dividing rapidly.

For neurons, we saw a dose response relationship as low as 500 nanomolars. This raised some concern in my mind, because we are using adult human frontal cortex. We know adult cells are much more resilient than infant cells. We know that there is some preferential brain uptake, as much as five fold compared to the blood levels, and we had a very short exposure time. However long you think thimerosal or methyl mercury hangs around in the blood and brain, it is probably more than 24 hours. Yet even at 24 hours we were seeing significant effect.

So these raised some concern, and I think put to bed in our mind the apoptosis hypothesis. Indeed, the primary mechanism of cell death from thimerosal exposure at least in this range seemed to be apoptotic cell death.

So we turned to do what I thought was the obvious experiment. But of course, it is still a work in progress. I do hope that the IOM will once again convene a meeting in about a year or so, because I think the science is still evolving.

We took the AGRE cells, the autism genetic source exchange, which is a bank of blood, DNA and immortalized cell lines, from autistic children who are very well characterized clinically, as well as blood from their

unaffected siblings. We asked the obvious question, if you take these two cells and you culture them and you put it in the same dose, do you see a difference.

These cells are immortalized with Epstein Barr virus, which is important, because it makes them very, very resilient. It turns out even though we wanted to start with a low dose, we were just at the limits of toxicity, because these cells are tough and they don't die. The virus actually injects its DNA into the nucleus.

So we took these cells, we expanded them in the laboratory. We treated them at different doses of thimerosal for 24 hours, and we did a very, very meticulous experiment, using 384 well plates. Each value had ten peets per measurement. We did it twice in the early experiment so that we could be relatively comfortable that whatever our results would be, they would be accurate, particularly since we didn't know that we would see the response at all to sib pairs. It might be that some children were more sensitive than others, so it was very important to use that we would have good data for each pair.

We used two assay detections, one on the caspace 3 detection, since that is the gold standard for apoptosis

detection in the world today, at least in the world of apoptosis. We used another assessment called calcine-AM, which is an assessment of esterase activity. Esterase activity in the cell correlates with general metabolic activity in the cell. It can be used to detect cell division, but it can also be used to detect just how metabolically active the cell is or isn't.

What we found was this. If you looked at caspace 3 activation in lymphocytes from autistic kids compared to siblings, we found three sets that had a difference, all in the same direction. The normal sib didn't seem to have any statistically significant increase in caspace 3 expression with increasing doses, but the autistic child did.

This shows another way, looking at the autistic child's response at the caspace 3 expression as a function of increasing doses of thimerosal. This is the normal and this is the autistic. The very interesting thing was that we saw the same effects in both assays. In other words, the same children that displayed the response to thimerosal as far as caspace 3 activation and apoptosis expression had the response in the esterase assay.

Look what happened here. Number 17 is autistic, number 18 is his normal brother. With an increasing dose

of thimerosal, the metabolic activity of the cell is depressed. This becomes statistically significant. Whereas it is random fluctuation, that at this dose of thimerosal, nothing happens to his unaffected sibling.

Here is another sib pair showing more or less the same thing, not much at 100 nanomolar, which is a very low dose, but at 501 micromolar we see differences, where here we don't.

So I must emphasize, this work is preliminary. It is not yet ready for final publication. But I present it here because it is an example of a quantitative assessment of live cells from autistic individuals using molecular biological tools, and it suggests -- and I underline the word suggests now -- that a subset of autistic children appear to be more sensitive than their unaffected siblings to thimerosal. We certainly need more work. We need higher doses and longer exposure times.

One of the things that I didn't realize was that Epstein Barr virus makes the cells so tough, that I think we are probably at too low of a dose. Normally lymphocytes and lymphoblasts are sensitive at this dose, but by immobilizing them with EB virus, you make them a little bit more resilient, so we need higher doses.

But this is an absolutely essential finding to the hypothesis that thimerosal in some way contributes to autism. There are so many children who have received a certain set dose of vaccination, and their brother or sister received the same thing. One child gets autism, one child doesn't. If there is no differential sensitivity in these children's tissue, you can't explain this. So this is at least supportive evidence to the hypothesis, and I think something that would be essential in order for the hypothesis to go forward.

There is an awful lot of things to say to try to tie things together, but time is short. I only have a little over five minutes. So I want to focus on the individual who really should be presenting here. It is brilliant work. It is Richard Dieth at Northwestern University. You probably heard his name several times before. He has looked at some very interesting changes in the folate pathway.

Richard would probably cringe with my oversimplification of his work, but he has basically focused on the enzyme methionine synthase, which as you probably instantly remember from your biochemistry takes homocysteine to methionine.

Now, methionine is a source of methyl groups. Why do we need methyl groups? We need methyl groups for several reasons. Number one, all of gene regulation, it turns out now, is based on methylation and acetylation. We silence the activity of genes by putting methyl groups on the DNA, and we activate them by pulling them off.

This is a fascinating exploding area called epigenetics. Most of the molecular geneticists in this country are now really furiously publishing on this subject. This is the main source of the methyl group for methionine. So if there is something wrong with this enzyme, if this enzyme activity is decreased, you have less methylation, and suddenly genes that are silenced will become activated.

So there is this balance between homocysteine and methionine, but there is another very important function for the methyl groups. The methyl groups bind to the phospholipid membrane and methylate the membrane. So is that important? It turns out that cell membranes have signalling characteristics, mediated at least in part through the DR receptor. This is a very popular receptor now that is involved in cell signalling. This process, this group of methyl groups and the DR4 receptors or how

many receptors there are per volume, has already been implicated in other psychiatric disease.

So the idea here goes, if there are less methyl groups available, not only do you disturb the balance in terms of gene silencing or gene regulation, but you disturb -- at least the hypothesis goes, you disturb phospholipid methylation and membrane signalling.

It turns out that studies recently have showed that methionine is decreased in autistic individuals, and preliminary studies again on these AGRI cell sets, a small number, shows that methionine synthase is also decreased.

What Dr. Dieth did, which is absolutely critical for this hypothesis, he looked at methionine synthase basal activities, showed that you could stimulate it either with IgF 1 or dopamine, and then if you add thimerosal to the mix, not only do you depress the basal rate, but you can no longer stimulate this with some of the normal cell stimuli that will stimulate the activity as the enzyme is needed.

In addition, he looked at phospholipid methylation and did the same sort of work, showing that both lead and mercury would suppress the amount of methylation of the membrane. So at least there is here now a potential mechanism for how this works, which is that

interruptions in gene regulation, genes' activation and silencing and changing membrane signalling, one can produce different states of the cell that are pathologic.

This is not a completely novel idea. You probably all know about Rett syndrome. This is an MECP2 mutation, which interferes with binding of methylated CPG sites. The protein necessary for histo modification and gene silencing doesn't form. And of course, Fragile X is a hypo methylation state with CCG repeats at chromosome 27, the X chromosome at 27.3, the Q region. So this is not a new idea. By the way, these are two diseases that are autism related, in which you see expression of autistic phenotypes.

We know that there are some other clues. I know that people are very interested about whether exposure to thimerosal could produce similar anatomy. Dr. Horning discussed some of their work with you, but she has also shown some loss of Purkinje cells in the cerebellum in her mice exposed to thimerosal, which is the single most reproducible finding in the neuropathology of autism. So that is kind of interesting.

Martha Herbert at Massachusetts General has done studies showing an increase in the white matter in the

first year of life, in parallel with vaccination schedules. Erica Shane has done this work in the past with MRI studies, corroborating the increase in head size.

So if I could try to put together some pieces of the puzzle, as someone said earlier, there is no genetic disorder that increases from one in 10,000 to one in 160 in one generation. There has to be some sort of environmental trigger. So I conceive of the etiology of autism as an interaction between environmental factors and genetic factors, genetic factors influencing methionine synthase. By the way, in data that I didn't have time to show you, Dr. Dieth showed that the primary influence on methionine synthase is through PI3 kinase, which by the way also regulates apoptosis. PI3 kinase is one of the main signalling pathways that starts apoptosis.

There is a whole lot of evidence about immune changes, which could either be due to methylation changes on the genes, then releasing or causing a change in the state which causes autoimmunity, or a direct effect of mercury producing autoimmune responses as you see on the lymphoblasts, which are much more sensitive.

The idea of an efflux disorder, you have heard the hypothesis, and certainly I think the studies on the

autistic brain need to be done. Then these genetic factors interacting with other toxins, whether it is mercury pre or post natal, or perhaps other toxins which are not yet discovered, and the fact that we have gone ahead and injected a neurotoxic substance into these children, on top of whatever other insult they have already received, which can't be zero, not in the environment we live in.

So what does this lead to? Once you get this regulated apoptosis, which I have shown you some examples of, you get mercury induced autoimmunity, pretty well described both in an article from Scandinavia recently, and by Eric Hollander at Mt. Sinai, who has shown antibodies to heatshock protein. It is not found either in patients with autoimmune disease or in control patients. The decrease in methionine synthesis and all those consequences, and another downstream effect on methionine synthesis is a decrease in glutathione, which is one of the cellular protections against reactive oxygen species and oxidative stress.

So from this initial humble beginning, you get all sorts of disregulation like what is seen in autism. None of this is speculative. All of this has been documented in good studies at good institutions.

So I implore you to consider the fact that this is still an issue on the table. It is still a plausible hypothesis. I believe it is moving more towards a causal link. We certainly need better epidemiology. I have sat in on some of the discussions at CDC, and I think these studies are very hard to do. No matter how you design the study, there always are some flaws.

I would implore the committee to consider what I mentioned earlier in the question period, which is, the best way to study this is to study it with populations at risk. That is how we found folic acid and neural tube defects. If you looked at folic acid and neural tube defects and did this epidemiology, you might not see it. I agree with the comment made by the previous speaker that it may not be that 100 percent of children with autism have this, so you would miss it if there is a subset of children that have it.

I would ask you to please ask for more funds to look at the mechanism, the pathophysiology of mercury as it relates to autism. This is still a huge issue, even though thimerosal is hopefully going away, because of all the coal power plants. That is our main source of energy. I would love to see the Institute of Medicine put some more

pressure on Congress to tighten up the regulations regarding cleaning coal and getting the mercury out of the atmosphere. It is so ridiculous. Why should we regulate fish consumption? We should regulate coal and power plants and how we clean coal, and that technology is there.

I think it would be a modest and wonderful thing if you would encourage in every way possible funding of studies that will look at treatment strategies to attempt to remove or reverse the effects of mercury.

Thank you.

DR. MCCORMICK: Any questions?

DR. JOHNSTON: in the experimentation on the system that controls apoptosis and autistic cells, you've got lymphocytes, you've got a different --

DR. BASKIN: Absolutely. PI3 kinase would be the place to focus, because it is a regulator of apoptosis. It does seem to feed in through the methionine pathway. So if this idea is correct, we should see very specific things and manipulate that system very well. So it is a good question. I have thought of it, but I don't have the answer yet.

PARTICIPANT: Of the ones you looked at, how many pairs have you actually tested to date?

DR. BASKIN: We looked at ten and we saw three. That is why I said it may be a small subset or it may be that my dose is too low.

I have had some people who are very experienced with cell cultures look at some of the others, and there are trends. I could show you curves, but they are not statistically significant. They don't go in the wrong direction, they go in the right direction. Everyone said, I think you used too low a dose. I didn't think about the Epstein Barr virus making the cell so tough.

PARTICIPANT: Can you help put that together, how that ties in with the idea that there might be an efflux disorder? Does your apoptosis stuff tie in with that directly or not?

DR. BASKIN: Well, no. There has to be yet a separate issue related to transport of mercury in and out of the brain in terms of ethyl mercury, whether it is elemental mercury or whatever happens in the brain.

My work would more say, given a set concentration of mercury, one person may go down a very disastrous pathway with apoptotic cascades and change in cellular metabolism and another may not, due to some other inherent genetic differences. It doesn't speak directly to the

efflux disorder.

But I think the evidence for the efflux disorder is there. You asked about clinical studies; I think it is a clinical study to cut hair and measure it. It certainly should be replicated, but I think the preliminary evidence is interesting. I agree with Dr. Aposhian; seeing another group at MIT replicate the same thing with very close data -- it is at least alarming and of concern.

DR. BAYER: Since there appears to be an undeniable increase in the level or rate or prevalence of autistic disorders, at least in those societies that can measure those things, industrial societies, and since the Danish data seems to suggest that the removal of thimerosal has not changed that epidemiology.

The question I have for you is, since we are in the area of speculation in general, what proportion of autism do you think could be linked to -- in your own thinking, to thimerosal, if given what we know about Denmark, given what we know about the increasing rates of autistic disorders?

DR. BASKIN: I think there are two separate questions in your question. The Denmark study, the doses are lower. Those children received less total mercury

burden than the U.S. So I don't know for sure that it shows that -- I don't know for sure whether what they received is enough to reach the threshold or not.

The bigger question which is much most important is what percentage. I don't know. I think that looking at stuff like autistic cell cultures is one way to get at it. You should expect it to be more or less whatever the percentage is you see. But until you do dose response and time courses, I don't know.

I suspect that it is going to be more than we think. I suspect that there should be interactions with other forms of mercury, and there may be interactions with other toxins. We may find out in 100 years that we are putting all sorts of toxins into ourselves, and the issue with mercury was, why did we want to inject yet even more.

I don't know the answers to any of it. It is pretty speculative. I suspect it will be at least 30, 40 percent. The incidence of regressive autism is about 40 percent, but I don't know whether regressive autism is the ones that get the mercury. You could argue the ones that don't regress may have a bigger hit. They never get good to begin with to get bad. So I don't know, it is speculative.

I think cell culture studies would be a way to start, with objective data, with good microbiological assays, to see what is different about the tissue on a sib pair basis.

DR. MCCORMICK: Any further questions? Thank you. I think we gained a couple of minutes. We will now hear from Dr. Polly Sager, who is Director of International Research for the National Institute of Allergies and Infectious Diseases. She was invited to discuss thimerosal exposure from vaccines and ethyl mercury accumulation in non-human primates. Dr. Sager will have 20 minutes for her presentation, and ten minutes for questions.

Agenda Item: Thimerosal Exposure from Vaccines and Ethyl Mercury Accumulation in Non-Human Primates, by Dr. Polly Sager

DR. SAGER: I'm Polly Sager with the National Institute of Allergies and Infectious Diseases. I've been there for 15 years. Before that, I did work in industry. I'm proud to say in my three years working in industry, I never once worked on a company compound. I had an odd job that allowed me to do basic research and publish whatever I wanted, so I believe I have no conflicts of interest here.

I'm going to talk to you about some studies that

NIAID and NIEHS have been involved in with thimerosal. Some of these first slides that I think people on the committee have seen before. The scientific question where we began was really looking at whether or not the guidelines that were developed for methyl mercury were appropriate for assessing the safety or non-safety of thimerosal, and to ask the question of how are the distribution metabolism and excretion of thimerosal and methyl mercury related.

The possibilities are three, at least, that thimerosal and methyl mercury are equivalent, that they are similar but the guidelines for methyl mercury might offer some additional margin or less margin of safety, based on how the two compounds were treated in the body, or that they were significantly different in their distribution metabolism and excretion.

This is just to remind you that the thimerosal exposure we are talking about now in the context of vaccine studies is by injection. It is intermittently spaced. It is primarily exposure of infants and children, whereas the methyl mercury guidelines for exposure, the numbers you have heard about, are based on methyl mercury in food. It assumes oral intake. It assumes continuing exposure to

steady state distribution. It looks primarily at the part of life that is most sensitive, which is prenatal exposure to methyl mercury, and the extrapolations are done for -- the prenatal exposures are done for maternal hair, not maternal blood. Those of you who know the studies from Iraq, they showed that it was maternal hair levels of mercury that correlated with damage to the infants.

We have heard prior reference to the study that was done a the University of Rochester. I just wanted to summarize two points from that study. One was that the blood half life of mercury after thimerosal exposure to vaccines appeared to be shorter than what is the half life for mercury in adults, possibly as short as six to eight days. These were again normal infants, as far as we know, although the children were at the time between two and six months of age, and I don't believe there has been a followup to indicate where those children are now.

The real surprise from that study was that infants excreted a significant amount of mercury in their stool. That was something that had not been seen or predicted from rodent studies of methyl mercury.

What I'd like to talk to you about now is a primate study. This is a study done at the University of

Washington by Tom Burbacker and Danny Shen. Tom Clarkson and Elsa Cinnisari at the University of Rochester were responsible for the analysis of these samples. The study was funded by NIAID and NIEHS, and that is why I am presenting it today.

The objective of the study was to compare the relative levels of mercury in brain and blood after exposure to mercury, either in the form of thimerosal or in the form of methyl mercury.

This slide shows a typical routine schedule of vaccinations. When the vaccines contain thimerosal, these are the levels of mercury that would be in a typical schedule an infant in the U.S. would receive. We used that as the starting point for the primate study. This shows you how the study was carried out.

The macaques were mated. The infants when they were born were assigned to two different groups. One group received methyl mercury as an oral gavage, the second group received thimerosal in the context of thimerosal-free vaccines. So the animals were vaccinated with human vaccines, and received a known dose of thimerosal.

The dose that was chosen was not chosen because of any particular level. It was simply that they wanted to

insure that there was enough mercury that they would be able to measure it. You don't do a study like this and find out that the levels are below the level of detection. So the animals were given 20 micrograms of mercury per kilogram, either in the form of thimerosal or in the form of methyl mercury.

You see here the various vaccines that were given and the timing of that. The reason that the vaccines and the dosing was done every week is because the infant macaques on a developmental scale is a week, and macaque development is approximately equivalent to one month in a human infant. So that is why these were done on a weekly basis. The animals were then sacrificed at two, four, seven or 29 days after the last exposure in order to be able to get information on brain levels.

This shows the body weights for the animals during the study. You see there is no significant difference between the controls, the methyl mercury or the thimerosal groups.

I'm going to show you the data in sets, the methyl mercury first, followed by the thimerosal data. These are the individual animals, looking at the total mercury in blood samples in the infants during the period

of dosing. So they were dosed at birth, seven, 14 and 21 days, and then groups of animals were sacrificed out through 49 days.

If we look at the mean mercury levels in the blood, you see here again that they were dosed here. You see it comes down a little bit. They received their next dose. So with each dose, you get accumulation of mercury in the blood, which is what we would expect to see, so this is a good control after the dosing has stopped and the blood levels begin to come down.

Now, for those of you who appreciate pharmacokinetic modeling, I have included the information here. Again we see that there is an accumulation with each dose in the blood before it begins to decrease after the last dose. The half life is on the order of about 25 days for this model, which is consistent with what is seen in other studies of primates with methyl mercury, and the clearance is given here.

These are the data from the individual animals that received thimerosal in the context of vaccines. The amount of mercury is the same for both the methyl mercury and thimerosal animals. You see the animals here. There are a few animals down here at the bottom line at the

dotted lines that received a lower dose of thimerosal. This was only ten micrograms per kilogram. Those animals were added to the information because they wanted to see just how low an exposure they could go to and still get measurable levels.

This is the plot of the mean blood levels for mercury in these thimerosal exposed animals. The purple bars are the ones that have the lower dose. The 20 microgram per kilogram animals are here. You notice that there is very little accumulation over the period of the dosing, and then the decrease after it at the end.

Now, I'm not a pharmacokineticist, so you will have to forgive me on this one. Danny Shen, who was the expert on this and analyzed the data, used two compartment models for interpreting thimerosal data. Therefore, we have two half times, an initial half time of pretty rapid elimination of the mercury during the -- when the animals were receiving their weekly vaccinations and thimerosal, and a slightly longer half time that helps account for some of the washout phase in the animals after the vaccination. You notice the clearance data are given here also.

Turning to the brain levels, these are the data for methyl mercury. Here we have the blood levels. This

is for the washout period, because we didn't have brain samples for the animals during dosing, so it is just during the washout period. So beginning after the last dose on day 21, this is after the last dose, the washout in the blood for methyl mercury has a half life of about 20 days, and from brain about 58.7 days.

The data for thimerosal are given here with the half life for the washout phase of 7.8 days, almost eight days, and the half life in gray of about 17.6 days.

You may have noticed that these graphs are on different scales, so I just want to review the data here for you side by side. The half life in blood of a single compartment model with methyl mercury, a two-compartment model for thimerosal. The thimerosal data was also looked at with the one compartment model, and the half life came out someplace on the order of five days.

You will notice that this half life for the second phase of elimination in the washout is just about the same as what was found in the study at Rochester for blood half life in the children that were receiving vaccines.

Washout from blood. This is the washout period, 7.8 days versus 20 for methyl mercury. Brain half life, 58

versus about 18. The blood-brain ratio, comparing how much is in brain versus blood, 3.6 for methyl mercury versus 5.1 for thimerosal. This is slightly higher. However, it doesn't make up for the fact that there is a great deal more elimination from the thimerosal than methyl mercury.

To show you here the model and the graphs, here I have put them on the same scale, so they are graphed in the same level. With methyl mercury, there is accumulation during the period of dosing, with thimerosal there is very little. The levels in the blood after thimerosal exposure, same amount of mercury, are not as high as you see after methyl mercury exposure. Again, the levels in blood, methyl mercury versus thimerosal for the washout period in brain after methyl mercury exposure versus thimerosal.

So the conclusions from the study are that the initial absorption and distribution of oral methyl mercury and mercury derived from I.M. thimerosal in a vaccine vehicle are very similar. There is a shorter terminal half life for mercury derived from thimerosal, both from blood and from brain. There is minimal accumulation of total blood mercury during I.M. injections with the injections of thimerosal. However, there is continued accumulation in blood after weekly doses of methyl mercury.

Although the brain to blood partition ratio is higher for thimerosal, it really does not make up for the much shorter half life and much greater clearance of thimerosal derived mercury from systemic circulation.

I would just like to thank Tom Verbock and Danny Shen and Elsa and Tom, who did the study, and Annette Kirschner and Cindy Lawler in the NIEHS that we have worked with, and who take care of the study on the NIEHS side.

Thank you.

DR. MCCORMICK: Do you have questions?

DR. BERG: Al Berg with the committee. Other than the comment that you made about the more rapid development of macaques, can you enlighten us any further on known characteristics of macaques compared to humans doing this kind of research, particularly with respect to heavy metals?

DR. SAGER: Yes. The group at the University of Washington has done a great deal of research on methyl mercury. That is one of the reasons we approached them to do this rather nasty study for us. They have looked at very low levels of exposure of pregnant primates, so it is prenatal exposure doing extensive followup on animals for behavioral and developmental effects from very low levels

of methyl mercury during parental exposure.

They find the same sort of effects of prenatal methyl mercury that have been well documented in human cases, where the poisoning levels were so much higher. So it is a very well characterized model for prenatal exposure to methyl mercury.

DR. BASKIN: This is really an important study, and I am glad it is being done. It really needs to be done. One of the things that we learned from Dr. Aposhian today is that as these various forms of mercury get into the brain, they are converted either to inorganic mercury or mercuric mercury or these other mercury species, which presumably are retained longer. Did you study that, or do you know how much of that ethyl mercury that gets in gets converted to other forms of mercury that stays, and then the rest of it comes out? Can you answer that question, or do you know?

DR. SAGER: We don't have the data right now. Part of the problem is that there are no good methods for actually detecting ethyl mercury. Part of the step of actually measuring mercury is to convert methyl mercury to ethyl mercury, so you need to work out different analytical methods.

I will say that we retained a lot of samples -- Washington has retained a lot of samples. These are very small animals, the samples are very limited, and the people at Rochester are trying to work out methods to actually be able to speciate the mercury in both the blood and the remaining brain samples. We don't want them to do it until they know the method has worked out.

DR. BASKIN: That is the critical question. Some obviously gets in, and then it looks like some comes out, but how much of what gets in gets converted to other forms and stays in?

DR. SAGER: We don't know that yet. I think there are people that understand the importance of that question.

DR. DETH: Richard Dieth from Boston. In your experience, you would think that these times and these rates of excretion had probably something to do with the ability of the mercury to be carried by thiol groups and cystine and glutathione and things like that. I suppose that the level of those metabolites might determine the speed of excretion? Or would that be a factor in determining those rates?

DR. SAGER: I think there are many factors. I'm

sure we don't know all of them, but I think that certainly might be one of them, yes.

DR. DETH: I think the Science study showed that in fish, most of the mercury exists as the cystine conjugate. We heard that before as well. So those numbers would probably be different if there were less available thiols to be able to carry out that inactivation and that excretion. So those might be normal values for normal macaque monkeys, I suppose. But if there were some sort of metabolic factors that altered the pathway, then they might be different and perhaps slower.

DR. SAGER: That is a possibility. We had to start someplace.

DR. HALEY: I have two questions. I am Boyd Haley from the University of Kentucky. Did you measure the mercury in the pituitary, and did they do it following the mercury in the feces to calculate is the mercury being excreted totally from the body, or is it just being relocated to somewhere else? Or do you know?

DR. SAGER: I believe that there are samples. I don't know if there are feces samples. I know they intended to try and collect those, as well as hair samples from the monkeys. I don't believe those have been analyzed

yet. The people at Washington tried to collet as many samples as they could to save everything, to make sure that when the methodologies are worked out, they will be able to go back and analyze additional tissues.

DR. HALEY: Did they do the pituitary gland, the mercury level in the pituitary gland?

DR. SAGER: The only samples that have been analyzed so far as these. I don't know specifically about pituitary, I'm sorry.

DR. REDWOOD: Thank you, Dr. Sager, for doing this important research and for listening to us when we ask for these type of studies.

I have two questions and one comment. When I was looking at our slides, the dosing schedule that you had was 20 micrograms, 20 micrograms, 20 micrograms. You had a slide up previously that had the infant dosing schedule that was 12.5 at birth and 62.5, and then 50, and then 62.5. That was a little bit more episodic in the study. Was there a reason that you chose to do a constant dose versus the more episodic dosing?

DR. SAGER: I think when you start a study like this, you want to do as simple and straightforward a study as you can, so you can interpret the data. I think varying

the dose and increasing it, or putting it up and down at different points would make it very difficult to actually interpret the data when you are starting from knowing nothing about ethyl mercury.

So I think that certainly is something that could be looked at in the future, but I think everyone decided to go with a very simple study to begin.

DR. REDWOOD: My second question, and then I'll end with a comment. You stated that there was a higher blood-brain ratio in the ethyl mercury group compared to the methyl mercury. Could you possibly comment on that? Could it be the fact that ethyl mercury more rapidly seems to change over to organic mercury, which would account for more accumulation in the brain? I'm just wondering about the significance of that, if you would please comment.

DR. SAGER: Right. I don't know. That certainly is one possible explanation. I think when they are able to analyze the samples to actually distinguish the mercury, the total mercury as being either inorganic or organic mercury, we may have an answer to that. But it certainly is one possible explanation.

The other thing you need to realize is that when you work with primates, you don't get huge numbers of

animals. So at any given time point, I believe there were three or four infants that were sacrificed. So these were very -- the N is very small. So I don't know if there were a larger sample if that difference would be more pronounced, or would be less pronounced, but we have the data we have.

DR. REDWOOD: My last is a comment. I understand that the primates were also tested for mercury. Their food was tested very carefully, they had no mercury levels before this. I guess I have to just comment that that doesn't really reflect real life, in terms of what we are exposed to.

Something else -- and this is a personal story -- that happened around the same time that we saw this increasing autism was also increasing exposure to thimerosal for rodium globulin products. That is oftentimes overlooked as being small and insignificant, but for myself personally, that was a 65 microgram exposure at 14 weeks gestation and 28 weeks gestation prenatally.

If you look at the recent data that is showing that the fetus accumulates mercury at anywhere from 30 to 200 percent more than maternal levels, I think we also need to step back possibly and look at a model where there is a

prenatal exposure on top of a postnatal exposure, where we may meet some threshold of not being able to further excrete.

So if you decide to do another study, I would really like to see something that had a prenatal exposure as well. Thank you.

DR. SAGER: Actually when we were designing this study and talking about it, we did talk about the possibility of that, and decided that the first study had to be simple. We didn't want to get data we couldn't interpret. So that certainly is something the people at Washington have thought about.

DR. REDWOOD: So you need more money to do your additional studies, correct? Thank you.

DR. MCCORMICK: Thank you. We are now at the point of taking a 15-minute break, which will bring us to five minutes to four to reconvene.

(Brief recess.)

DR. MCCORMICK: I think we will get started. Our next presentation will be from Dr. Boyd Haley, who is chairman and professor of the Department of Chemistry at the University of Kentucky. Dr. Haley has been invited to discuss his paper, which we have heard about several times

today, on the reduced levels of mercury on first baby haircuts of autistic children. We have allotted 20 minutes for Dr. Haley's presentation and ten minutes for questions.

Agenda Item: Reduced Levels of Mercury in First Baby Haircuts of Autistic Children, by Dr. Boyd Haley

DR. HALEY: I'd like to thank IOM for inviting me here. What I am going to talk about, and I'll try and make it short -- what I want to convince you of is that when we start talking about who is susceptible to exposure to mercury toxicity, primarily with autism in this issue, we are going to talk about people that are genetically susceptible.

I am showing this one slide. What this is a slide of is an amalgam. It is 50 years old. It is emitting mercury vapor. This is a test that I have my students do in a class. You can see it on Uninformed Consent. The reason I bring that up is, it is necessary for you to understand part of my talk.

In essence, my talk is going to be the amount of mercury in the birth hair of autistic versus normal children plotted against the amalgam count of the birth mother.

What we see here, if we look at the non-autistic

children with a mean of 3.7, we see a huge increase, or a wide variety up to almost 19 parts per million of mercury in their birth hair, compared to the autistic children, which are near one or less parts per million. We had 94 of these and 34 of these.

When I first saw this data, I have got to tell you, Dr. Holmes and I are reasonably well acquainted after a period of time, but when I first met her, she did not like the idea necessarily of mercury being involved, with thimerosal being involved, because she had an autistic son, and the mercury in his birth hair was nearly zero. Whereas, the mercury in her own hair was quite a bit higher. So when she listened to my research on thimerosal toxicity with neurons, et cetera, she said, how can I explain that? I said, it is simple. The mercury in the birth hair is the mercury that is being excreted. If you cannot excrete mercury because you are putting something like 12 to 25 to 50 micrograms, millionths of a gram, in a body that weighs thousands if not ten-thousandth of a gram, then the mercury would immediately partition into the mass of the body, which is over 80 percent of the area, and that mercury that you see in the blood after a day for certain, mainly after an hour probably, is the mercury that is being

excreted. It is excreted in the glutathione complex form, and that is the form that ends up in the hair.

When we did an algorithm -- and this was done by Mark Wachsel, he set this up. We plotted the expected mercury level following this line, and you can see that the controls more or less follow this. This incudes looking at amalgam volume, fish consumption and vaccines. You can see that it makes no difference if the mother ate a lot of fish or not, whether she had a lot of vaccines or not. The autistic children still stayed down where they did not excrete mercury very effectively.

What is unusual about this data, and I have been doing research as a professor for 30 years, is that there are no significant outliers. These autistic children when we plotted them, the mercury in their birth hair, versus the number of amalgams in the birth mother, essentially every one of them stayed down here, one parts per million, whereas in the control children the level went up dramatically. After you get above greater than ten amalgam fillings, there was a huge increase.

I would point out in this study, the number of people down in this range is considerably more than the number of people with no amalgam fillings. The level you

are seeing here is primarily the level in the hair of control children whose mothers had no amalgam fillings.

This slide is the most telling. It will answer a question that was brought up earlier. If we plotted the mercury in the parts per millions versus the mild, moderate and severe autism case, this was done by Mark Wachsel, because he pointed this out to me, as we came across, you see that as the disease gets more severe, the level of mercury in the birth hair drops. That is the first significant thing. So you see that there is in my opinion an inability to excrete mercury that is paramount in this disease.

The second thing. If you look at the female versus male ratio, as you see in this slide, the males will get this disease or become affected with autism, even though they are better excretors than the females. As we go across here, we see that the number of females drops. Invariably they are below the line, indicating that they have to be much poorer excretors to become autistic than the boys. When we get to the very end, out of this whole case there was only one female in the group, so this does have a gender property as well as a biochemical property, so you have to have a sex link trait and an inability to

excrete mercury.

I think this slide will show it, and I will present it first. What we found, this is neuron survival. We take hippocampal neurons in culture, and we can grow them out for 24 hours and see the number. They are live, most of them will live for some time. If we add 50 nanomolar of thimerosal, we get this intermediate killing rate that will kill over 60 percent of them in 24 hours. With this we add other components initially.

The most dramatic thing that we found is that if we added testosterone at this point, by the time we hit three hours, without testosterone present, with the thimerosal, the 50 nanomolar thimerosal, there was no killing, and yet all of them were dead if testosterone was added.

I would add at this point, if we added estradiol, we had the opposite effect. The estradiol would take this killing rate and bring it up to this level, depending on how much we added. So we have observed with neurons in culture, being exposed to nanomolar levels of thimerosal, the female hormone estradiol is protective and the male testosterone enhances the toxicity.

This is the reason I say you have to worry about

the synergistic toxicities and the effects of other compounds on the toxicity of any compound, but primarily mercury. If we look at neomycin, which is an antibiotic, it is not very toxic at this time point. If you add that to thimerosal and put the two together, you see an enhancement of toxicity that is quite marked.

With aluminum, if we have 50 nanomolar aluminum, it is not very toxic at all. Back at this point you can see no significant killing in the presence of aluminum, and a slight killing in the presence of testosterone, pardon me, thimerosal. But when we put these together, we get enhancement. So children that are exposed to other heavy metals are much more sensitive to thimerosal toxicity than anyone else.

There is this old study, where they showed that if you took an LD 1 level of mercury and an LD 1 level of lead and mixed them together, you ended up with a solution that had an LD of 100. So these things are not one plus one equals two, it is one plus one can equal 100.

We talked about the effects of thimerosal in mercury. If we put in mercury with these neurons in culture, we see significant killing. With thimerosal we see the same thing. If we add these two together, we get a

greatly enhanced toxicity. So I would say children coming out of the mother that has been exposed to a lot of mercury from either her amalgams, eating fish or some other exposure, are much more sensitive to the thimerosal and the vaccines than otherwise one would expect.

Acrodynia is a disease that is caused by mercurous chloride. Mercurous chloride is probably the least toxic form of mercury. The cause of this disease was found, and it affected one in 500 children, and the cause of this was found to be teething powders that the more wealthy parents could afford for their children. It was proven in Cincinnati. It only took the medical community ten years to accept this hypothesis, but once the teething powders were removed from the market, the disease disappeared. It was as contentious, from what I understand, as the thimerosal issue is today.

People say there is no proof that mercury collects in tissues. This was published in the Journal of American Cardiology in 1999. It is looking at the level of mercury and idiopathic dilated cardiomyopathy disease. What we find in this disease is, or what was reported, it has 178,400 nanograms of mercury per gram of heart disease, whereas controls who died of vascular ischemic heart

disease only had eight. That is a 22,000 fold difference. I would say that this would suggest that under certain conditions, tissues in the human body can retain mercury at a level that you may not see elsewhere. You have to ask, where does this mercury come from, and why couldn't these people get rid of it.

There was a question earlier, but there have been studies done with rats. If rats are on a milk diet, it takes them ten times longer to excrete mercury than rats that are not on a milk diet. If you put antibiotics in with that, you see the same effect. The rats on antibiotics have a much more difficult time excreting mercury than rats that are not on antibiotics. And of course, that is specific with certain antibiotics.

Someone asked the question earlier, is there any other data, and this is where we are going now -- is there any other data to indicate that this is a process that we really need to think about, or that we have overlooked.

In a paper by Baumann and Nelson -- and I use this because they were saying this is not associated -- they quote in the Saychelles Islands study of over 700 children, and exposure was to murine fish only, and boys to higher levels of hair mercury performed better on some

tests, including the Boston naming test and two tests of visual motor coordination. This observation has led the American Dental Association and others to say a little bit of mercury is good for you, it makes you smarter. I am serious, that is the comment that came out of that.

What I would point out to you is, it is more than likely in light of the autistic observation, the boys with the highest mercury hair level were those who were capable of excreting the mercury they were eating. I would also point out that there is a casopea study going on, where they are studying the amount of mercury in the blood and hair of children. Half of them get composite fillings, half of them get amalgam fillings. The last time I heard these people talk, the children that had gotten the amalgam fillings had less mercury in their blood than they did before they placed the amalgam fillings. That seems almost an impossibility, unless you start talking about the fact that perhaps different kinds of mercury can inhibit the processes more and prevent the emission or excretion of mercury from the body.

This is a study that was done at the University of Kentucky by one of our professor emeritus, Bill Ehmann and Markesbery, who is in the Alzheimer's Research Center.

They had done earlier studies showing elevated mercury in the brains of Alzheimer's patients, so they wanted a good outside easy biopsy diagnostic, so they went after the nail tissue. They said mercury is decreased in the elevated subjects compared to controls, exactly the opposite of what one would expect. The reason that this worked, it was published back in 1990, it was done earlier than that, it didn't seem to fit the paradigm. They said, mercury tended to increase in the nails with increasing age of the patient and with the duration and severity of the dementia. This decrease is counter to the elevated levels of mercury in the A.D. brain as compared to age matched controls.

So I submit to you that here is a case where the lack of being able to excrete the mercury in your blood prevents it from getting in your nail tissues and is more likely to lead to a neurological problem.

A study done in the Journal of the American Dental Association, looking at the brains of 101 humans, mostly nuns -- it is called the nun study -- did a statistical analysis. I have a lot of objection to this, but I do know the student who measured the mercury in the brain. If we look at this, we found six of 110 subjects with mercury levels above 200 nanograms per gram weight.

This represents between 1.2 and 3.5 micromolar, ten to the minus six molar. That is a huge concentration.

Anyone sitting here who knows anything about mercury would consider that an extremely toxic level of mercury. If we go down to a half micromolar, or 100 nanograms per gram, this increases to about 15 percent of these subjects with highly toxic levels of mercury in their brain. I would again say -- this is a suggestion -- 15 percent of elderly Americans die with elevated mercury in their brains. They are no more exposed if they are nuns. They were all in the same place. They were selected because they lived almost the same type of life. You would have to say 15 percent of these people for some reason, I would think mainly genetic, but it could be genetic plus having antibiotics or other exposures to other toxic materials, collected in their tissues a level of mercury that a reasonable person would consider to be very toxic.

The reason that I am probably standing here is probably based on this slide here. We compared two Alzheimer's patients and two control patients with and without mercury added to the brain tissues. Alzheimer's disease is characterized by the inability to label this protein called tubulin, which holds the axon in shape.

This is tubulin, this is actin. They are the two main components of brain tissue, the two main proteins. If you have seen the film from Calgary, they are the two proteins that are involved in the extension of the axon and holding it in its shape. What we found many years ago is, if you added mercury in increasing concentrations at low micromolar levels, within just a few minutes -- this is not an extended study, this is done on a lab bench -- you can make a control brain look exactly like an A.D. brain, by the basis that you wipe out the tubulin viability, and have no effect on actin.

I put this up for a reason. The reason is, when you use thimerosal as a toxicant, it is quite different.

Two things that we need to know here. Thimerosal with and without light, and especially with light, because it is photosynthesized, it breaks down with UV light, is extremely toxic, more toxic than mercury against tubulin. It also wipes out actin, which mercury doesn't do. What this proves, and what the whole status shows, is that you do not expect toxicity from ethyl mercury or thimerosal to behave like mercury toxicity from mercury vapor or inorganic mercury. They are not the same.

Thimerosal or ethyl mercury does not have to

break down to inorganic mercury to inhibit these enzymes. In fact, ethyl mercury is much more toxic to enzymes than is regular mercury, based on the fact that the ethyl group can penetrate and interact with di-thiols of the enzyme. So there is no reason that one would say this should be an exact mimic.

I would point out that there was a paper published by Sally Bernard and Lynn Redwood and other people in Safe Minds, that pointed out many similarities between autism and micro mercurialism, so those statuses are there. But mercury is quite diverse in its effects that it can cause, so it is very difficult to say that it is an exact mimic.

The contrast between birth mercury in hair levels and body mercury levels. Autistic children have much lower mercury levels in the birth hair. I didn't even know about that study that Dr. Aposhian talked about, but I can tell you that Bill Walsh at the Fifer Institute at Illinois redid our studies in a retrospective way. He went back and looked at all the mercury levels in the autistic children and compared it to those who had schizophrenia and others. He said, even with older children it was correct. So this also confirms what we are seeing.

The second thing he said, though, if children were put on chelation and certain reductive antioxidant therapies, that the mercury in their hair would go up. So this does give us some hope that we might be able to come up with something that would increase the mercury removal from the brain.

Dr. Bradstreet is going to talk about this. There are people -- and I have looked at I don't know how many metal analyses of urine before and after DMPS and after treatment of autistic children. They not only seem to have a lot of mercury, but they seem to collect a lot of heavy metals, which I would expect. As mercury gets in and becomes toxic, what it will do is, it takes the homeostasis of compounds like copper and zinc and wrecks them. They are not normal. So any metal coming in that you should be able to get rid of under normal conditions such as lead or cadmium, you probably will retain.

So the conclusion that I have is, there appears to be a subset of the population that cannot effectively excrete mercury, and they are at greater risk for exposure in the general population. The presence of other heavy metals, antibiotics, may enhance the toxicity of thimerosal. We have this estrogen-testosterone effect that

I think explains the four to one boy to girl ratio and sensitivity in mercury.

I'm done.

DR. MCCORMICK: Thank you.

DR. BERG: Say we accept this hypothesis. We apparently live in a soup of all kinds of toxicities. One of the things that strikes me is your second conclusion, the presence of other heavy metals. One of your graphs for example could be interpreted that mercury is fine unless you add aluminum, and it is aluminum that is the problem.

Can you comment in the context of where you put your research in mercury toxicity with the many other things that are known to affect neurodevelopment?

DR. HALEY: It is not an area where I am a total expert, but I would point out that when you take mercury and you try to mimic the diagnostic hallmarks or the apparent biochemistry that you see in an Alzheimer's disease brain from a control brain, the only heavy metal that will do that is mercury, and only mercury. However, if you add lead or cadmium or aluminum in the amount of mercury it takes to form an neurofibrillary tangle or to strip or knock the tubulin out, inhibit creatine kinase,

inhibit glutamine synthetase, the amount that is required is much lower.

So they are synergistic, but they don't mimic mercury. You cannot chelate mercury with any organic acids such as EDTA, citrate, et cetera, and inhibit its ability to depolymerize tubulin. You can do that with all the other metals. Chelators will prevent them from being toxic. Mercury when it gets in the brain, the organic acids in the brain that are normally there, that normally chelate things like iron, lead, cadmium, they do not prevent mercury toxicity. They prevent or reduce the toxicity of the other heavy metals.

DR. CASEY: Can I just ask, in Wilson's disease, we spoke earlier about having a carrier protein and understanding the mechanism of transport somewhat. if I could transpose this for a second and say, do we know anything about the transport mechanism at all? Could you ever think of it as damaged nervous system, albeit in an Alzheimer patient or an affected autistic child or adult, where those brains for some reason just suddenly not be able to transport mercury?

DR. HALEY: The simplest explanation is, mercury leaves the body as a glutathione complex. When you get

mercury in your blood, the liver doesn't remove it in the form of mercury. It has to go inside a cell, because that is where the glutathione is located, in millimolar levels. That is where glutathione transferase is located, so to remove toxins through the glutathione transport mechanism, you have to be inside the cell, be modified and have the glutathione attached to it.

The glutathione is the binding site for the transporter that transports the mercury out of the cell back into the blood. When it gets to the liver, the biliary transport system, it removes it in the feces through the biliary transport system using bile.

Se if some of the work by Dr. Dieth is correct, which I think it is, you won't have the glutathione to assist in this clearing. Not only that, mercury would inhabit is own extrusion.

DR. MCCORMICK: This is my own question. For many babies, when they are born, the hair that they are born with falls out. So the hair that you would be measuring an average of 18 months later was hair that grew in subsequently. I just don't know enough about the metabolism to say what is being picked up. That was a question, since so many kids lose their hair immediately

after birth.

DR. HALEY: That is a good question. What you find is that it didn't have to be birth hair. I was the one that insisted on that, because they haven't been out doing anything that would cause the mercury to come in.

Dr. Walsh has found that in older autistic children, they don't have the mercury in their hair unless they have been under some sort of therapy. So I think that is a good question.

I would point out, we never had that hair. Amy sent them boxes, and the parents --

DR. MCCORMICK: It is just the metabolism of what would be picked up when the first set of hair actually has fallen out.

DR. SHAYWITZ: Bennett Shaywitz for the committee. One of your slides showed birth hair by severity of autism. I was wondering, how would you measure severity of autism?

DR. HALEY: I'm a Ph.D and I didn't, Amy Holmes did.

DR. SHAYWITZ: Do you know how she did it?

DR. HALEY: I talked to her, and I would rather you asked her. Just however a physician determines how

severe a case of autism is. I don't know. I didn't do that.

I got the sheet from her before we did this, with the amalgams, the severity of the disease, sex and everything else, but I don't know how they did the severity. That was her part.

DR. BAYER: I know that epidemiology is not the flavor of the month today, but let me try this again. Given your characterization of mercury, one would have to assume that thimerosal vanishes from vaccines and medications, that the level of autism should experience some decline. My understanding of the epidemiology of autism is that that is not the case. How do you account for that?

DR. HALEY: You're talking about the Danish study?

DR. BAYER: Yes.

DR. HALEY: That is the reason I asked the young man the question. Our work is comparing apples to cows when we compare the American autism situation to the Danish situation. They are starting out at a very, very low rate of autism, right from the very start, compared to what we have in this country at this time. So I think probably

there are other factors that may happen in utero that induce autism. But the rate of autism in Denmark is somewhere -- I looked at the chart in the paper, and the highest level was around five, and younger age groups it was even lower, down to less than two. In this country, I see numbers where it is 67 per 10,000. So I don't think that comparison of that piece of research is really very relevant.

DR. BAYER: Great Britain?

DR. HALEY: Great Britain is quite a bit. I am kind of bemused by that. It reminds me of the line by Mark Twain, liars, damn liars and statisticians. I sat here baffled as a basic Ph.D scientist; you can get different answers from epidemiology. I know people in England that say the rate is quite higher.

There is a man over there by the name of Tony Bateson who is president of one of the autism societies. I challenged him to find one autistic that wasn't vaccinated, and he couldn't find one. He found one that they thought wasn't vaccinated, of all the autistics in their group. In England, at one point in time, I think about 14 percent of the people were not being vaccinated.

So I can't really get into the epidemiological

thing. I am baffled by it, just like everyone else. You see such different results from people using the same database.

DR. BAYER: Are you implying that the epidemiologists lie?

DR. HALEY: I imply that some epidemiologists lie, yes.

DR. FOXMAN: I have a question regarding your data. It sounds to me, in terms of the stuff that you have presented, it is most consistent with a genetic hypothesis, that if children at birth are less able to excrete mercury when they are autistic, then they are already predisposed at time of birth, is that correct? Is that the proper interpretation? And if that is true, then how do you fit thimerosal in there?

DR. HALEY: Yes, I think it is a genetic susceptibility. I get upset when people say they have genetic abnormalities. An example would be the Alaska American versus the German's ability to drink and detox beer.

DR. FOXMAN: Presume that I said it was a genetic polymorphism. The fact is, it suggests a high degree of susceptibility in some proportion of the population, which

seems very consistent if your data are correct. The question is, how does thimerosal go in there, if these people already have a predisposition to not be able to process mercury?

As has already been said repeatedly today, there are multiple sources of mercury all over the place. I don't understand how genetic variation is somehow manipulated by this environmental exposure.

DR. DALTON: The difference is, the ones that are being exposed to mercury from the mother's amalgams, that is a very low rate that gets in there. So they are getting a very low rate over a long period of time. When the child is born, on the day he is born, he is given a bolus dose of ethyl mercury at a time when his biliary transport system isn't working, at a time when his kidneys are not working, and they are very susceptible at that time.

I would also point out, on the testosterone level, Baron Cohen, he won't tell me his data, but Baron Cohen did an analysis of the amniotic fluid of the mothers who gave birth to autistic children, and the only difference he found is that they were excessively high in testosterone.

DR. MCCORMICK: We have time for two quick

questions.

DR. CLARK: Dr. Alan Clark from Carthage, Missouri. A question, Dr. Haley. On that slide about the large amounts of mercury in the heart tissues in the athletes with idiopathic dilated cardiopathy, these guys I know don't eat a lot of sushi. Is it possible that they are injecting testosterone as a body building agent, which has 25 micrograms of thimerosal to get this level? Is that a possibility?

DR. DALTON: I think that is a very distinct possibility. A lot of young athletes are using testosterone, and many times it is being preserved by thimerosal.

DR. MILLER: Dr. Miller from the U.K. I think it might be useful for the committee to have clarification on the current prevalence of autism that we see, and the exposures. From what you said, the exposures are too low in the U.K. to be in the range of the instance that you see here.

In fact, from the latest study that we did, the prevalence is now in children aged five to 15 three per thousand. That is about half the rate which you quoted as the highest rates in the U.S. Clearly we have seen an

apparent increase in the rise of diagnosed autism to levels approaching those seen in the U.K. over a period when, according to the exposures you are talking about, exposures have been too low in the U.K. to be associated with the kind of levels that you see in the U.S.

So I think we need to be clear. We have seen changes in the epidemiology of diagnosed autism in the U.K. over periods when the thimerosal exposure has not changed, and moreover, has been significantly lower than in the U.S.

The issue about -- that there hasn't been an autistic child who hasn't had a dose of vaccine, with all due respect, that is not an epidemiological observation. We have very high coverage of vaccines in the U.K. Virtually every child will have had at least one dose at birth, and for DTP containing vaccines, about 95 percent will have completed the three dose course. Inevitably, you will see most autistic children will have had a dose of vaccine.

Also, most children who had a dose of vaccine do not become autistic. These observations are not helpful in the debate, because you need to do epidemiologic population-based studies to interpret them.

DR. DALTON: Can I ask you a question? When you

did your study, you were talking about DPT vaccination. Do you not have single dose DPT vaccinations in England?

DR. MILLER: No.

DR. DALTON: No one ever uses a single dose in England for DPT?

DR. MILLER: What do you mean, single dose? We have single dose, and they contain thimerosal.

DR. DALTON: Not added as a preservative.

DR. MILLER: Yes.

DR. DALTON: In England, unlike the United States, they add mercury in the single dose vial?

DR. MILLER: Yes, we do. I showed you the doses of thimerosal per dose, the amounts of thimerosal per dose of vaccine. They are equivalent per dose of vaccine. In DPT, we have 25 micrograms of mercury per dose.

You may say, why is thimerosal added to the single dose vial. That is a different question. But the fact is, it is added.

DR. DALTON: I'll have to take your word for it.

DR. MILLER: No, you don't have to take my word for it. There is an electronic medicines data sheet compendium available on the Internet which will tell you what the additives are in the U.K. vaccines.

DR. MCCORMICK: Our next presentation will be by Dr. Jeffrey Bradstreet, from the International Child Development Resource Center in Florida, where he is the Director of Clinical Programs and an adjunct professor at Stetson University. Dr. Bradstreet was invited to discuss his studies of mercury burden in children, again studies that we have heard about repeatedly, with autistic disorders, and of measles virus genomic RNA, which is new data, in cerebral spinal fluid in children with regressive autism. He will have 20 minutes for his presentation, followed by ten minutes for questions.

Agenda Item: A Case Control Study of Mercury Burden in Children with Autistic Disorders and Measles Virus Genomic RNA in Cerebrospinal Fluid in Children with Regressive Autism, by Dr. Jeffrey Bradstreet

DR. BRADSTREET: Thank you very much for inviting me. It is a privilege to present to this committee. I would add that in part, I am just going to be refining what I told you in the past, because I presented the preliminary data on the mercury set of the DMSA challenge two and a half years ago. Actually, when I was thinking about, it really was two and a half years ago. It is hard to believe

it was that long ago, it seems quite a bit sooner. This is obviously a sign that I am aging rapidly.

In addition to that, I am going to be giving you some new data and, given the amount of time that is available, I won't have potentially the time to develop the subject completely. We will go into some of these as best we can.

The title of the talk is, Biological Evidence of Significant Vaccine Related Side Effects Resulting in Neurodevelopmental Disorders. I hope to be able to defend that.

I do want to thank some of my sponsoring institutions, the ICDRC, the Southwest College of -- Medicine and Health Sciences, and Stetson University, for allowing me to make these comments today.

Dr. Adam Wakefield has joined our staff and our team; he is our Director of Research. I would very respectfully ask that both Dr. Wakefield and Dr. O'Leary's team be given an opportunity to present in much greater detail the allelic discrimination and genomic measles virus in the spinal fluid of these children at some future date, at the convenience of the organization. It is absolutely critical for your understanding to fully develop this

argument.

I have absolutely no data about populations of children with autism. I have evaluated carefully and done histories and physicals on about 1500 children. I have examined them, their entire pediatric records. We have an electronic database that we share with two other universities, in terms of trying to sort through these data sets. Those Universities have IRBs to allow them to investigate our data set, and there is quite a bit of research that will be coming out from the ICDRC in the next few months to years that I think will help to answer some of these questions.

I am not an anti-vaccine zealot. All of my children have been fully vaccinated. I think that a safe vaccine policy is part of public health, and has been for my entire medical career, which is 26 years now. That is another bit of time that flew by rather quickly. I would encourage the appropriate safe use of vaccines, and I hope that what I saw today can in fact refine safety for vaccines.

I have been asked specifically, and been given permission in writing, which has been forwarded to the committee, to present Dr. Jill James' data. Dr. James

recently left the FDA, where she was a biologist looking at the toxicology of various substances as it relates to methylation and sulfation, and has specific data that I think is valuable, and helps to answer the question about how do we get into this mess, and who are the vulnerable subsets of children that seem to be affected by mercury.

I do want to give credit where credit is due. I'd be taking way too much credit for myself if I didn't mention parents, usually the mothers, who brought us these concerns. The scenarios of thimerosal and MMR are largely maternal complaints that were passed to clinicians and researchers, usually falling on deaf ears, and I would include myself in that. It took me actually quite awhile to get the connection, probably three years of hearing it over and over again, before I started to believe that there could be something wrong with vaccines that could be affecting a subset of children. I want to qualify all of my comments as it relates to a subset of children. So persistence on the part of moms is why I am here today. I think they deserve most of the credit, and hopefully they will get most of the blame if you don't like my talk.

So this is our approach, something I learned from my pediatrician, my mentor, Lou Barnett, who many of you

may know. Lou is a brilliant pediatrician, who taught me to listen to the mothers, and to examine the kids specifically. Then I want to ask answerable questions. I am a clinician, my background is as a family practitioner. I am a globalist. I tend to think about the whole package, the immunology, the cardiology, the toxicology, collectively to try and solve various questions. I think my family practice background is an advantage in this scenario, because I'm not hyper focused on one specific pathophysiology.

So as we do that, as we look at the biochemistry, the immunology, the virology, the toxicology and the genomics, which is an exciting field which is exploding rapidly and giving us some very impressive insights, we do see patterns. We can assemble all the pieces as Dr. Baskin did in his puzzle, and I think we see something very exciting.

This however is critical. Timing is everything in pediatric neurodevelopment. The sixth layer neocortex of a human being is very carefully orchestrated. It is like a symphony in the way all these different layers come together. I have forwarded to the committee this entire paper by Dr. Landing, who unfortunately has passed on, but

it is impressive.

If you look at this very carefully, what you will see is, at the time of maximum accumulation of mercury burden in the first six months is when the explosion in the primary motor context takes place. Thee is a rapid development after that. Intriguingly enough, there is a reversal in that whole process at 15 months, which is the time when most of our children in the U.S. get MMR vaccine.

That is just an interesting pattern. That is the way it is suppose to develop normally. We looked at hundreds and hundreds of pediatric brains at autopsy, and did some very careful calculations to actually establish that. That doesn't prove cause and effect, but that tells me that there are critical times and stages when toxins or various other agents, viruses that could be exposed, could actually cause some rather significant differences in the pattern that we see.

So the mercury question. If mercury is associated with autistic encephalopathic neurodevelopment disorders -- and I don't like the term autism, because it doesn't really define anything for me -- can we find elevated levels in children? That was a very simple question, and one that I felt our current laboratory

methods can answer for us.

If those levels are elevated -- this is the further question -- is it related to abnormalities in the methionine trans sulfuration pathway, with result in low cystine, low glutathione and low metallothione. These are the things that regulate mercury, as best we know today, and those are easy questions to ask as well. I'm going to show you the data.

Some of how this is being portrayed, perhaps inflamed in the media, which is why it is incumbent on the IOM to come to some sort of credible decision to help to guide decision makers and the public media. As an example, this is referring to Dr. Dieth's paper. A lot of U.S. researchers peg mercury based chemical as the smoking gun in the development of autism and ADHD. We can talk about that probably for two or three days, but we won't.

This is a comment from Dr. Gold in Canada. This is a pattern of deception that is built into the media. The media is unwittingly passing along what is clearly from a scientific perspective not true. This is Dr. Gold's comment: There is no evidence that low doses of thimerosal that the researchers tested would even cross a child's blood-brain barrier.

I would ask you, is that true based on all the data that we have today. The answer is no, that is not true. In fact, we know both from the people in thimerosal or methyl mercury, depending on which account you want to read, that it was not only fatal, but accumulated rapidly in the brain.

We can go back to some excellent work by Fagan, Pritchard, Clarkson and Greenwood that showed that ethyl mercury in the form of thimerosal rapidly can distribute to the brain. So we have these careful historical facts.

I like this one comment from Fagan et al., that says that the fact that mercury is highly toxic seems to have been forgotten in the form of thimerosal. I hope that we don't continue to make this mistake.

Also, there is a pattern of prenatal exposure to thimerosal from anti-Rho which has been systematically ignored in estimating pediatric risk. It is not in any of the epidemiology or the considerations at the point in time. It is not part of the IOM issue, because it is not actually a vaccine issue, but it is something that I think you have to consider in looking at this. We have an inordinate representation, and this is not an epidemiological study at this point in time, but it is a

very easy one to do. It appears anyway in our data set that there are too many moms who received anti-Rho containing mercury.

This gives you an idea of why I am worried about anti-Rho mercury infusions or flu vaccines given to moms. We know it rapidly redistributes through the placenta to the baby. Based on the size and kinetics of children, this is preterm kids, which you can say are similar to when you might be giving potential mercury exposures. So there is a rapid accumulation and a much higher potential burden in the little ones than in the older children who are full term.

This is very concerning to me as well. I have done work with Indonesia that I have shared with the IOM last time. I am very familiar with the complexities of trying to do safe vaccine policies in a hut in the jungle. I met with those people, I met with the Indonesian officials.

This is a document from the WHO as of 2002, where they are going to be lobbying for the continued use of thimerosal in vaccines to the Third World, and they are trying to discourage if you will the First World from removing thimerosal from our vaccines, because it sends a

bad message to the Third World that in effect, it is not good for our kids, but it is okay for your kids. I know people in Indonesia and Argentina, and they love their children absolutely as much as we do, and they do not want to expose them to potential neurotoxins. I would encourage the IOM, because you are looked at from around the world as the authorities, and what you say about this subject will be taken as though it is the gospel handed down on tablets in places like Indonesia and Malaysia. I know that because when I travel there and talk to them, they refer me back to the IOM on a regular basis.

I would say that you have been systematically ignored in your recommendations, as has the American Academy of Pediatrics.

So I sum that up that any kind of thinking is bound to lead to confusion and unhappiness, by my favorite humorist, James Thurber. I feel that sometimes, some of you may be a little confused and probably unhappy by the end of the day.

I have always felt, as I presented the very first time, that I am talking about mercury. I am not talking about just thimerosal, or thimerosal as it is called in Europe and England. So I am very concerned about mercury

in general, and I am even more concerned, after 600,000 children have been added to the at-risk group of mercury from dietary exposure in fish eating.

This just says that Dr. Banberg was very concerned about the CDC's failure to state a preference for thimerosal-free vaccines. I understand the CDC's rationale for that. I understand how it is integrated with who. I understand world policies on safe vaccines in multiple countries where refrigeration is not available. However, I would say that wherever possible, exposing children to neurotoxins even at a theoretical level is an unwise practice. I will tell you, from a genomic perspective, it is a very unwise practice, and I will give you that data here shortly.

This is our study, a case control study of mercury burden in children with autistic spectrum disorders. We controlled for amalgam, but we did not control for fish in the diet. But I can tell you that children with autism themselves actually are not big fish eaters. They eat chicken nuggets, they eat junk food. They are carboholics. They have a very difficult time getting them to eat any protein whatsoever, and if you don't believe me, you can go do some dietary

epidemiological studies on autistics and find out what they are consuming.

The relative increase was significant, about six times more mercury on average in children with autism, but not all children. Some children with autism, we couldn't find any mercury whatsoever with a DSMA challenge. This is not a therapeutic DSMA challenge, this is a diagnostic challenge, in an effort to see what the relative body burden of mercury is, compared in retrospective when we did this to those who are neurologically normal. I'll go into more details, but the paper largely speaks for itself.

You can see that the range of mean mercury in the entire lot was about four plus or minus eight, which would take you to a negative number, but obviously it doesn't. But essentially that runs from zero to 58.65 micrograms per gram of creatinine, very large in some kids. A huge amount of mercury is excreted on a DSMA challenge in some children, compared to a maximum amount of 6.2 in the neurologically normal kids. So both go from zero to something, but a lot more in the autistics on average.

You can compare this anyway you want. While I do see individual cases where there is high cadmium or high lead in special situations, like if mom watched home

improvement shows and decided to take her 50-year-old bathroom apart, we can find some very high lead in those cases. Those are the exceptions, however. In fact, it is not the norm. While lead almost made it to statistical significance, it didn't, but cadmium clearly did not. But those metals are present. As any toxicologist will tell you, their presence combined with high levels of mercury have a compounding effect that is not additive, it is not one to one, it is a very significant amount of potential increased toxicity.

We compared this, vaccinated against unvaccinated. I wish we had a lot more controls. If you can find them for me, I'd be happy to look at them. However, unfortunately, in this country largely this study cannot be reproduced. This is a one of a kind. Our vaccine policy in this country has changed to the point where we don't have the same exposure to thimerosal anymore. We can doa lot of other things that can help us to define the kinetics of autism in mercury, but we can't go back and look at this historical reference, and I think this makes this a very important study for the committee to consider.

So the answer is, yes, there are relative burdens

of mercury in children with autism spectrum disorders.

The methionine trans-sulfuration issue is extremely important, because it is going to result in a deficiency in the detoxification pathways that are alluded to today. We know what the detoxification pathways are to some extent, not entirely, but we don't know completely how mercury gets out of the body yet.

For here, I had major help from Dr. Jill James, and we have submitted her entire paper in the pre-press form. This is a complicated pathway, but I just want to point out a couple of things. There is methionine synthase. That is what Dr. Dieth has been talking about, but this is an area that we have been very interested in, based on some other observations that folic acid metabolism is abnormal in the autism population.

This is MTHFR. I refer to it as the mother-father gene in autism, because methylene tetrahydrofolate reductase controls the conversion of 5,methyl tetrahydrofolate, which donates the methyl group to be 12, which then donates that back to homocysteine under some assistance here. It leads you to methionine. So you get your methylation out of this part of the pathway, but eventually you have got to come down to cysteine, which

makes metallothionine. There is a paper that has been presented in the American Academy of Psychiatry that says that these kids have a metallothionine deficiency. I'll show you some data that they are glutathione deficient, and I'm going to show you some data that they are cysteine deficient.

That enzyme, cystothionelyze, does not exist in the brain. If you are cysteine deficient in the liver and you are exporting cysteine to the brain, the brain will be deficient. The brain is completely dependent upon cysteine as transported in the form of glutathione to the brain, to remake it back into glutathione for the neurons.

I gave you a paper that was reviewed -- that reviews the importance of glutathione to brain cell function and survival, and it is absolutely critical.

This is Dr. James' information as presented at the last DAN meeting in October 2003. It is headed to press. She was kind enough to share it because of the importance she has felt it has had.

What we see is that methionine is at a relatively high level in controls and significantly less in cases. Methionine is critical, and it has been discussed already. SAMI, which is one of the main methylators of DNA,

contributes to the function of neurotransmitter development. It is significantly low. SAH, which is potentially inhibitory in the intermediary, is elevated in autism. You could go down the list. Everything that should be high is deficient in autism, and everything that should be low is up in autism, and it results in a cysteine deficiency of about 22 percent in her study, and I'll show you ours, which is 21 percent. Oxidized glutathione is elevated, and we see that we have a significant potentia issue in handling heavy metals of all types. This is the primary mercury pathway.

This is our data. We will be headed to press shortly, probably within the next six months. This shows that the percent difference between normals and autistic individuals on plasma cysteine is a 21 percent reduction, almost identical to Dr. James' data, so we have reproduced that in two separate populations, two separate laboratory methodologies, and completely unrelated researchers. I had no idea we were doing this until I stumbled across it and we had already accomplished it. These were independent data sets of different children.

So as of the Human Genome Project, all bets are off. Things are really different now. There are 1.4

million nucleotide polymorphisms. Those are single base substitutions. So you can be one base different from your neighbor and be at risk for a potential disease.

I'll show you what we know about this. If you look at the methylation data on the MTHFR pathway that I provided to you, it occurs in a maximum of 15 percent of Europeans. Fifteen percent is a very interesting number in this, when you look at the potential risk factors of people that are involved. It is variable however, and it doesn't occur the same in all populations. That makes it very complicated unless you do genomic based epidemiology. I would suggest that that is where epidemiology needs to go. There are a lot of people who are starting to do that.

Dr. Boris and his assistant Goldblatt and others looked at 413 individuals with autism in ADHD, and 89 percent had a polymorphism in the MTHFR pathway. It didn't matter if they had ADHD or if they had autism. In our data set, it was 14 out of 15, and in Dr. James', glutathione transferase and other glutathione enzymes or the genomes for those enzymes were abnormal as well.

This would be a specific mercury vulnerability factor. It is not an autism gene, in the sense it doesn't build an autistic brain, but it makes you vulnerable to the

potential effects of mercury, and it makes you likely to have autoimmune disease, and Dr Singh will talk about that later.

I don't really need to present Dr. Deth's paper, except to say that in his findings, a single dose of thimerosal got you into trouble. I want to at least show this. MTHFR is the most common genomic SNP that we found on autistics so far. It is upstream from methionine synthase. This is inhibited by thimerosal. This is already inhibited if you are autistic. If you give this population mercury in the form of thimerosal, it may be 15 percent of the population, and I think you are asking for trouble.

So the answer to the mercury methylation question is, yes, we have defined a genomic biochemical abnormality that would put the kids at risk. I want to show you this, because I think it may tie things together. It looks like I'm not going to get to the measles virus data very quickly, I may be a little bit over, but this I think is really critical to consider.

We have a very large population, 85 percent of individuals who do not have a trans sulfuration methylation defect. We have about 15 percent of the population that

do. If we do an entire population and look at them as the effect of thimerosal, I think you are going to have a dilution effect, where we will lose the significance. However -- and we are going to do this, and we will give you the data within six months -- we are going to look at this population and look at the effect of thimerosal versus no thimerosal in that population of genomically predisposed individuals. Our hypothesis is that you will see something quite significant.

But the other question is, could you see a protective effect in the folks who have proper methylation and proper sulfation. The answer is, potentially, through induction of various genes that are along the methionine pathway, or methionine itself. I don't have time to go into that.

I'm not going to critique Dr. DeStafano's paper, because it is in my handout and you can read that. I would say that these two papers need to be presented, just very briefly, as a background We found measles virus in the G.I. tract of kids with autism. It was typed to the vaccine strain. That told me we needed to look at the CSF. This paper has been accepted for publishment and it will be in the Journal of American Physicians and Surgeons, the

summer issue, coming up.

I want to tell you what we found. We looked at three boys who had autistic regression following MMR vaccine, who had enterocolitis symptoms that were proven at endoscopy. We had proven that they had measles virus in their G.I. tract, two of three had measles virus in their blood. We looked at their CSF; all three had measles virus in their CSF. We got three controls from Tulane, of children who had a shunt for hydrocephalus. They did not.

Moving on to our next data set, we have 28 children -- and this is headed to publication as well, with Dr. Sheils as the lead author, who we have looked at, who we have done spinal taps on. Essentially 70 percent of them have measles virus RNA in their CSF, and one out of about 40 plus controls of a leukemic individual have measles virus in their RNA. Interestingly enough, leukemia is an MTHFR pathway defect.

Let me just go down to the answer to the MMR question. Yes, measles virus RNA is present in the bowel and the lymphocytes. I gave you the paper where it shows that it is in the lymphocytes in the CSF of ASD children, and when AD is possible, so vaccine origin. Always when you find measles virus in two locations, it is presumed to

be an active replicating virus. I gave you that paper as well.

The conclusions are, while there is a great deal of additional research to be accomplished, the data are now sufficient and provide evidence of significant adverse events in a subgroup of at-risk children. There are additional data supporting the role of mercury from all sources in this population requiring mercury reduction and screening to be advised in these children. A likely genomic biochemical link has been discovered within the methionine trans sulfuration and methylation pathways.

With that, I thank you very much for the time and the extra two minutes you granted me.

DR. BERG: It would be helpful if you could recalculate some of your P values in the work from Dr. James and your unpublished work. They are not mathematically possible, given the differences you have in the table.

DR. BRADSTREET: Well, fortunately I told Dr. James to recalculate her P values, and I'm not a statistician. That is one of the values of peer review, and I'm sure in the process of peer review those things would come to light.

These are preliminary data, and oftentimes those sorts of things are picked up in the process of going through peer review. But given the immediacy of getting the data into your hands, I think that the pattern is still quite clear, regardless of the P value calculations. I will have Dr. James recalculate those, and pass them on to you in revised form.

DR. JOHNSTON: You went really fast over some of this because you had a lot to tell us. But in particular in the abnormalities related to methylation, since the MTHFR is very well defined across various populations, what are the data without the Boris and Goldblatt, and in particular the C to T?

DR. BRADSTREET: The C to T homozygous rate is about 30 to 40 percent in the autistic population. It runs about five percent in the non-cardiovascular at-risk population, and in the cardiovascular at-risk population as much as 15 percent. If you look at the literature that is published in people who have heart attacks and strokes, it is 15 percent, but the background rate is about five.

It is a very complicated subject to look at because of the differences in the populations, but it is variable. When I say about five, it may be seven or eight

in different populations, or even lower.

DR. CASE: I realize you were rushing at the end, but could you give us some more detail about the clinical condition of the children who had the spinal taps done? Were they done just for the purposes of this study?

DR. BRADSTREET: No. These were children who had previously had measles virus determined to be present in their inflamed intestinal tissues. So 100 percent of them have been endoscoped and have been shown to have an enterocolitis pattern at the same time they had measles virus present in that enterocolitis. I won't make any presumptions about causality at this point in time.

A significant portion of them had measles virus in their CSF, and they all had a presentation of an encephalopathic regression, almost always associated with autoimmunity to their brain in a pattern that is not inconsistent with measles virus at the time of the spinal taps. The spinal taps were accomplished because the technology had improved to the point that we felt we could find measles virus if it was present. We felt it would be of both diagnostic and clinical significance.

DR. CASE: Did they have CSF cliocytosis, elevated protein, anything else?

DR. BRADSTREET: The full characterization of the CSF is under way in a couple of institutions including Wake Forrest. I can't presume those data at this point in time.

PARTICIPANT: I have two questions. One relates to your study design in your case control study. What sort of study was that? Was that a randomized trial?

DR. BRADSTREET: No. Let me explain how we did the case control. Again, time was not sufficient to go into all the details, and I didn't want to take up the entire afternoon.

What happened was, we had looked at mercury for a great number of years in children who were presenting with encephalopathic or abnormal childhood development disorders, as well as lead and other heavy metals. So it was part of a toxic screening study that we were doing clinically to see was there lead associated with this, because lead presents with an encephalopathic picture as well, and DMSA is an excellent lead chelator. So it shows DMSA looking for lead and possibly mercury.

Then the mercury hypothesis emerged on the scene. So what we did is, we went back and looked at our collected data to see whether or not there was a statistically significant amount of mercury compared to 18 neurologically normal

children whose parents had been concerned about fish consumption and fish mercury exposure in their children, but were neurologically normal.

PARTICIPANT: I had a second question that in our IRB we would call a human subjects concern. That is, the safety of giving this chelator to --

DR. BRADSTREET: DMSA.

PARTICIPANT: Yes, giving it both to children affected and controls. What are the risks?

DR. BRADSTREET: Actually it is a retrospective look at our database. We weren't doing this in a prospective way. DMSA has been available for decades as a chelator, the safety of which has already been approved by the FDA. The FDA has approved Succsimer, which is a DMSA drug on the market for lead in encephalopathy. That is a 19-day course, where toxic lead exposure is treated with 19 days of DMSA.

But if you look at the literature, DMSA for mercury exposure is widely discussed and widely practiced in medicine. So it was not an unusual practice to do that as a diagnostic challenge. We were very intrigued that Frumpkin had published his data in Environmental Perspectives, where he used DMSA in adults in an effort to

look at past exposure to mercury, and found that it wasn't a good predictor of past exposure to mercury in his adult population. But it has been out there as a way to look at mercury relative and total body burden for some time.

PARTICIPANT: Two questions. In those youngsters with the measles mRNA in the gut and CSF, were attempts made to look for expressed measles specific protein in either the gut or the CSF?

DR. BRADSTREET: Yes, not in CSF to my knowledge at this point in time, but measles specific protein has been detected and previously published. You should have all that data. Dr. Wakefield as presented and published that on several occasions, that measles virus expressed proteins are present in the G.I. tract.

PARTICIPANT: CSF has not been demonstrated?

DR. BRADSTREET: Well, measles virus antibodies are present in a significant number of those children, but that would indicate that -- there are multiple ways to get antibodies into the CSF. That becomes an extremely complicated discussion at this point in time to work through the methodologies of measles virus in CSF. There is clearly not time to do that. Dr. Lieber has done a fairly good review.

There are other reviews on measles virus behavior in CSF. Those are all wild strain measles, not vaccine strain measles. I think this is something new that is being characterized, and I'm not exactly sure what it means at this point in time.

PARTICIPANT: Second question. Has there been any direct demonstration that the polymorphism in glutathione transferase or the other enzymes involved in metabolism of mercury removal are reduced in those with polymorphisms?

DR. BRADSTREET: The MTHFR metabolomics, if you look at the metabolomics of MTHFR, it is fairly well characterized in the literature. Various polymorphisms affect outprocessing of the conversion to 5,methyltetrahydrofolate under different circumstances. It is a fairly complicated subject to go into.
But somewhere between 15 and 60 percent reduction in actual enzyme efficiency occurs in different circumstances, how much folic acid you are loading and other sorts of things that are present.

PARTICIPANT: I'm speaking about specifically with those with the polymorphism, to see if that supports the hypothesis that the polymorphism is going to be more

sensitive to mercury, which you could also demonstrate in vitro mercury sensitivity in the presence or absence of the polymorphism.

DR. BRADSTREET: Yes, it is a very easy study to do. I think it would be an easy one. There are cell lines of MTHFR --

PARTICIPANT: That has not been done?

DR. BRADSTREET: No. What we have done -- to try and help you with that, the understanding of how we are developing this argument is, we found elevated mercury. It has been observed since Dr. Panguen first presented the data approximately 20 years ago that a subset of children with autism had cysteine deficiency. Cysteine is the rate limiting stuff in the production of glutathione and metallothione.

We know that those are important to mercury metabolism. So when we found that, if you go back up in the biochemical chart, it gets you back to the folic acid cycle and the thioneine cycle, so we just decided to start looking at those. The findings have been presented, and you can start looking through that data.

DR. MCCORMICK: I think we are going to have to quit now. Thank you. Our final presentation will be from

Dr. Vijendra Singh, who is a research associate professor at Utah State University. Dr. Singh was invited to discuss his work on autism vaccines and immune reactions. Again, 20 minutes have been allotted for his presentation and ten minutes for questions.

Agenda Item: Autism, Vaccines and Immune Reactions

by Dr. Vijendra Singh

DR. SINGH: Ladies and gentlemen and members of the IOM committee, distinguished members, it really is a privilege to be here. I am very happy to come and speak to you a little bit about my research.

First of all, I was invited by the same committee about five years ago. There was a very nice discussion about vaccines and autism. I think since then, what I see is happening is that there is a change in the agenda, if you like. There seems to be overemphasis on the mercury problem in autism. I think we have to remain very open minded about biological factors. At this stage in autism research, which I think this is in very infancy stages, I don't think anybody has any clear answer to any problem.

Having made those couple of simple remarks, what I would like to do today is spend a little bit of time -- I won't be able to cover everything out of my ten years of

research, but I'll talk about the issue of autism, vaccines and immune reactions that the committee asked me to come and talk about.

Let me talk a little bit about autism. I tend to think that autism is a complex biological disorder. A lot of these things have been implicated. We have been focusing our research on immune factors since day one for one reason only. At that time I was working at Children's Hospital in British Columbia, Vancouver, Canada. In my pediatric neurology and pediatric immunology research, I came out with one observation. There was seemingly some connection of rubella virus with neurological manifestations described as autistic disorder. Since then, I have been very much interested in neurodevelopmental disorder, but I really pursued autism about ten years ago as a main focus of research.

All these things are important. As I said, I don't think anybody has any handle on what is really the important one factor.

Our question has been very simple, is autism an immune disorder? That is what we have been investigating. Most of the research that I have been able to publish by now over the last ten years or so, the vast majority of it

has been replicated worldwide when we talk about immune analyses. I am not going to dwell too much on that issue today.

At that time so many years ago, my simple hypothesis was interaction between virus and immune system, could that affect the nervous system function and lead to autoimmune problems in autistic children. Today I will stand here with you and share that there is a viral component and there is an immune component. That seems to be an autoimmune component.

In all this research, the specificity of the observation is of utmost importance. When we do biological research, if you cannot demonstrate the specificity, I don't think you are really finding cause and effect relationship. Quite frankly, I am very stunned at some of the specificities that we are seeing today in our research in autism. I'll show you some of that.

Let me give you the upshot of that. We are finding a serological problem of measles virus. We are finding an autoimmune problem of antibodies to myelin basic protein, which is a very important constituent of the myelin sheath in the vein. As I will present to you, I raised the question, are these two things related, and they

seemingly are. So that is the kind of basic observation after so many years of research is coming along.

At this point, let me also add very important information. That is, in all of the autoimmune diseases for the last 35 to 40 years of research, people are searching for a viral connection without any success. So is it possible that coincidentally I might have found something here that is telling us very important for autoimmune process in autism, which may be virally triggered?

That is the kind of message, purely on a scientific basis, that I am delivering to you. I am not here for one group or the other. I am a very strong advocate of vaccine programs, because I strongly believe, and I have known that for years, that vaccines are the most important preventive measures that we have today in our hands, and we should not play around with infectious diseases.

I won't talk about everything. I will cover three, four, five and six. This is new research, which is telling us something very important, and of course immune modulation therapy is very important in autistic children.

This is an immune profile that one can do to

examine immune problems. Everything on the right-hand panel basically has come out of my research from the last ten years or so. The left-hand things are very standard clinical immunology testing programs available through any hospital and so on.

Subjects in these studies are autistic children. Diagnoses were made by clinicians in our collaborative effort. I am only talking about my research study, not something I might have done for other families, so please keep that in mind, in normal children. When I say autism, I am really talking about children who have been diagnosed only with autism. Very rarely do I included a PDD or ASD or PDDNUS. So these are as best as a clinician can define to be a firm diagnosis of autism.

Occasionally I have used other disease controls with some children and some normal adults, just to see what we really see. All children in the study have had their full MMR and DPT vaccination. A very important thing is, in our research I always look for baseline profile. I am not looking for any subjects randomly given some other intervention, because I think we have to understand what is the basic profile of these children. We should not worry about everything else.

Immune abnormality; there is so many of them. There are a couple of them which are very important, and I will show you in the next couple of slides. I don't want to go into that.

This is a slide which I think is quite important, cytokine profile. All the data from different labs, my own research, as well as other peoples' research is basically telling us that in autism there seems to be a TH-1 profile. That is based on the finding that interleukin 12 and interferon gamma that pushes the part of the immune system really are markedly elevated in these children, both production-wise, which was shown by other people, and in the plasma that we found ourselves. All these things ar published in journals that I have listed in my handout to you.

Then we have also investigated a whole variety of brain autoantibodies. This molecule seems to be the hallmark feature of autoimmune process that we have investigated so far. What I mean is, we have investigated several of them, seven or eight of them, and very recently we have found a problem of involvement of caudate nucleus. That is the first time we have found anything, anybody has ever described that. The major significance of that I will

privately discuss if anybody wants, because I have to remain focused on time.

When we say autoantibodies, we are talking about a profile which looks like this. I don't think you need a microscope to see that sort of reaction. If it is not there, it is not there. We see that present in roughly about 65 to 80 percent of autistic children, not in control, not in normal adults, not in Downe children; there were some diabetic. So here is an illustration of so much specificity for this marker, with disease and normal subjects. I think it is very important.

In this study we were trying to see if other adult markers, two of the major markers of myelin sheath, if they will have anything. What we found is that this enzyme marker is basically no antibodies, but antibodies to myelin basic protein are still present. This one was also present in normal. I tend to think this is a secondary issue in the autoimmune process.

Remember my hypothesis, viral interactions? I was looking now to see which virus has any connection with autism with autoantibodies. There are several of them, but we raise two questions. If we do virus serology and MMR afterwards, the question was, is there a change, is there

an abnormal level of distribution? Secondly, is it related with autoantibodies? If there is a virus serology and other autoantibody disturbance, we should see some correlation. Otherwise it would be a non-specific finding, and I will not pursue the research any further.

As I showed, the answer is yes for measles and MMR, answer is yes for measles and MMR. They correlated with MBP autoantibodies.

Virus serology we did for five different viruses. We did not see any significant difference for these four. These are measles antibody, which are significantly elevated. We have seen that in at least three different studies that we have conducted so far.

This is one illustration where measles antibody tends to be -- it is not a robust observation, it is not a robust increase but it is significantly different increase. Mumps was no difference, rubella virus, no difference.

So at that point we had published the article. We raised the possibility that it is measles virus causing autism. It was a speculation based on our serological finding of measles antibodies. The idea was, if these children have elevated levels of measles antibody, there was also the presence of this autoimmune marker,

specifically myelin basic protein. We studied other neural protein autoantibodies, they did not show that correlation.

So we posed that question, and then we decided to look at it a little further. So we were obviously trying to figure out, is this coming from a measles virus, viral screen, which unfortunately I don't have lab facilities to grow measles virus, so I didn't want to do that research. So I thought, let me see what I can learn if I simply use vaccine strain which I can use in my lab.

Ladies and gentlemen, I was so surprised that we have seen this observation. No difference of antibodies to DPT, but we see consistently significant increase in the MMR antibodies. This was published in this journal.

Here is the illustration, actual data. You can see the dilution in three different groups, normal children, autistic and other disease children, no difference. Same thing here for DT. Same experiment done with MMR vaccine. This is the autistic group. The other two groups are way at the bottom. When I saw this result, for almost a week I just didn't want to go back to my lab, because I was very disturbed. I don't want to see any flaw, any problem with the vaccine. Then it became even more of a concern for me when I did an analysis trying

to identify what protein in the MMR is actually doing this antibody response. We are finding consistently that this is a protein that looks like HA protein of the measles -- particle.

We have characterized it. It seems to be like this, MMR, measles virus HA, where the protein is the one which is reactive. No reaction with MP protein, no reaction with rubella, no reaction with mumps antibodies.

You could follow a little bit more closely if you want in the journal; more details are given in our publication, but the idea is that we are seeing this reaction on a consistent basis. I was frightened, but as a researcher, you must continue a little more.

So the second question was, is there a correlation? We see correlation with MMR MBP antibody nearly 90 percent or so, they show that correlation. The idea is, if you have the antibody to measles or MMR now, do you see also the presence of any neuronal autoantibody, and we see that for MBP.

As I said, this is published. A more recently published article, here I also included some siblings. This is the autistic group and this is the normal group. But results for mumps or rubella, no statistical

difference, but measles antibody level are statistically significantly elevated in the siblings as well as normal children.

This is a point of interest for all of you. Trivalent vaccine, was this monovalent vaccine. It took me a long time to finally find a couple of vials that I was able to purchase, and we found same reaction with measles vaccine only. This is monovalent vaccine only. So trivalent as well as monovalent, it is a measles sub-unit that seems to be somehow inappropriately showing a misguided immune response in autistic children. I wish we knew why it is. We probably will in future. This is not published, as I showed you.

Another interesting thing was that immune manifestations of measles virus, this is wild type measles infection versus autism. Number two through four, there were four immunological abnormalities. They are parallel, they are common.

This was really fascinating. This was a study published by another group. MMR vaccine, measles component HA antigen, it stimulated the TH-1 response via interferon gamma production. Way before that, I had published that in autistic children, we are seeing this TH-1 response through

higher levels of this and that. It is so exciting on the one side to see this correlation, and at the same time somewhat of a major human concern, that with vaccines we should not see these things.

So it looks like this H protein, which is on the membrane of the viron particle, seems to be somehow the culprit if you like. My thinking is, the measles infection in autism is an atypical measles infection, without the typical measles rash, but it causes neurological manifestation.

An example of that has recently been published in a study of four cases where that has been shown. So my thinking is that there could be a wild type of measles latent infection, or there could be something uniquely wrong with the MMR or MBP vaccine that is causing this problem.

This is a very new result, from the last three or four months. I want to share this, because it is very important. I decided to look what is present in the MMR vaccine, and this is the MMR vaccine. All these things are common, human albumin is present. It struck me, because if you look at the molecular weight of that protein that we are seeing, it is closer to the molecular weight of this

protein. So I wanted to see if this immune reaction is because of human albumin.

We have now reached a preliminary observation that autistic children do not have significant increase of antibodies to albumin. Secondly, this is very important, I took about 30 samples of autistic children, which were positive for MMR antibody that I showed you a couple of slides ago. They are all negative for albumin.

Moreover, we know that there is albumin by using positive control and MMR antibody in the patient. There seems to be a difference in the molecular weight. This is bigger than this one. So I think based on these observations, I can safely say that MMR vaccine reaction that we are seeing is not due to albumin.

Very quickly, one or two last slides. What I want to show you is that there are two hallmark features that causes autoimmunity. These are the antibodies which are well known in the literature. We did some investigation. We do not find any evidence of these antibodies in autistic children. They are not significantly different.

So in conclusion, autoimmunity is the core of the problem in many autistic children. I tend to think this

represents up to three-quarters of the autistic population. We have to remain open minded; it could be 60 percent or it could be 80 percent. But I tend to think -- and secondly, I postulated that in a typical measles infection without the rash, but shows neurological symptoms, might be etiologically linked to autism. This may come either from a mutant strain or from MMR vaccine, which might potentially cause autistic regression.

I would like to propose at this time that I think it is time to re-evaluate vaccine safety and the way we practice vaccines today.

This is the funding support disclosure, which I have already made in my report. I do not have any conflict of interest from anybody. I have never received any funding from anybody who is asking me to do this experiment and don't do that experiment. If anybody ever did ask me, I will never do that work. So I just want to make sure that folks will understand that all my work is privately funded on a small scale seed grant basis. I continue to share that information.

Last but not least, just to show you, in the lab I do look like a research scientist. Thank you very much.

DR. MCCORMICK: Thank you. Your second to last

slide answered my first question, which was your funding. We now have time for some questions.

DR. BAYER: Since so much of our attention this long day has been focused on the issue of mercury and thimerosal, and you raised a somewhat discordant perspective, I am wondering how you viewed the presentations of the day, which so stressed the role of thimerosal and mercury as etiologically responsible for autism.

DR. SINGH: That is a very, very important question. I was hoping nobody will ask me that.

With due respect to the distinguished members of the committee of IOM, I would like to simply point out that I think mercury or thimerosal still is a very important factor, but I don't see the real science that I would like to see someday, that is telling me that it is causing autism. I hope in brief I can say that answer to you.

DR. MCCORMICK: Any further questions?

DR. SINGH: If I may just add to that, this does not mean that I have found the answer by finding measles connection. I think all research of autism as I view it is still in very early stages. Autism research from biomedical side is in infancy stages. We need more

people. When I started out on autism, there were only one or two names that I can remember, but now there are quite a few physicians involved in taking care of these children. I feel happy about that. There are a few more researchers that are now coming into this field, pharmacologists, toxicologists, biochemists. Ladies and gentlemen, from a scientist's point of view, I think everybody is so much welcome, the more the merrier. Eventually we will find answers for these children. That is how I am looking at th problem of autism.

DR. JOHNSTON: As you know, we usually think of antibodies to myelin basic protein and other antibodies to myelin proteins as related to multiple sclerosis, motor decline, eventually some other neurologic abnormalities. But how do you relate what you see here in these children to the phenotype of autism?

DR. SINGH: Dr. Johnston, that is a very important question. I hope someday I will get a million dollar grant to address that.

But let me give you an answer, what I have found. This is a very important question for all of you to know that, but remember, I was saying earlier that there has to be a certain level of specificity. If you don't have

specificity, it is all secondary issue.

With the help of a seed grant, in fact, it was awarded on behalf of one of my undergraduate students when I was at the University of Michigan, what we found was that this antibody reaction is coming from a different epitope on the molecule, myelin basic protein. It is not the same epitope which is in the MSREAE model of multiple sclerosis. There it is known as encephalitic epitope. It is more on the N terminal, whereas the epitope that we are recognizing and we are trying to identify in autism is coming more from the C terminal.

But that is based on still indirect peptide binding competition studies. It is not direct evidence. So I tend to think that this other antigen somehow might be coming from the heart of the structure. Maybe we need to do genotyping of different forms of MBP proteins.

So the idea is, in children it would be a developmental defect. That means myelin is not formed correctly in children. Quite frankly, there is an MRI observation to provide some evidence in that area. All these areas, nobody is looking. But now there are some people who are following my research. Last November I was at the Society for Neuroscience, and there was a

presentation by Martha Herbert. She had the poster next to mine, and she was waiting just to give me a hug. Dr. Singh, can I give you a hug? I said, why? What did I do? She says, I have been following your work for so many years, but look at what we are finding.

Remember our observation of another person, Eric Coshane in San Diego, of changes in the brain volume in the cerebellum and so on? What she has found through her MRI investigation is that this is due to th white matter changes in the brain of autistic children. It is a wonderful observation from a neuropathology point of view.

So I hope I have given you some perspective in why in autism myelin basic protein autoimmune reaction would be different than what it would be in multiple sclerosis, as an example.

DR. MCCORMICK: We have time for one last question.

DR. HALEY: I want to talk about cause and effect. One thing we know that mercury does and ethyl mercury is totally disrupt the axon structure. Then the cause and effect is, a toxicity such as that, that would destroy the formation and the integrity of the axon could easily lead to a subsequent autoimmune effect, because the

myelin basic protein is going to be released in that destruction, and it might do that.

So while I agree with your research, it is very nicely presented, I think that one has to say that it is not likely that an autoimmune disease just jumped out without some other external causal effect. The one thing that would fit into this would be mercury and ethyl mercury disruption of the axon.

DR. SINGH: Dr. Haley, as you know, I respect your work very much. Quite frankly, I have nothing against anyone who presented the mercury presentations. But all my concern is where is exactly the objectivity of that research, where are we coming from. I am hearing in tissue culture cells, could you translate that nanomolar concentration, but how many cells do you have, what is the cell culture? Do you have a million cells, 100 cells or 2,000 cells? Those are the toxicological amounts that you are using to deal with. You are going to see that. You should be able to translate that into what might happen in vivo. Do these enzymes at a single molecule level in vivo actually ever see that amount of mercury?

Pardon me for being so difficult here, but I remain very open minded. Mercury is also very important.

Please don't misunderstand me.

DR. MCCORMICK: I think we are really going to have to break now. Thank you very much.

DR. SINGH: Thank you.

DR. MCCORMICK: We will now open the floor up for public comment. I would ask that the first three people on our list start moving up towards the microphones while I am comment on this. They are Alan Clark, Richard Fisher and Barbara Loe Fisher.

I would like to say that I really strongly urge you to keep your comments to two minutes. We have to leave here -- the committee has to leave here at 6 o'clock. If you are not part of the list we talked about, we would love to have your comments in writing.

I would note for those of you who are listening to the webcast, we have had several e-mails sent into the committee. The committee will review those tomorrow at our meeting. If there are questions that need to be addressed by the speaker, please be assured that we will follow up with the speakers to get their answers.

Please use the microphones in the aisle, and please state your name and affiliation. As I say, if you are not able to provide your comments today, please submit

them in writing to the committee by February 16.

The first person is Dr. Alan Clark.

DR. CLARK: Dr. Alan Clark, physician in Carthage, Missouri. Comments here are concerning autism and its relationship to mercury toxicity.

Not since Dr. Semmelweiss first advocated hand washing before patient examination in 1847 has a medical issue experienced more confusion and misrepresentation, I believe. The pervasive theory that ethyl mercury and thimerosal is a benign and acceptable additive for human consumption is as erroneous, I think, as claiming that an ethyl group on the plutonium atom makes it a proper food preservative.

Credible researchers that we have heard today have repeatedly reminded us that there are well over 5,000 articles detailing toxicity of thimerosal, dating back to 1953. The Romans slowly poisoned their society with the toxicity related to the lead in their underground water systems. Our leaders perhaps have ignored warnings of toxicity of leaded gasoline in the past, published as early as the 1920s. Not until the Clean Air Act did our population's lead levels drop by 80 percent.

I have been a physician for 30 years. I perceive

that the loss of credibility in the area of vaccine research alone will reverberate through the profession for many years to come. For the truly dedicated medical professional, it is discouraging to me to endure a loss of confidence in some of our present vaccine safety research. It is difficult to fathom the reasoning of those individuals who willingly expose children to a known neurotoxin. So it is time for men and women of true science to stand together and demand accountability from our peers and policy makers. The actions of a few now pollute the good work of those dedicated to maintaining ethics in publication. This must be a call to arms to demand our government researchers to restore their moral and social consciousness.

Thank you.

DR. FISHER: Thank you. My name is Richard Fisher. I am a practicing dentist, and have been doing so for about 30 years. For the first eight years of my practice, I placed mercury fillings in the teeth of children and mothers and fathers. Twenty-two years ago, I stopped doing that when I recognized that there was research out there that the mercury came out of the fillings.

What I am here to talk to you today about is, one, I am here as a representative for the International Academy of Oral Medicine and Toxicology, which is a professional group which has sponsored some of the landmark studies on the physiological effects of specifically mercury derived from dental fillings. I am here today to talk to you about body burdens, however.

It has been shown that the predominant source of body mercury in humans today is from dental fillings. That is not my opinion, that is the research of mercury toxicologists, including the World Health Organization.

I have given for your perusal some data that I presented to a Congressional committee on this. What I showed is that the largest contributor particularly to the fetus and the neonatals is from the mother's fillings passing through the placental barrier into the child. In fact, the average fetus when it is born has more mercury in it from the mother's fillings than it gets from the first five years of life, from all the vaccines it receives.

So dentistry is the largest and the earliest exposure of mercury. At that age, of course, the effects of the neurotoxicity to the developing brain stem is the most vulnerable.

I would ask for the Institute of Medicine to broaden their focus from looking at the 20 percent of body burden from diet, vaccine and environment, and broaden it to look at the 80 percent from dentistry.

Thank you.

MS. LOE FISHER: My name is Barbara Loe Fisher. I am co-founder and president of the National Vaccine Information Center, which has represented parents of vaccine injured children since 1982.

Today we join with a third generation of parents in the past 22 years to ask for scientific acknowledgement that vaccines can cause some healthy children to regress physically, mentally and emotionally after vaccination, and be left with brain and immune system damage, including autism.

As the mother of a son brain injured by the DPT vaccination in 1980, I remember back then that no scientific body had ever analyzed and published information about vaccine risk for the public. The National Childhood Vaccine Injury Act of 1986 asked IOM to do that. The reports you have published since then have been historic documentation of the fact that vaccines can cause brain and immune system dysfunction in children. You have been the

first scientific body in the world to call for more credible basic science research to answer outstanding questions about vaccine risk.

That being said, you know from our testimony before your committees over the past years that we have not agreed with all your conclusions. Dr. Harris Coltrane and I were the first to report vaccine induced autism in our 1985 book, DPT, A Shot In The Dark, which was used by your 1991 committee as a reference. However, that committee declared that vaccine induced encephalopathy could not cause autism, an early conclusion which was later called into question by IOM's 1994 re-analysis of NCS, because many of the DPT damaged children in NCS have autism spectrum disorder symptoms.

You said in 2001 that it is biologically plausible that vaccines could cause autism. Causation was once again rejected. In coming to that conclusion we questioned the preferential treatment given to published retrospective epidemiological studies for clinical and biological mechanism evidence.

What you do at this critical moment in time may well determine vaccine safety research priorities in this country, including whether or not studies will be funded to

find out which children are genetically vulnerable to vaccine reactions so their lives can be spared. We hope you will do what needs to be done, to not only foster public trust in the impartiality of the Institute of Medicine, but to light the way for the medical community to do the credible scientific research that will prevent future generations of children from suffering the kinds of vaccine damage that our children have suffered over the past quarter century.

Thank you.

MS. G. CLARK: I would like to relinquish my two minutes so that Dr. Deth may finish his statement. Thank you.

DR. MCCORMICK: The next person I have is Lisa Sykes.

MS. SYKES: Dr. McCormick, just a clarification. Is it my turn at the mike? Our of respect for a physician being here and I am merely a parent, I thought it more important for the committee to hear from a colleague than from a parent.

DR. DETH: That was very gracious. I am Richard Deth from Northeastern University. The paper that has been recently released has caused some stir and interest, in the

results that we found that insulin-like growth factor and dopamine can regulate methionine synthase, a previously unrecognized signaling pathway, the pathway that regulates the methylation of DNA and also provides methylation of the membranes of nerve cells, which seems to play a role in attention.

The D4 dopamine receptor is involved in ADHD. I handed out the supplementary sheet here with some figures, if you are interested in that. The D4 receptor has been linked to ADHD by over 50 papers in the psychiatric field. It relies on methionine synthase. What we found is that the number of repeats of the gene for this receptor makes a difference in its ability to carry out methylation. The repeat that has been associated with ADHD, that is, the seven repeat form, is low in its ability to carry out methylation. So there is a link between ADHD and methionine synthase in that form.

Furthermore, in the six months since this paper was published and the work was done, we now understand exactly what thimerosal does. Thimerosal interferes with the PI-3 signalling pathway that controls methionine synthase. It interferes with the glutathione dependent synthesis of methyl B12. That is to say, the methionine

synthase in the cells that have the D4 dopamine receptor is missing one domain. It is missing the domain that normally repairs the B12. Without the B12 repair mechanism, new B12, methyl B12, is required. That new methyl B12 has to be synthesized in a glutathione dependent manner. What we have found is, when you treat cells with thimerosal, there is no methyl B12 synthesis.

So I just want to let the committee know that we have actually gone a further step in clarifying how that works.

Finally, I would like to say that if thimerosal interferes wit IgF 1 signalling and perhaps can fit into autism the way it appears to from this discussion today, the committee might think about other consequences if thimerosal interfered with IgF 1 signalling. For instance, you might consider that if you asked diabetologists the most common cause at a molecular level of type two diabetes and obesity, they would say interference with IgF 1 signalling.

It would be fair to say that another population, not the one here today, experiences type two diabetes and obesity in association with separate risk factors. So you might be careful; there could be other issues besides

autism where thimerosal's toxicity is involved.

Thank you.

DR. MCCORMICK: Is this what you had signed up to say?

DR. DETH: Yes.

MS. SYKES: I have brought for the committee copies of chelation reports from my son that have been plotted, and a graph that everyone here can see. The graph shows mercury dumps, the provocative agent for these were DMSA. Where the green line comes in, you will see ALA has been added. These were urine samples. So the total burden probably still was five times what the red line shows, once we introduced ALA.

Those are copies for you to take with you. I might mention too, only after several of these large mercury dumps did my son break out with the measles. He had been immunized on a regular schedule. Wesley is autistic.

I am not anti-vaccine. I have got a passion right now to be intently and doggedly anti-thimerosal until this does not happen to other children.

The Greeks tell a story about a young man named Icarus, who was given a great gift by the gods, a pair of

wax wings. He soared so well and so high on these wings that he thought he could reach the sun. As he neared his goal, those wings melted and he plummeted to earth.

I fear now we are realizing that we have been too daring and too proud in our vaccine policy, and that we have soared too high. Poison delivered as a vaccine preservative, thimerosal is poison nonetheless.

The article just referred to by the physician at the microphone talks about methionine synthase. I might mention to you that the article details, it creates a nanomolar solution of ten to 30 in our newborns. We do not need to bathe the brains of our newborns in mercury.

The article also states that the potential for thimerosal to cause adverse effects on MS activity at concentrations well below the level produced by one individual thimerosal containing vaccine. I might add to you that after we got all this mercury out of my child, who is now eight years old, we began methyl carbollum shots injected that I give to him each day. He is finally starting to learn. We are addressing a serious deficit that was caused. He was not born with it.

I wanted to ask the committee, as I have asked the office of investigations that has oversight over HHS,

to look into Simpsonwood, a meeting that occurred there on June 7 in the year 2000. Verstratton was there. The quotes in the transcript of Simpsonwood are damning. He said, quote --

DR. MCCORMICK: You are going -- there are other folks behind you.

MS. SYKES: May I please finish? This quote is so important, Dr. McCormick, I would appreciate being allowed to share it with you. The quote is that we have found statistically significant relationships between the exposures of thimerosal and outcomes, first at two months of age, an unspecified developmental delay which has its own specific ICD-9 code, exposure at three months of age, exposure at six months of age, and attention deficit disorder, exposure at 36 months of age, language and speech delays, which are two separate ICD-9 codes, exposure at one, three, six and nine months of age, the entire category of neurodevelopmental delays, which includes all of these plus a number of other disorders.

I am alarmed that what has been said at Simpsonwood has not been brought here to this committee, and I must ask why. We have gotten rid of infectious disease, we think, but I would posit to you that we really

have exchanged it for lifelong and widespread developmental disorders that our children may never recover from. We are decimating an entire branch of the genetic family tree, where infectious disease was random. We have targeted one branch, and many researchers think we are taking out our brightest kids, those who would be our proteges, our engineers, our scientists for the next generation. That is why there is this issue over maternal education. It would be tue of paternal education too. These kids come from very bright families.

I will close now. Going back to the issue of the Greeks which someone else here has raised. They practiced infant sacrifice. Unintentionally, I would assert to you as a theologian that we have also. Every day we wait, eery day we study, every day we debate, we lose more children. I have lost one. I have a healthy younger son. You can more easily put a gun to my head and pull the trigger than you could inject thimerosal into that baby.

Thank you.

DR. MCCORMICK: Just as a matter of fact, the committee has seen the complete Simpsonwood transcript, and we also have all of the concerns and complaints documented in our briefing book in terms of addressing those issues.

So the reason it didn't come up today is because the committee has been well aware of it.

Deborah Darnley-Fisch.

MS. DARNLEY-FISCH: I am Debby Darnley. I am a physician parent from Detroit. I have submitted my son's data. He is now eight years old, and he is one of the children who had apparently normal development until he received his MMR at 15 months. It was only in the past year that I have really started to understand my son. It was very concerning to me after reviewing Fields Virology. At the time he received his MMR vaccination, he possessed at least four of the five risk factors for measles persistence.

This is a child who has lymphoid nodular hyperplasia. He also has significant counts of measles virus genome in his CSF as a progressive inflammatory encephalopathy, now his second year of adrenal cortical insufficiency. He is basically not going to do very well. His IgF-1 is also very low.

I am very concerned. I am very pro-vaccine. I am a mainstream physician. I do not want us to stop vaccinating and then have not only vaccine injured or developmentally disabled children as well as children

suffering wild measles and wild viral complications. But I am wondering if there is any way that we can look at these children on an individual basis prior to vaccinating them, perhaps the children that have active eczema and allergies, we can test their GH-1 competence. This is a society where we have far more TH-2 disease. I think we are basing our immunologic readiness on old data. I'm not sure the children today are immunologically ready on an individual basis. Overall, hopefully they are.

Secondly, I expressed in the data I submitted that I am concerned that in Fields Fourth Edition of Virology, there is apparently no human reservoir of measles virus. I am very concerned that future editions will have to speak otherwise.

I think my son is a human reservoir for measles virus. It is replicating. God knows, I hope it is very low infectivity, but I really hope that we do not let this get ahead of us.

Thank you.

DR. MCCORMICK: I would simply note at this point, we only have time for two more people to make their comments. Robert Krakow is the next person.

MR. KRAKOW: Thank you. I have a four-year-old

boy who was diagnosed at two and a half with heavy metal toxicity. He is one of the lucky ones, because he started getting treatment right away.

The research burden that you have heard about is being carried by practitioners, by clinicians. They are doing it as they go, and it is working. I want the committee to understand that these kids are being treated and they are getting better. My son is getting better. So what you do here is so significant, because it could lead to more research and more treatment.

For my son and every child who is getting treatment, there are a hundred, maybe a thousand, who are not getting any treatment at all. That is why it is so critical that you come to the right decision based on what you heard.

Thank you.

DR. MCCORMICK: Thank you. We have time for two more. The next name I have is Laurie McElwaine.

MS. MC ElWAINE: I'm going to try to be real quick, too. My name is Laurie McElwaine. I serve as the executive director for the National Autism Association. I also have a four-year-old child with vaccine induced autism.

I just wanted to say to the IOM today, thank you so much for listening on this important issue. I am not going to touch on the science as much as I am going to touch on public opinion and what that might dictate in the future.

I hope that you will consider that obviously, thimerosal is still in these vaccines. Dr. Offett himself told me that it shouldn't have been in the rogam. Obviously if it was as innocent as water, it wouldn't matter. He does not think that is the case, obviously. He said it shouldn't have been in that rogam shot. It shouldn't be in any of these shots. I would hope that you would find a way to require -- not request, but require -- these drug companies to get it out of there, period, because if you do not, proactive parents will make sure by way of media that it will. We have been able to do that with homeland security. They fell flat on their faces when they inserted that bill. That worked against them.

I hope that you will see that by sweeping it under the rug, by not talking about it, it is going to backfire.

The last thing, I am just going to point out that the parents that developed Lorenzo's Oil were discredited.

Dr. Needleman with lead discredited it. Dr. Betteheim, practically given a trophy. Who out of all of those people now have more credibility?

Listen up, parents. Thank you.

DR. MCCORMICK: Scott Bono.

MR. BONO: My name is Scott Bono. I am the father of a 14-year-old autistic child, Jackson Bono. I believe in safe vaccines. I wear my helmet if I ride a motorcycle, and I wear my seatbelt in my car.

I think we have made a mistake, and we need to fix it. Once it is acknowledged that it is poisonous and it is doing harm, the best and brightest minds under your influence can start looking for the cure, rather than trying to find ways of proving what we have seen in our own children are wrong. So please, as you deliberate research that you have seen today and heard today, Jackson Bono passed the seventh grade standard course of study test for the state of North Carolina. I just got word of that on Monday, my birthday. We have been chelating for three years.

I was told when my son was three that he would probably be dead by five. So much for that doctor's opinion. Institutionalization was an alternative one

physician suggested, leaving me with my greatest hope that my son wouldn't be sexually abused in a state institution.

I want my son to be paying taxes. I want my son to be working. Most of all, I just want my son to fall in love, to have a friend. You hold the public trust. The greatest threat to the trust in our public immunization program lies with the people who profit most from it. They are the greatest threat right now. You are looked up to and looked to for scientific advice, reason and logic.

Thank you very much for your balanced approach today. I am completely impressed with both sides that you have allowed to speak. You are to be congratulated. But please, it is the Jackson Bonos, it is the Connor McElwaines, the Hunter Pipes, it is the thousands of children who may not ever go to that human condition that each of you already have. You have fallen in love, you have had a friend.

That's all I want. Thank you very much.

DR. MCCORMICK: Our last speaker is Liz Birt.

MS. BIRT: Thank you. I provided to Dr. McCormick the results of my son's most recent colonoscopy and endoscopy. He was originally treated at the Royal Free Hospital in 1999. Three weeks ago I received a call from

the school nurse that she was going to have to call an ambulance because my son was in pain on the floor for two hours, grabbing his stomach and pounding on his chest. So I called the gastroenterologist in Long Island and my son was scoped. His disease has progressed to the point that he has terrible esophagitis, lesions in his bowel, his colon is diseased, and it is a progressive meltdown of his G.I. tract. He also has measles virus in his spinal fluid.

I do believe that there are a subset of children who are at risk for this type of thing. I think thimerosal plays a role in that it skews the immune system and makes their bodies so they can't accept a live virus. But I have laboratory documents documenting all of this. I live with this every day.

I think that the epidemiology won't answer this question. I agree with Barbara Loe Fisher; it is going to be the science looking at these children individually to see what caused this and how to fix it.

Thank you.

DR. MCCORMICK: Thank you. The meeting is now adjourned.

(Whereupon, the meeting was adjourned at 6:00 p.m.)