UNITED STATES
COURT OF FEDERAL CLAIMS

IN RE: CLAIMS FOR VACCINE INJURIES RESULTING IN AUTISM SPECTRUM DISORDER, OR A SIMILAR NEURODEVELOPMENTAL DISORDER	)	
FRED AND MYLINDA KING, PARENTS OF JORDAN KING, A MINOR, Petitioners,	)	
v.	)	Docket No.: 03-584V
SECRETARY OF HEALTH AND HUMAN SERVICES, Respondent.	)	
GEORGE AND VICTORIA MEAD, PARENTS OF WILLIAM P. MEAD, A MINOR, Petitioners,	)	
v.	)	Docket No.: 03-215V
SECRETARY OF HEALTH AND HUMAN SERVICES, Respondent.	)	

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Courtroom 402
National Courts Building
717 Madison Place NW
Washington, D.C.

Tuesday,
May 20, 2008

The parties met, pursuant to notice of the Court, at 9:00 a.m.

BEFORE: HONORABLE PATRICIA E. CAMPBELL-SMITH
HONORABLE GEORGE L. HASTINGS, JR.
HONORABLE DENISE VOWELL
Special Masters

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C O N T E N T S

WITNESSES:	DIRECT	CROSS	REDIRECT	RECROSS	VOIR DIRE
For the Respondent:					
L. Jackson Roberts	2153	2187	2195	--	--
Jeff Johnson	2198	2248	2262	--	--

E X H I B I T S

RESPONDENT'S EXHIBITS:	IDENTIFIED	RECEIVED	DESCRIPTION
6	2154	--	L. Jackson Roberts, II slide presentation
7	2197	--	Jeff Johnson slide presentation

P R O C E E D I N G S

(9:00 a.m.)

SPECIAL MASTER HASTINGS: Good morning to all. We're here for another day of proceedings in the omnibus autism proceeding, Theory II trial. We've got a couple of matters to talk about, at least one before we go on the record.

(Discussion held off the record.)

SPECIAL MASTER HASTINGS: We have a housekeeping matter that we need to take care of. As you heard the other day, we had some presentations by both parties about how to handle the rebuttal evidence in this case and the issue of how to handle the testimony in the third case and the rebuttal.

Last evening, after the close of proceedings on the record, we did have additional discussion of that. Both counsel for both parties made presentations on that and we discussed it, and Special Master Vowell is going to explain our ruling on those issues.

SPECIAL MASTER VOWELL: Okay. Essentially, our ruling is this. We've asked the Respondent to continue presenting its case in chief on both the Theory A and Theory B of Theory II, the second theory of causation, to put on all of their witnesses now

with the exception of the two that we've already discussed. Drs. Clarkson and Magos were not available until July.

Petitioner will put on their rebuttal case in this coming week and a half at the conclusion of the government's case to what they've already heard from the government. We are scheduled to have further proceedings then on the Theory II omnibus matters, the general causation matters, and to hear the third test case case-specific information in the third week of July.

We will allot two days to testimony on the specific new test case, two days to the government to present the two toxicologists, and whatever is left of the second day that Petitioners have or the beginning of the third day to hear their case-specific, I assume pediatric neurologist, whether it's Dr. Rust or someone else.

Friday will be reserved to the Petitioners to provide rebuttal to Drs. Clarkson and Magos, but we will strictly enforce that it has to be rebutting what Drs. Clarkson and Magos testified to, not the gamut of what we've heard or what we will hear in the next week and a half.

Does that comport with my recitation of the

ruling last evening?

MR. POWERS: From Petitioners, that's an accurate recitation, Special Master.

SPECIAL MASTER VOWELL: And, Mr. Matanoski, you looked a little confused.

MR. MATANOSKI: No, ma'am. Actually, that comports exactly with what I understood the ruling to be when we spoke last evening. I just wanted to, for the record, as far as in another proceeding earlier in this omnibus trial, we had rebuttal close at the end of that proceeding and then there were some written submissions thereafter.

I just wanted to make sure that that's not the process that we're adopting here today, but rather, in this process the rebuttal is going to end at the end of this trial.

SPECIAL MASTER VOWELL: That is my fervent hope, Mr. Matanoski.

MR. MATANOSKI: Thank you.

SPECIAL MASTER HASTINGS: All right. Well, with that matter having been discussed, I guess it's time to go to the next government witness. Mr. Matanoski?

MR. MATANOSKI: Thank you. At this time, the government is going to call Dr. Jackson Roberts.

SPECIAL MASTER HASTINGS: Dr. Roberts, please have a seat, and we'll ask you to raise your right hand, please.

Whereupon,

L. JACKSON ROBERTS, II

having been duly sworn, was called as a witness and was examined and testified as follows:

SPECIAL MASTER HASTINGS: Please go ahead, Ms. Renzi.

MS. RENZI: Thank you. Good morning.

DIRECT EXAMINATION

BY MS. RENZI:

Q Good morning, Dr. Roberts.

A Good morning.

Q Could you please state your name for the record?

A L. Jackson Roberts, II, M.D.

SPECIAL MASTER HASTINGS: All right. Dr. Roberts, we wondered if you could, to the extent possible, sit to your right there, please? Don't go off the edge of the podium there, but it would be easier for us to see you. And do keep your voice up so we can hear you.

THE WITNESS: Can you hear me now?

SPECIAL MASTER HASTINGS: Ms. Renzi, go

ahead.

MS. RENZI: I think you're fine.

THE WITNESS: Okay.

SPECIAL MASTER HASTINGS: Now, I notice that we've just been given a handout to go with Dr. Roberts' testimony. We should mark that, I assume, as Respondent's Trial Exhibit 6, Ms. Renzi?

MS. RENZI: Yes.

SPECIAL MASTER HASTINGS: All right.

(The document referred to was marked for identification as Respondent's Trial Exhibit No. 6.)

BY MS. RENZI:

Q Dr. Roberts, could you briefly describe your educational background starting with your Bachelor's degree?

A I got a B.A. degree from Cornell College in 1969, and then I went on to the University of Iowa where I got an M.D. degree. I subsequently went on to Washington University in St. Louis where I did internal medicine residency and was board-certified in internal medicine.

And then I went to Vanderbilt University to take a fellowship in clinical pharmacology where I've

remained since.

Q When did you become a full professor at Vanderbilt?

A I think it was around 1986.

Q And then in 2006 you became a T. Edwin Rogers Professor of Pharmacology. Could you please explain what that is?

A That's an endowed chair that was given to me by the university. Endowed chairs give money to particular professors who had a long track record of accomplishments to give them some latitude, so it's a very prestigious thing.

SPECIAL MASTER HASTINGS: Dr. Roberts, if you could do your best to speak up.

THE WITNESS: Okay.

SPECIAL MASTER HASTINGS: Perhaps a little slower, too. We're having a little tough difficulty hearing you. Speak up as loud as you can, please.

THE WITNESS: Okay.

BY MS. RENZI:

Q And, Dr. Roberts, I'm going to put on the screen, it's right there in front of you, a portion of your curriculum vitae that was filed as Respondent's Exhibit DD, and this section is called the academic and professional honors. Could you please describe

your awards?

(Away from microphone.)

A Well, when I was in medical school I was elected in Alpha Omega Alpha, which is AOA, Society, which is like Phi Beta Kappa in normal universities. In 1983, I received the Burles Open Scholar award in clinical pharmacology, and then in 2001 I received a Sidney Kolowick faculty research award from Vanderbilt.

Then I was elected to two of the sort of prestigious societies in medicine, and you have to be elected, which is the American Society for Clinical Investigation and Association of American Physicians. 2001 I received a merit award from the National Institutes of Health. That's a very, very prestigious award.

They only give out a few of those, and they give them out to certain scientists who have had a long track record of very successful funding and accomplishments to allow them normal research grants. The maximum you can have is five years, so this goes for 10 years and it doesn't have to be renewed after five years, you just tell them what you're going to do.

So they're putting their confidence that

because of your track record they give you this latitude for 10 years of funding. Then in 2006 I got the Discovery award from the Society for Free Radical Biology in Medicine. This is basically for our discovery, which I think we'll talk about a little bit later, compounds of liquid oxidation called isoprostanes.

Then the same year I received the Earl Sutherland prize for achievement in research in Vanderbilt University. This is not just a medical school competition, it's universitywide. It's a very, very prestigious award. Then we talked about receiving the T. Edwin Rogers chair in pharmacology.

Then last year I also received a distinguished alumni award from the University of Iowa School of Medicine.

Q Thank you. And, Dr. Roberts, do you currently sit on any editorial boards?

A I'm associate editor of the journal called *Free Radical Biology in Medicine*.

Q And what are your duties as the associate editor?

A Well, journals have editorial boards, particular individuals who will usually agree to review papers. The editors sort of sit above the

editorial boards. When somebody submits a paper for publication it comes in to the journal, and it's assigned to, I think we have four associate editors, one of the associate editors.

So it comes to me, I take a look at it, I decide a couple of very excellent potential reviewers to review this paper, including myself looking at the paper, we send it out for review, we get the reviews back from experts, I look at those reviews and my opinion about the paper and I make the ultimate decision whether this paper should be published or rejected.

Q Now, Dr. Roberts, we're going to show you a slide up on the screen. This is Slide 2, and it's a list of your current funding and grants. Could you please go through those?

A The first grant is a very large grant that's been going on in our division with Jason Morrow, who is head of our division. He's the principal investigator. You know, it's been going on for about 25, 28 years now I believe. So there's multiple investigators involved in this and I have one project in that.

The next grant, that 5R37 GM40256, that's my merit award, which I've already discussed. The next

one is a grant from National Institute of Aging and that's another grant where I'm principal investigator in that. I'm dealing with investigating the role of oxidative injury in Alzheimer's disease.

The third one I'm also principal investigator on and that involves investigating the role of oxidative damage in cardiac arrhythmias. The last two are just grants from other people in which I'm sort of a coinvestigator on.

Q And the amounts on that funding chart, are those annual amounts?

A Yes.

Q And next to the annual amounts we see 1.2 calendar months, six calendar months. What do those numbers represent?

A Those are sort of if you add up, for example, if you look at the second one, six calendar months means that I'm spending half of my time a year on that grant and then it's split up by 1.2, et cetera, et cetera, et cetera.

Q Okay. And do you run a lab at Vanderbilt?

A Yeah.

Q And do you supervise people in your lab?

A Yes.

Q How many?

A I've got four research assistants, I'm also mentoring a junior faculty member and one Ph.D. student.

Q You also hold several patents, which are listed on pages 6 and 7 of your CV. I think we're going to bring up another slide, which is Slide 3. Could you briefly describe just the patents that are related or relevant to your testimony today?

A Well, these are all sort of general titles but they all have to do with specific patents related to oxidative stress/injury.

Q And, Dr. Roberts, you've published over 340 peer-reviewed articles, abstracts and book chapters, is that correct?

A Correct.

Q And of these papers and book chapters, approximately 180 are in the area of oxidative stress?

A Correct.

Q And since 1990 have all your papers been or almost all your papers been in the area of oxidative stress?

A Correct.

Q And do you give presentations on the topic of oxidative stress and oxidative injury?

A Many.

Q Could you just describe where you've been asked to present?

A I've been asked to present at several international meetings regarding oxidative stress. About four years ago I was also the keynote speaker on the first European workshop on isoprostanes, which is in France. We have annual meetings for the Society of Free Radical Biology in Medicine. I've presented there numerous times.

Q Now, prior to you exclusively studying oxidative stress, your research focused primarily on prostaglandins. I'd like you just to tell us how your research in prostaglandins led you into the area of oxidative stress.

A Well, to describe a prostaglandin though, prostaglandins are small lipid molecules. Prostaglandins are made by the enzyme that's inhibited by drugs, like aspirin. We had discovered that one prostaglandin called prostaglandin D2 is metabolized to prostaglandin, what we call an *F* type ringer, *F* type prostaglandin.

For various reasons there should be several different isomers of these. When we were looking and trying to analyze for these, these *F* ring metabolized to prostaglandin D2 by mass spectrometry in normal

humans. The levels consistently, drawing blood from several people in the lab or whatever, were consistently around 30 picograms per ML.

We happened to take a plasma from a normal person that had been in our -20 freezer for several months. Of note that at -20 freezer things are not solid ice, and chemical reactions can happen even in the freezer. What we saw with mass spectrometry, we saw the identical same compounds by mass spectrometry but now the levels were in the thousands.

This was, what in the world is going on here? And so we were running around the department getting samples from the freezers and they were always in the thousands, but consistently if we drew blood from normal people they were always around 30 or 40 picograms.

To make a long story short, I even submitted a grant to the NIH with a rational hypothesis to explain this still related to prostaglandins, thinking these were still prostaglandins. That turned out not to be the right hypothesis, but we did figure it out. What we figured out was what was happening in the freezer was simple oxidation of the precursor, which is a lipid arachidonic acid.

So we were generating these prostaglandin-

like compounds in the freezer without this psychooxygenase enzyme that normally makes these that drugs, like aspirin, inhibit. So basically this was just a free radical oxidation of the arachidonic acid going on in the freezer.

So then the obvious question is is this happening in our body? What we subsequently showed, yes in fact it is. So at that point I sort of looked back on the prostaglandin field, looked ahead in the oxidative free radical field. I said, the free radical field, it looks more interesting and it's probably more relevant to human disease, so I switched fields.

Q And during that process is when you discovered the isoprostanes, correct?

A That's what these compounds that we termed -- yeah.

Q And what has been the impact of your discovery on the area of oxidative stress and the research that has followed?

A I think it's been pretty profound in that at the time -- let me get the glass of water. So sort of about the time that we made this discovery, people in the free radical field, a lot of chemists were in the free radical field trying to understand in a test tube

all of these chemical reactions that free radicals can do.

It's a lot of elegant chemistry. Then people started asking the question: does this chemistry happen in humans, and is it involved in the pathogenesis of various and sundry human diseases? So people developed certain analytical methods to assess that, and the problem is at the time most of these methods were very unreliable or nonspecific.

So there was a problem at that time with translating all of this elegant chemistry to see whether it actually happens in the body, and secondly is that it plays a role in the cause of any human disease.

So when we discovered these compounds, these F2 isoprostanes, for the first few years after this discovery it sort of became and it appeared that maybe measuring these compounds appeared to be probably the most reliable way to assess oxidative stress status or oxidative injury in the body.

It was about four years ago it was published, so this was then independently confirmed. We actually asked National Institutes of Environmental Health Sciences. They set up an independent sort of study which they called the BOSS study, biomarkers of

oxidative stress status, where essentially they set up a very well-accepted model of very, very, very severe oxidative injury to the liver in vivo.

They took samples of liver, et cetera, and plasma and sent these around to investigators all around the country who all had different methods of assays to assess oxidative stress. What came out of that study clearly was that measuring these F2 isoprostanes is far and away the most reliable way to assess oxidative stress status.

Some of these sort of assays that people had been using, such as what's called the TBARS assay -- I mean, this is a Hiroshima bomb model of oxidative injury, so the F2 isoprostanes in this model go up like 80 fold. Some of these other assays that people had been using, they didn't go up at all and a couple, as I remember, even decreased.

So basically I think what this discovery of these F2 isoprostanes was is it really helped move the field from the test tube to what's going on in humans and what's going on. Is oxidative injury really playing a role in human disease?

Q Now, doctor, you don't consider yourself to be an expert in mercury toxicity or the diagnosis or treatment of autism, is that correct?

A Right.

Q And do you consider yourself to be an expert in the area of oxidative stress and oxidative damage as it relates to various diseases?

A Yes.

Q Now, in addition to your report filed as Respondent's Exhibit CC, you also listened to the testimony of Dr. Deth that was presented to this Court on May 13?

A Yes.

Q Now, I want to move on to if you could just explain in simple terms, and we have some slides, about just what oxidative stress is?

A Well, we've heard that term so I thought it might be helpful to actually sort of define what is the concept of oxidation. I'll start with just saying molecules are made up of several different atoms.

SPECIAL MASTER HASTINGS: Doctor, let me interrupt here.

THE WITNESS: Sure.

SPECIAL MASTER HASTINGS: I think we're on Slide No. 4 here, so as we go from slide to slide let's record the number. Also, as we get into the substance of your testimony do bear in mind that you're talking to laypeople here. When we get into

difficult terms or terms that laypeople may not understand, make sure you explain them to us.

These words that are new to us, pronounce them as precisely as you can so we can understand and follow what's going on. Go ahead, Ms. Renzi.

SPECIAL MASTER VOWELL: If I could add to that, if you would slow down just a little bit it would help me.

THE WITNESS: Okay. I'm a fast talker. So I tried to make this concept of oxidation, which we've heard of during these trials, exactly what it is, why it happens and what's going on. So I think everybody knows what molecules are. Molecules are made up of atoms, okay?

So there's electrons that orbit the nucleus of atoms and they orbit them in pairs. One is spinning one way, one is spinning the other way, and so everything is happy because everything is balanced. Now, if something comes along and extracts a single electron from an atom, that's termed oxidation. So extraction of electron is termed oxidation.

So what that leaves behind is one unpaired electron and that electron now is not happy because its lost its partner. So what it's going to do is try and find another electron somewhere. It's very, very

unstable when you have an unpaired electron. Very, very unstable, and these compounds are very reactive. They really want to go find another electron.

So they'll go find, extract an electron from another atom or atom in another molecule and that actually causes a chain reaction that propagates the further oxidation, and it just keeps going, and going and going.

BY MS. RENZI:

Q Yes. And a free radical then is a molecule that only has one electron, the other one is gone?

A Exactly.

Q Now, your next slide, which is going to be Slide No. 5, is on free radical produced lipid peroxidation and the mechanisms for oxidative damage, is that correct?

A That's correct.

Q And could you please go through that?

A So this just sort of depicts what I have just said. The *L* here is a lipid, and an *H* is a hydrogen atom and the *R* is a radical. So this radical will extract this hydrogen, which is an electron, from the lipid and so that will make an RH. What's left behind now is a lipid radical that doctored the radical, an unpaired electron.

There's a lot of oxygen around in the body so that Ldot or that lipid with an unpaired electron will immediately react with oxygen. That still leaves a radical. It's LOO. So that LOOdot, now it's still trying to find another electron so what it will do is oxidize like another lipid, so that makes LOOHdot. That's okay, but what it leaves behind is another Ldot, which we can see. Then you start back up here again. So this just keeps propagating itself until something stops it.

Q And how do you stop this chain reaction?

A I think we go to the next slide.

Q And we're on Slide 6.

A So how do we stop this process? Because it's just a chain reaction. And so the way this can be terminated is by donation of an electron, which is termed reduction. So oxidation is taking an electron away, reduction is donating an electron from, for example, an antioxidant molecule.

So you might think, well, that's just going to set up continued propagation. But that's not the case because antioxidant molecules have very unique properties in that the unpaired electron remaining after donating electrons in the antioxidant molecules is not highly reactive and therefore will not extract

another electron from another molecule.

So there, the chain reaction is stopped by this antioxidant. So accordingly, the REDOX status, we've heard that term in these proceedings before, really represents the balance between reduction and oxidation and it's a balance and where you are in that balance. So oxidative stress is defined as an imbalance between oxidation and reduction in favor of the former.

Q Thank you. Now, every human being undergoes oxidative stress, is that correct?

A Right. Our normal process of metabolism, everything, we have a certain amount of oxidative stress, but the damage caused by that is kept in check.

Q And what are some examples that will elicit oxidative stress?

A Well, several things. I mean, you bruise your finger, you're going to have oxidative stress in your finger until that's repaired. Exercise is even associated. That's well-known to actually cause a modest oxidative stress. Actually, a modest oxidative stress can be actually beneficial because what we have, we're balancing things, like I said, between the amount of oxidation and the amount of reduction.

We have a battery of protective mechanisms, which we call antioxidant enzymes, and superoxidate -- catylates (phonetic) through the found peroxidates.

SPECIAL MASTER HASTINGS: Can you say that again? You really started to go fast in that last part of the answer.

THE WITNESS: Okay. So we have a balance, you know, there's a certain amount of oxidative stress always going on. So why doesn't that just go rampant, because we have a battery of protective antioxidant enzymes that keep that in check at a certain level. I can name these enzymes. So it's how much oxidation is going on and how much of this protective mechanism.

That's why we don't just oxidize ourselves to death. We have this constant low-level degree of oxidation going on but we have these incredible antioxidant defense mechanisms to keep that at bay. Does that make sense?

MS. RENZI: Yes.

THE WITNESS: Okay.

BY MS. RENZI:

Q So if exercise causes oxidative stress it doesn't mean I shouldn't go running when it stops raining this afternoon?

A No. I mean, most people think that's what

is behind the beneficial effects of exercise. It's a modest oxidative stress which then leads to upregulation of all these antioxidant defenses so that then if you have some oxidant going on you're already more than prepared to take care of that.

Q So you're saying basically that oxidative stress can have a protective effect?

A Well, in a modest degree.

Q Then we'll move on to Slide 7. Now, oxidative stress does not necessarily mean no oxidative damage, is that correct?

A That's correct.

Q And if you could just go through this slide, please?

A So you have to say oxidative stress and oxidative damage are two different things.

SPECIAL MASTER HASTINGS: Can you say that one again?

THE WITNESS: So oxidative stress doesn't necessarily equate to oxidative damage. Your body senses that this REDOX status is altered and what it's trying to do is keep important molecules from becoming oxidatively damages, so it will upregulate these defenses to keep this at bay.

So there's ways you can assess oxidative

stress, but that doesn't necessarily equate to oxidative damage, per se, or it will be a minimal damage that can be readily repaired. So the second one is the fact that moderate levels can be protective, when the body senses, it upregulates to synthesis enumerant antioxidant enzymes which can prevent oxidative damage to --

SPECIAL MASTER HASTINGS: Doctor, when you start pointing at the screen and reading your voice doubles in speed and we can't get any of it, so when you get to that part slow down.

THE WITNESS: Okay. So I actually think I've just covered these things on the slide.

BY MS. RENZI:

Q Okay. Does the finding of oxidative stress in the periphery, the plasma, indicate that there's oxidative stress in the brain?

A No.

Q And why is that?

A You know, the damage done by free radicals happens where they're generated. As I said, free radicals are very, very reactive. They don't travel around the body and go to the brain. They're going to try to get this electron from what's next door and they will get it. They don't travel around because

they're very, very extremely reactive.

So there's all kinds of diseases which we and others have, you know, well-documented: severe sort of oxidative injury in peripheral organs, you know, and these people don't have any central nervous system abnormalities.

You know, we even looked at graduate students who have elevated cholesterol levels. These are 20-year-old kids who have cholesterol levels above 200. They have higher levels of isoprostanes for another reason. Obviously they're in graduate school, they don't have any problems thinking.

Q And if there is oxidative stress in the brain, can that be detected by measuring oxidative stress in the periphery?

A No, it can't, and for the same reasons I said before. These free radicals don't move around. They're going to react locally with other macromolecules. In fact, we've done a lot of research into the role of oxidative injury and Alzheimer's disease.

We can detect this by looking at brains taken from patients with Alzheimer's disease at autopsy or we can see this elevation in these isoprostanes in cerebral spinal fluid taken from the

lumbar spine which is draining the brain. We've published this as well, that we cannot pick up this oxidative injury that's happening in the brain when we measure isoprostanes in plasma or urine.

I've got ongoing studies with some people in Dallas where we're looking at people who have very, very severe, traumatic brain injury. Indeed, the amount of oxidation going in the brain and the spinal fluid that's draining the brain is enormous in these patients, and we cannot pick that up in the periphery.

We're also studying patients with subarachnoid hemorrhage where they bleed into their brain. The amount of oxidation there we can pick up in the cerebral spinal fluid is very high, and we cannot pick that up in the periphery.

Q Now, Petitioners and Petitioners' experts here have talked a lot about various biomarkers that they say indicate ongoing oxidative stress. I just want to ask you a few questions about some of those tests, well, of some of the indicators of oxidative stress.

The first is the glutathione and oxidized glutathione balance. Could you just explain what glutathione is? I think it's referred to as GSH?

A Yeah, GSH. And so, GSH, if you remember

back on my first slide, if you extract electrons from GSH, that *H*, that hydrogen, you're left with GSdot that's an unpaired electron. So that then, if you've got two GSdots, they will combine and form GSSG. I think Dean Jones who is going to testify will get into this in more detail. So the ratio of GSH to GSSG is the ratio of oxidized glutathione to reduced glutathione.

Q And in this case, the altered GSH and GSSG ratios have been used to argue that children with autism have chronic oxidative stress, which Petitioners have causally implicated in the etiology of autism. Can these altered ratios be indicative of oxidative stress?

A They can be indicative of oxidative stress, but they're not necessarily indicative of oxidative damage. Furthermore, these measurements were done in plasma, which I've said what's going on in the periphery doesn't have any relevance, very little relevance, to what's going on in the brain.

Q I think you've talked about this earlier on when you were talking about your research and what led you to the discovery of the isoprostanes but what is the most accurate way then to measure oxidative injury?

A I mentioned this before, that this independent study by National Institutes of Environmental Health Sciences essentially sort of evaluated the validity of almost every method that's ever been developed to assess oxidative injury, and the analysis of F2 isoprostanes far exceeded other things.

I mean, some of these assays, like I've said before, you know, the isoprostanes went up like 80 fold in this well-established animal model of oxidative injury. In some of these other methods that had been developed, I mean, they went up 20 percent or something like that.

Q And so, again, if you did find an elevated level of these F2 isoprostanes in the plasma, would that reflect anything that's going on in the brain?

A No.

Q And were there oxidative damage in the brain, could that be detected by measurements in the periphery?

A No.

Q Now, in your expert report you reviewed some of the papers relied upon by Dr. Deth that proved that there was oxidative stress going on.

A Yes.

Q And I would just like to go through a couple of those with you.

A Okay.

Q The first is the Chahan 2004, which was Petitioners' Master List 481. The authors of that paper measured levels of MDA and plasma by TBARS assay. I'd like you just to describe that process and let me know if that's a reliable test to determine oxidative damage.

A It's totally unreliable, and there's two reasons for it.

SPECIAL MASTER VOWELL: I'm sorry, you're going to have to repeat that. I cannot pick that up.

THE WITNESS: Okay. It's totally unreliable. The reason for that is MDA, or malondialdehyde, is not a specific product of lipid peroxidation, and the TBARS assay used to measure MDA is not specific for MDA. There's a real problem with using the TBARS assay in plasma. The reason for that is so TBARS measures malondialdehyde and some other things.

SPECIAL MASTER VOWELL: Measured? What was that word?

THE WITNESS: TBARS.

SPECIAL MASTER VOWELL: TBARS measure

something?

THE WITNESS: Measures MDA, but some other things as well. It's not specific for measuring MDA.

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: Okay? Malondialdehyde.

SPECIAL MASTER VOWELL: That was the word I couldn't pick up.

SPECIAL MASTER HASTINGS: That's the word, and that's what?

MS. RENZI: MDA.

SPECIAL MASTER HASTINGS: That's MDA. Okay.

THE WITNESS: MDA is malondialdehyde, right. And the other problem is they measured this in plasma. I'm going to try and make you understand this because it may get a little complicated, but I'll go through it as slow as I can.

So does everybody know what platelets are?

SPECIAL MASTER VOWELL: Yes.

THE WITNESS: Okay. When you draw blood to isolate plasma you cannot draw blood without activating platelets, okay? It just happens. There's little platelets, and, you know, they come squeezing through the needle, and they don't like that, they get activated. So one problem is there's this enzyme in platelets called thromboxane synthase.

So when you activate platelets they start making thromboxane by this enzyme. The problem is for every molecule of thromboxane that this enzyme makes, it also makes one molecule of MDA. Has nothing to do with oxidation. It's an enzymatic product of this enzyme and platelets which is activated when you draw the blood.

So, as I said, measuring MDA in plasma, if you had a very, very specific assay for MDA, would not be reliable as a measure of oxidative stress. The TBARS is not even reliable to measure MDA. So there's big problems with measuring the TBARS assay in plasma. Totally unreliable.

BY MS. RENZI:

Q And the other study that you reviewed was Ming 2005, which is Petitioners' Master List 124. This also is relied upon by Dr. Deth. In that Ming test they measured F2 isoprostanes with immunoassays as evidence of oxidative damage in autistic children. If you could just go through the type of test that was performed and whether that is reliable?

A Well, we do our analyses for isoprostane by mass spectrometry. I'm sure there's many people who don't know what mass spectrometry is. It requires a very sophisticated instrument which costs several

hundred thousand dollars, but it's the most accurate way to probably measure anything.

So immunoassays is where somebody develops an antibody against some analyte, whatever that is, usually it's proteins, and that binds to it. So you can measure certain compounds using an immunoassay. This gets back to, as I said, these isoprostanes are small lipid molecules. They're not big proteins.

Antibodies against big proteins are usually much more specific than trying to make antibodies to small molecules, such as lipids. These isoprostanes are lipids. So when I was in the prostaglandin field they developed immunoassays for prostaglandins. Remember, the isoprostanes are prostaglandinlike compounds.

SPECIAL MASTER HASTINGS: Would you say that last again?

THE WITNESS: Okay.

SPECIAL MASTER VOWELL: I think if we could just take a short recess here. We're going to try to swap out your mic, doctor. We're getting feedback, which is also interfering with our hearing. So if we can go off the record for five minutes or so.

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: I'll just ask

again, doctor, please try to slow down. We know you're giving us some important testimony here. We want to actually understand it.

THE WITNESS: Okay. Do my best. So we were talking about immunoassays for isoprostanes. So when I started this I wanted to make it clear, I hope I made it clear, that isoprostanes are prostaglandinlike compounds, okay, which are very small lipid molecules.

So when you have immunoassays you have an antibody, and it binds to a certain analyte, and you can measure how much the binding is, and therefore, quantitate whatever you're trying to measure with an antibody. Most antibodies are against things, big molecules, such as proteins or something.

So but isoprostanes are small lipids, very small molecular weight. When I was in the prostaglandin field people tried for years and years to try to develop a reliable, accurate measure immunoassay for measuring prostaglandins, and essentially it never happened.

They tried all kinds of distortions and all kinds of ways to do this. Sometimes they would extract samples, and sometimes that made it worse, sometimes it made it better. So there's a lot of interfering substances and biological fluids, such as

plasma and urine, which can interfere with this antibody binding.

So basically companies sell these immunoassay kits because they make money, but their reliability is very, very questionable. Aside from that, given the fact, which I've talked about before, measuring something in the plasma tells you nothing about what's going on in the brain.

So even if this immunoassay that they used measuring isoprostanes in the plasma was reliable -- which it probably, I won't say it wasn't because I don't know for sure, but most immunoassays for small lipids and isoprostanes that have been developed, and we've shown that, are not reliable -- that tells you nothing about what's going on in the brain.

BY MS. RENZI:

Q Thank you. Have you done in vitro studies on oxidative stress?

A Yes.

Q And you've also done animal studies on oxidative stress?

A Yes.

Q And human studies?

A Yes.

Q And do you have an opinion on the value of

extrapolating in vitro data to what occurs in vivo?

A It's very, very, very difficult. I think we've already heard that at this is you can use cell culture systems as an initial hint of whether you might want to do something else, but you can't extrapolate. These cells in culture are already transformed.

You know, if you took a cell out of your brain, a neuron, and put it in a culture, it's going to die. So these are not normal cells and the culture conditions are not normal. So if you're extrapolating what you might find in a cell culture system to what happens in vivo, you can't. It's just initial hint, should we look further or not?

So, you know, that's a lead. Then usually, you know, you will go, if you've had animal model -- for instance, in some disease we'll set up an animal model of that disease -- a lot of animal models in disease don't actually accurately mimic, at least in toto, what's going on in a human disease, and that's known to be problem.

So that's a stepwise sort of fashion. Then you sort of evaluate if there is one in an animal model if that's human disease. If things look positive, then what you really absolutely in the end

need to do is look at humans. I'm sort of a translational scientist, I do very basic research, but I go to humans as fast as I possibly can because that's where the real answer is.

Q In your opinion, do the studies relied upon by Dr. Deth show more likely than not that autistic children have oxidative stress in the brain?

A No.

Q So even if Dr. Deth and Dr. Mumper are correct, namely that there is evidence of oxidative stress in children with autism with finding in the periphery, is there any way to discern the cause of that stress?

A No.

Q Why is that?

A As I said, I mean, it can happen from all kinds of different things, such as even modest exercise. And so all we can say is in these kids, and it may be poor nutrition, these children aren't normal children. I can imagine their dietary habits or intake is not completely normal.

They get up from the table, and they go somewhere and, you know, it could be -- we have to take in certain small antioxidant enzymes such as we've all heard about, vitamin C, vitamin E.

We have to take these in from our diet. And so it's conceivable, it's all speculation, that maybe some of these children, their nutrition is not adequate, so their amount of vitamin C, vitamin E and other antioxidants they have to ingest may not be normal.

Q And is oxidative stress indicative of oxidative injury?

A No. What I might say is that, you know, an oxidative stress may cause a minimal amount of oxidative injury, but that can be protective because then that leads to this upregulation of all these antioxidant defense enzymes and mechanisms that keep this whole process in check from getting out of hand.

Q And would that finding tell you anything about what is going on in the brain?

A No.

Q Are there any specific biomarkers for mercury-induced oxidative stress?

A Not that I know of.

Q And finally, doctor, in your 20 years of experience working in the field of oxidative stress, in your opinion, is there reliable evidence to conclude that autism is caused by oxidative stress?

A I see none.

Q In your opinion here today, do you believe that reflects what's generally accepted by researchers in the area of oxidative stress?

A Yeah. I mean, our sort of Free Radical Society and Society for Free Radical Biology in Medicine, I can't even remember, I can't say that there wasn't some abstract submitted on that, but it's certainly not something that's even discussed in people in the field.

MS. RENZI: Thank you. I have no further questions.

SPECIAL MASTER HASTINGS: Petitioners have any questions here?

MR. WILLIAMS: Very few.

SPECIAL MASTER HASTINGS: Please go ahead.

CROSS-EXAMINATION

BY MR. WILLIAMS:

Q Good morning, Dr. Johnson.

A Roberts.

Q Let me ask you, is there any way that researchers trying to treat autism could measure oxidative stress in the periphery to see if it was going on in the brain?

A No.

Q No way to do it at all?

A (Nonverbal response.)

Q Spinal fluid maybe?

A Yes.

Q Do you understand that the general theory that Dr. Kinsbourne presented here is that the persistence of inorganic mercury in the brain of these children leads to active chronic neuroinflammation which then can explain the symptoms of autism according to a number of published papers? Do you agree that neuroinflammation can explain the symptoms of autism?

MR. MATANOSKI: I want to object. This is beyond his scope of his direct. He is offering opinion on Dr. Deth's testimony.

SPECIAL MASTER HASTINGS: Mr. Williams?

MR. WILLIAMS: Well, let me withdraw the question then.

BY MR. WILLIAMS:

Q Can neuroinflammation in the brain cause oxidative stress?

A Yes, it can, up to a certain extent. It depends how severe the inflammation is, and, you know, that doesn't this oxidative stress may not, depending on the severity. There's a gradation, as I've tried to explain, and we have defense mechanisms that may

not necessarily translate into oxidative damage.

Q And oxidative damage could be a loss of function, not just cell death, correct?

A Yes.

Q Would inorganic mercury inside neurons cause oxidative stress in those neurons?

MR. MATANOSKI: Again, beyond the scope of his direct, and also, he said that he's not an expert in mercury.

SPECIAL MASTER HASTINGS: Well, that question, let's see if he can answer. If he can't answer it then he'll tell us that.

THE WITNESS: So repeat your question, sir.

MR. WILLIAMS: Can inorganic mercury inside neurons cause oxidative stress to the neuron?

THE WITNESS: I don't know that.

BY MR. WILLIAMS:

Q Okay. You mentioned autopsy studies of Alzheimer's patients which do show oxidative stress in those people, correct?

A And oxidative damage.

Q And oxidative damage. Have you reviewed any of the autopsy studies of autistics?

A No.

MR. WILLIAMS: Thank you. That's all I

have.

SPECIAL MASTER HASTINGS: Any redirect for this witness?

MS. RENZI: No. Thank you.

SPECIAL MASTER HASTINGS: All right. Dr. Roberts, we're sorry we stuck you with a bad microphone for the beginning of your testimony. I thank Special Master Vowell for figuring that out. I didn't. We thank you very much for your testimony. You're excused at this time.

I'm sorry, I'm sorry, you're not excused. My mistake. Special Master Campbell-Smith?

SPECIAL MASTER CAMPBELL-SMITH: Dr. Roberts, I did have a question. I heard you testify on cross that neuroinflammation can cause oxidative stress, and your testimony was depending on the level of neuroinflammation?

THE WITNESS: Uh-huh. Yes.

SPECIAL MASTER CAMPBELL-SMITH: Can you give some general, without perhaps specific numbers, but when you say depending on the level, would that have to be substantial neuroinflammation that would cause oxidative stress?

THE WITNESS: I mean, it's a continuum of things where a small amount can be protective. The

only way to answer that question would be if you had absolutely firm evidence that there was oxidative damage at some level of inflammation because, you know, leukocytes try to kill bugs by making hydrogen peroxide.

SPECIAL MASTER HASTINGS: Can you say that sentence again?

THE WITNESS: Okay. So when you have inflammation, like leukocytes, I mean, they try to kill bacteria by making oxidants, like hydrogen peroxide, but the point I tried to make across, there's a continuum of all this. So your question is I think by and large a little bit unanswerable because where does this amount of inflammation turn into oxidative damage rather than not?

SPECIAL MASTER CAMPBELL-SMITH: Well, you're anticipating a question, and maybe you're thinking a step ahead of me. What we've been hearing is there is a chronic level of neuroinflammation --

THE WITNESS: Right.

SPECIAL MASTER CAMPBELL-SMITH: -- that leads to oxidative stress, oxidative stress that is sufficient to cause damage that looks like a loss of cell function as opposed to cell death.

I've heard you testify that there is a

chronic level of oxidative stress that exists at all times that can be handled by upregulated antioxidants, and that does not necessarily translate into damage which would be, in your definition damage is alteration of cell function and cell death?

Because it appeared when you said that you can cause damage but if the body can handle it and the cell protective mechanisms can repair it you don't really regard that as a damage.

THE WITNESS: that's correct.

SPECIAL MASTER CAMPBELL-SMITH: Okay. So I think you might have been anticipating my next question. I recognize it may be unanswerable but we're floating in a level of where is the difference between just what is oxidative stress that the body's own system is able to handle and when do we begin to veer into what you would regard as damage.

THE WITNESS: Well, the only way to know where you're on that curve is to actually measure and quantify the level of oxidative damage. So you don't know, and it may vary between one person or another person -- so there's a continuum, and you don't know where you are on that continuum unless you measure a product of oxidative damage.

It's too hypothetical to know where you're on

this curve. Does that make sense?

SPECIAL MASTER CAMPBELL-SMITH: It does.

THE WITNESS: Okay.

SPECIAL MASTER CAMPBELL-SMITH: It does. Okay.

THE WITNESS: You know, you have to see, is there oxidative damage and actually actively quantify the level of oxidative damage and see if it's increased. You don't know where you are on that curve because how much in this person, your antioxidant defenses are upregulated because of this low level of stress. You don't know. The only way you'd know where you are on that curve is to measure how much damage is being done.

SPECIAL MASTER CAMPBELL-SMITH: And you cannot do that in the periphery and get an understanding of what's happening in the brain?

THE WITNESS: That's correct.

SPECIAL MASTER CAMPBELL-SMITH: Thank you.

SPECIAL MASTER HASTINGS: No. Wait a minute, doctor.

MR. WILLIAMS: Well --

SPECIAL MASTER HASTINGS: Yes, please.

MR. WILLIAMS: One more quick line of questions.

BY MR. WILLIAMS:

Q When you speak of oxidative damage in the brain, there's been evidence here that monkeys exposed to inorganic mercury leads to neuroinflammation and astrocyte death. If astrocytes die as a result of neuroinflammation, can that lead, as Dr. Deth told us, to a lowering of glutathione in the brain?

MR. MATANOSKI: I'm going to object again because it's beyond the scope of his direct and I think it's beyond the scope of the questions from the bench as well.

SPECIAL MASTER HASTINGS: Well, I'm going to let him. Why don't you ask the question again. I'll let him answer that.

MR. WILLIAMS: Okay. One of the things Dr. Deth testified to was that neuroinflammation in the brain can lead to dysfunction, even death, of astrocytes. Dysfunction and death of astrocytes can lead to a lowering of glutathione, which then leads to a chronic inability to restore the REDOX balance in the brain.

My question to you is do you agree that that is a plausible mechanism?

THE WITNESS: Well, I would ask do we know what caused the cell death? Is it really oxidative

damage? You know that cells are dying in the brains of these children with autism but do you know the mechanism?

MR. WILLIAMS: Well, I'm asking you.

THE WITNESS: You would have to have measurements of oxidative injury in those brains.

BY MR. WILLIAMS:

Q And you haven't reviewed the autopsy studies?

A No.

MR. WILLIAMS: Okay.

SPECIAL MASTER HASTINGS: Anything further, Ms. Renzi?

MS. RENZI: Just one question.

SPECIAL MASTER HASTINGS: Please go ahead. Doctor, you stay there until we tell you. Each time one of us asks a question that means the rest get to respond to that.

THE WITNESS: That's fine.

REDIRECT EXAMINATION

BY MS. RENZI:

Q I'll make this very short. Is there a difference between oxidative stress, oxidative damage and oxidative damage that has consequences?

A Yes. As I said, I mean, these isoprostanes,

which are indicative of oxidative damage to lipids, I can measure those in your plasma and there's a normal level of oxidative damage to all of our macromolecules, proteins and everything else, but that's kept at a level that doesn't hurt us.

It's only when we can't keep it at that level and it starts to sort of fly away, then we get more damage and that's when you can get into trouble with cells dying, et cetera, et cetera.

MS. RENZI: Thank you.

SPECIAL MASTER HASTINGS: Mr. Williams, anything further?

MR. WILLIAMS: No. I'm finished.

SPECIAL MASTER HASTINGS: Special Masters, anything?

THE WITNESS: Can I leave?

SPECIAL MASTER HASTINGS: Going once. Going twice. Doctor, thank you again.

(Witness excused.)

SPECIAL MASTER HASTINGS: All right. Is the government ready to call its next witness?

MR. MATANOSKI: They're in the building, as I understand, they're just not in the courtroom right now. If we had about a 10-minute break and switch out here.

SPECIAL MASTER HASTINGS: Let's take a 10-minute break.

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: I imagine we have Dr. Johnson on the stand here. Ms. Babcock, when you're ready, please go ahead.

MS. BABCOCK: Good morning.

MR. JOHNSON: Good morning.

MS. BABCOCK: Could you please state your name for the record?

MR. JOHNSON: Jeff Johnson.

MS. BABCOCK: I suppose we should start by saying we have a slide exhibit.

SPECIAL MASTER HASTINGS: All right. Yes.

MS. BABCOCK: Should we identify it as the Trial Exhibit 7?

SPECIAL MASTER HASTINGS: Yes.

MS. BABCOCK: Take a moment.

(The document referred to was marked for identification as Respondent's Trial Exhibit No. 7.)

SPECIAL MASTER HASTINGS: Thank you.

MS. BABCOCK: I'm sorry. Special Master, did you want to swear the witness in?

SPECIAL MASTER HASTINGS: Yes. Dr. Johnson, would you raise your right hand, please?

Whereupon,

JEFF JOHNSON

having been duly sworn, was called as a witness and was examined and testified as follows:

SPECIAL MASTER HASTINGS: Please do speak up. Go ahead, Ms. Babcock.

DIRECT EXAMINATION

BY MS. BABCOCK:

Q Could you please state your name for the record?

A Jeff Johnson.

Q And what is your profession?

A I am a Professor in the School of Pharmacy at the University of Wisconsin in Madison.

Q And could you briefly describe your collegiate and graduate education?

A I received my bachelor's of science degree in biology with minors in chemistry and philosophy from the University of Minnesota in Duluth. I received a master's in pharmacology from the University of Minnesota in Duluth. Then I moved on to a Ph.D. at the University of Wisconsin in environmental toxicology.

I did a postdoctorate fellowship at the University of Washington, the other U-dub, for about three years, and there I worked on molecular neuroscience and single transduction.

Q And do you hold teaching positions at the University of Wisconsin?

A Yes, I do teach at the University of Wisconsin. I'm a Professor there, yes.

Q You teach both graduates and undergraduates?

A Yes, I teach both graduates and undergraduates and also professional students in the pharm-D program.

Q And you also have a laboratory at the University of Wisconsin?

A Yes.

Q And what is the primary focus of your research?

A The primary focus of my research is neurodegenerative diseases, so we work on Alzheimer's, Parkinson's, ALS and Huntington's disease. Specifically, we're interested in ways to protect from cell loss and neuronal cell death in those diseases.

Q And have you published on topics in the field of your research?

A Yes, many times.

Q And are you a reviewer for any publications?

A Yeah. In fact, I just got an email this morning to review an article while I was in my hotel room, so I get asked about every week to review an article from, I can't even -- there's, you know, 20, 30 different journals.

Q Pardon me. Did I cut you --

A No. That's okay.

Q Okay. Sorry.

A Did you want me to recite the 20 or 30? I don't want to.

Q I think for the sake of brevity this morning we will let your CV, which has been filed into the record.

A Okay.

Q Have you received any significant honors for your work?

A Well, one of the ones that I received early in my career was the Burroughs Welcome award, which is a new investigator of toxicology award. Why I consider that an honor is it's a very competitive award.

Essentially every institution in the United States that does research, like the University of Wisconsin, picks one candidate from their institution

that's an assistant professor and they're allowed to submit one application. So you can imagine there's probably 50 to 100 applications on one of those rounds and they select four of the top people in their field that get that award, and I was that.

The other one that I'm proud of recently was I was given the Community Humanitarian award by the Huntington's disease Society of America. That came from my research which got a bunch of publicity. I got involved in, you know, groups, and family groups and things like that.

So I'll go and speak to family groups, and, you know, groups with ALS and things like that to talk to them about what we're doing in research-wise. That's been, actually, a very rewarding part of my research that's come out in the past couple of years.

Q And did you review the expert reports and literature in this case as it related to your area of expertise?

A Yes, I did.

Q You've also prepared an expert report which has been filed in this case?

A Yes.

Q And were you present in Court last week to hear some of the expert testimony?

A Yes, I was.

Q And as part of your research you study neurodegenerative diseases, correct?

A That is correct, yes.

Q Now, comparing the general symptoms of neurodegenerative diseases and the ultimate end point, does autism bear any resemblance to the diseases that you study?

A No. If it did, we'd be studying it. Again, in the case of neurodegenerative diseases, the outcomes are essentially neuro death and the patients die, and so that would be my focus. If that was occurring in autism, it would probably be under that list of research areas that I gave you at the beginning.

Q And in general, do most neurodegenerative diseases occur much later in life?

A In general, yes. At least the ones I've listed begin later in life. They can begin early in life depending on the genetic components that are made up of that. For example, Huntington's disease is a genetic disease which can occur earlier in life.

Q Have you ever been a reviewer for the National Institutes of Health?

A Yeah. I served on study section for five

years.

Q And study section is the group of academics and researchers who review grant applications, correct?

A Correct, yeah. So when I submit, the same happens to me. It's like peer review of a manuscript, only it's peer review of a grant.

Q And study sections have particular focuses? You know, is your study section based on your area of expertise?

A Yeah. My study section was titled Neurotoxicology and Alcohol, so it basically dealt with the neurological effects of alcohol in the brain as well as neurotoxicology.

Q Now, when you review grant applications for your study section are decisions based on the scientific validity of the proposed study or potential political policy considerations?

A We don't consider politics and political considerations. The reviews, and as specifically, I can comment on this in response to something that Dr. Deth was saying in his testimony, our study section actually reviewed a Deth grant in 2003 and my recollection of that grant review was that it was a weak hypothesis, preliminary data didn't support it

and there was not a good experimental design set up in the grant.

And so the reason that grants and study sections don't get funded, or get rejected, or get low scores is not due to the politics, it's due to the scientific merit of the application. Absolutely.

Q Now, in your review of Dr. Deth's expert report you identified a number of concerns. I wanted to just talk about a few of them here. I think we're switching to Slide 2 now. In general, did you encounter instances where Dr. Deth used in vitro laboratory work to extrapolate to the in vivo situation?

A Yeah. In his expert report he did that I think over and over.

Q Now, are there complications that can arise from in vitro studies?

A There's complications and there's dramatic limitations. I mean, in vitro studies are in vitro studies. I mean, I tell the people in my laboratory and postdocs and grad students if you're working with a cell line, the interpretation that you can make on the data that you generate in that cell line, it has to be held within that cell line.

I mean, you can form hypotheses based on

maybe what you get from the cell line, but you can't extrapolate that data without actually doing experiments.

Q Now, are there protective mechanisms that happen in vivo that are not present in vitro?

A Once you take out the cell and put it into, you know, an environment that's not natural, a lot of things change, and so, yeah, you're changing all kinds of aspects of that, so you don't have the cell/cell communication anymore and you also are looking at, you know, different things that are occurring in the cell, so things are completely different. You can't even compare it.

Q And the particular cells used sometimes have an effect. This is Slide 3.

A Yeah. I put this slide up to try to, again, help clarify what the cell line is and how we look at a cell line. So a neuroblastoma cell line is basically a self-renewing cell line that grows spontaneously and they're usually from tumors. They often have aberrant numbers of chromosomes. They don't have the normal number of chromosomes.

They also contain in a number of situations multiple genetic mutations because of the way the tumors are growing, and self-renewing cells tend to,

you know, have mutations that they carry through. Again, as I've mentioned earlier, they're constantly expanding and dividing and they have uncontrolled growth.

If you transplant them into mice they form tumors. In most situations, especially when we're talking about neuroblastoma cells, and I think this is a real critical point, is that we get what we call a dedifferentiation. So, for example, the neuroblastoma cells that are being used in most of these studies is they also have characteristics of glial cells.

So they don't have just the neuronal proteins in them anymore, they also express glial proteins, and they have glial characteristics and you can see all these other kinds of things. So they almost take a step back away from this differentiated state that we see in vivo to more of a primordial state where they have different proteins turned on that are not normally there in neurons.

That's I think a major issue that people need to understand is these are not like neurons, differentiated neurons. The reason they're used is because they're easy to use and they're cheap, and so you can do a lot of experiments, you know, fast in them, but you do have to remember that their

interpretation, it's impossible to extrapolate to humans.

The other system that I want to talk about briefly in vitro was this primary neuronal cultures. In this situation they're very different.

SPECIAL MASTER HASTINGS: Can you say that again? The what?

THE WITNESS: Primary neuronal cultures. They're very different because what you do in that situation is you remove the brains from mice and you're able to dissociate and plate them out in a dish, and they are terminal basically. They differentiate, like you would in vivo.

So if you look at the culture, they have markers of glial cells in the glial culture and the neurons don't have those glial markers and so on. SO they do maintain, "a little more of their phenotype", in that kind of context.

So the next step, at least what we've done, is if you find something in a blastoma cell or a cell line, we instantly move it into a primary culture system because it's more representative of what's the in vivo situation is. Again, because it's not in the context of the brain, its extrapolation is limited.

You can't just say what's happening there

will happen in vivo, but at least they're normal cells, although they may not be, you know, in this context of the brain, but they do have normal phenotypes, and so they're a better thing to use in a dish than a cell line for sure.

They give you a little bit more insight and they maintain much more of the normal function of the individual cells you're trying to study.

MS. BABCOCK: Now, on page 3 of his report in the second full paragraph Dr. Deth states that thimerosal is toxic to human cortical neurons in neuronal cells grown in culture. Is it okay if I just generally describe where that is? Do you need me to give you a more pinpoint identification of the location?

SPECIAL MASTER HASTINGS: No, that's fine.

MS. BABCOCK: Okay. He cites to three references, which I believe are Herdman, Baskin and Parran.

THE WITNESS: Yes.

MS. BABCOCK: Were these in vitro studies?

THE WITNESS: Yes, they were.

BY MS. BABCOCK:

Q And what type of cells were used in these --

A Cell lines. They were the neuroblastoma

cell lines, and they were not the primary cultures.

Q So they -- I'm sorry.

A Yeah. This again bothers me because when people talk about cell lines and they use the word "neuronal" that infers to people that are looking at it or that are reading it that it's actually neurons and they are not. So there is a distinct misrepresentation of using that kind of terminology, cortical neurons or neuronal, when you're looking at neural cell lines.

Again, if a student gives me a paper that they're working on and they use the word "neuronal" and they don't use the word "neuroblastoma," it's changed, it's wrong. It's just a misrepresentation. It's misleading to say that.

Q And were the doses used in these studies similar to what's used in vaccinations?

A Oh, no. You know, based on the couple of papers that have looked in mouse and primate at, you know, this mimicking this vaccination schedule, the Berman paper and then the Burbacher paper, but I looked at those papers just to kind of get a dose of what would be in the brain.

Q And let me be clear, I'm not asking you to go into specific toxicology of mercury because that's

not --

A That's not my area, no.

Q -- what you're here to talk about.

A All I did was look at those and say, okay, here's what they're saying is a dose or a level in the brains of these animals. As it's been presented before, that's in an animal range. So when I looked at the papers then of course they were micromolar range, and so that to me said that they were much, much higher than what would be at least in those two studies.

Q Now, Dr. Deth also cites a paper by Mady Hornig in support of his arguments, correct?

A Yes.

Q I believe that's PML 15. Now, you mentioned, this is in your expert report, that the mouse strain Dr. Hornig used was selected because it had a stronger immune response.

A Right.

Q But took issue with Dr. Deth's explanation of the rationale behind the use of the strain, correct?

A Right.

Q And I believe he stated that hers was a mouse strain harboring genetic deficits and REDOX-

related enzymes. This is from page 4, the first sentence of the paragraph preceding the bolded effects of methylation. What strain of mouse was used?

A That was an SJLJ mouse. The interpretation of that or the way that that was written by Dr. Deth in his report was inferring that there is a REDOX enzyme differential or some kind of differential, and that's absolutely not true. I mean, the mice have this increased immune response and that's why the mice were selected.

They've been used in these studies a lot by other groups. So there is absolutely no data supporting the fact that there is a REDOX enzyme differential. Now, I can understand the reason it's in there is because it supports his hypothesis in the sensitivity, but that's not an accurate representation of the mice.

MS. BABCOCK: And in general, do you have confidence in Dr. Hornig's reported results? Here, actually we're switching to Slide 4. I apologize. We inadvertently switched Slide 4 and Slide 5, so really what we mean to be switching to is what is marked in the trial exhibit as Slide 5.

SPECIAL MASTER HASTINGS: All right.

THE WITNESS: Will you repeat that?

MS. BABCOCK: If you have confidence in Dr. Hornig's reported results?

THE WITNESS: No.

BY MS. BABCOCK:

Q And part of that has to do with the hippocampus section, correct?

A Yeah. I mean, the quality. I mean, I don't know if I can point this out but if you look at these images on the right side of this slide --

Q Again, they are the sections from the Hornig paper.

A Yeah, these are from the Hornig paper. These are the vehicle and this is the thimerosal treated mouse.

SPECIAL MASTER VOWELL: Which ones were your referring to when you --

THE WITNESS: This. The upper right and the lower right are the two images from the Hornig paper. The *A* is the vehicle and the *B* is the thimerosal.

BY MS. BABCOCK:

Q And what do you mean by vehicle?

A Vehicle in this case, whatever they were dissolving the vaccine that they were giving in. So it's basically the same solution, same volume, without the thimerosal in it, all right? what you can see

when you look at these images is to me these images are absolutely awful.

Now, the staining here is hematoxylin and eosin, and it's supposed to stain for architecture, and cell integrity and a variety of other things. The tissues are very diffuse, there's not clear neuronal fields. Right here there are deep staining.

Q You're pointing to the top slide.

A Yeah, top slide. If you look at those images, the cells that are dark right there, those are the neuronal fields. The quality is just extremely low.

SPECIAL MASTER HASTINGS: I'm not sure what you mean. The quality of the photography is low?

THE WITNESS: No, no. The quality of the sections themselves is really -- in the next slide I'll specifically talk about what I think, you know? Put it this way. I've seen this in my lab before, I've seen people come to me with images like this or with sections and stains like this and I'll say something's wrong, okay, the tissue wasn't prepared right.

There's something that's definitely wrong with this because these do not maintain the nice cellular architecture that you should see if the

experiment is done right and the tissue is harvested correctly.

BY MS. BABCOCK:

Q So it's fair to say that problems with these slides and these sections caused you to question the ultimate findings of this paper?

A Absolutely, yeah.

Q And has the recent paper in fact refuted Dr. Hornig's findings?

A Yeah. We've talked about the Berman paper. That's come up in the Berman paper. These sections on the left side of this slide, the upper and lower sections, are comparable sections from the Berman paper and, I mean, to me they're absolutely beautiful.

When you look at the cellular architecture and the structure of the hippocampus, which is this region, the cells look very nice. The stain is different, that's cresyl-violet, but that wouldn't make any difference. You're still looking at the cell structure, the cell architecture and the way that the tissue was prepared, and it looks very, very, very good.

Q Now, strictly discussing dose, what dose of thimerosal was used in the Berman's paper?

A Well, Dr. Berman did the same dose that was

used in the Hornig study but then that group also did a dose that was 10 times higher. In both situations there was absolutely no pathological outcome with regard to the dosing with thimerosal.

Q And did both papers use antibodies on brain tissues?

A Yes, and this adds another factor that I wanted to point out. Yes, staining with antibodies. So antibodies, now, what antibodies do is they stain specific proteins in the tissue sections. What I want to point out here is the distinct difference between the Berman paper sections and the Hornig paper sections using the same antibody. Now, this is exactly the same staining and the same antibody.

SPECIAL MASTER HASTINGS: Now we're moving back to Slide 4?

THE WITNESS: Back to Slide 4, right.

MS. BABCOCK: What was, yes, originally identified as Slide 4. I apologize.

THE WITNESS: So, again, what I want to point out is if you look at the architecture of the tissue, it's really nice in the Berman study and you see this nice staining in the hippocampus which is validated, which has basically been shown in many, many other papers prior to this, this type of staining

where you see --

MS. BABCOCK: If I can stop you for a moment. I'm sorry. I think we need to clarify.

SPECIAL MASTER VOWELL: Right. Dr. Johnson, I'm going to be listening --

THE WITNESS: But you can't see. Okay.

SPECIAL MASTER VOWELL: -- or reading your testimony again and I'm not going to be able to see where you're pointing. You have to tell me.

THE WITNESS: All right. So the Berman sections are the two panels on the left side of the slide, here and here, and then the comparable little boxes in those panels on the left side are enhanced on the lower portion of the right side of the slide. So these little squares are put down here.

BY MS. BABCOCK:

Q And this is the bottom right-hand --

A At the bottom right hand corner.

Q The two bottom panels.

A The two bottom panels. Again, they're labeled vehicle and thimerosal plus vaccine. What you can see is there's very nice staining in the field. The neuronal field, which are the neurons -- the clear layer that goes through that section, those are the neurons, and they are not staining intensely but the

cells around that field are staining very intensely.

Again, that's been published multiple times by other groups, that pattern. Now, if you'd look at the upper four panels on the right side, these are the similar, comparable panels from the Hornig study. The first thing that I want to point out is if you look at the tissue it's full of holes.

So if you look at this enhanced image right here, the bottom two panels, *C* and *D*, from the Hornig study, you can see that the tissue almost looks like it's disintegrating. It's breaking down. There's holes all over in the tissue. Now, I know from experience that when you see tissue like this the amount of nonspecific staining with antibodies could be intense.

Basically, if somebody came to me with this kind of staining or this kind of tissue in my laboratory, I would say go back and do the whole experiment again because: (1) these are unpublishable to me; and (2) the potential for artifactual data generated from this kind of degenerated tissue is extremely high.

And so they would need to go back and redo these studies to ensure that they were doing the tissue preparation correctly. I mean, this is very

important, especially when you're trying to do histological section and making conclusions from histological data like this.

You know, you can do whatever you want after you have the tissue, but it's the process of getting the tissue to the point where the tissue quality is extremely good, then you can make the correct interpretations. In this situation, I don't see that.

Q And is it fair to say that you do see that in the Berman tissue --

A Yeah. The Berman tissues look absolutely perfect. It looks like their preparation is done extremely well and the sections are beautiful.

Q And as a result, do you place more weight on the Berman findings?

A Yeah. Absolutely.

Q Now, Dr. Deth also relies heavily on the 2004 Waly paper which has already been the topic of some of our expert testimony, PML 257. Do you also share concerns about this paper?

A Yeah.

Q And what are dose curves, and how are they important here?

A Well, dose curves are critical to understand the differential sensitivity of different toxins that

you're actually using so you have to lend dose curves to make sure that you're running in the range where you're seeing the effects that you want.

If you're trying to compare between different toxicants, I mean, you have to have dose curves so that you know the differential sensitivity of the toxicants with regard to what you're measuring.

Q And Dr. Deth, did he use dose curves?

A No, he did not.

Q And what type of methionine synthase was being measured, and how is that significant? This is Slide 6.

A And this is Deth Slide 15. The type of methionine synthase in the SH-SY5Y cells is actually a mutated form of methionine synthase that lacks this blue domain in the upper left-hand corner of the slide, as he pointed out.

Q And you're circling the area with the *X*.

A Yeah, with the *X* through it. This goes back to the point of cell lines. I mean, cell lines are not normal, so if you look in normal cells, and normal neurons and normal astrocytes you don't see this form of methionine synthase. This methionine synthase is a result of this tumor transformed to a cell line that's being used in these studies.

Now, how that affects the interpretation of the amount of methionine synthase data remains to be determined, but clearly, you can't assume that the data that's generated with regard to methionine synthase in this cell line is going to represent what's going on even in the dish with the normal neuron because the protein's different.

Q Now, how is the reported MAP 3 kinase and PI 3 kinase inhibition significant? Why do you doubt the results reported in the Waly paper?

A Well, again, Dr. Mailman talked about this quite abit, too, is that there are a lot of things that you can do. For example, what was used in the paper were chemical inhibitors. Chemical inhibitors are not specific, they're selective. By that I mean is that they can inhibit lots of different things at different doses if you're not careful with how they're used.

So today, in this age when we have a variety of other new novel techniques where we can selectively target and knock out specific proteins -- so, for example, in addition to using a chemical inhibitor we often use, you know, a technology called SRNA technology where you can say, okay, IRP 1, or this one protein, this one kinase we think is doing this. We

have an inhibitor but it inhibits like 10 kinases.

We want to just see if that one, okay, if we can inhibit that one or take that one out of the cell, do we block what's going on? So what you can do is you can actually do that with these new technologies. You can go in and selectively target specific proteins and get rid of them in the cell and then come back and show that the effect that you're looking for is gone or eliminated.

So good, quality papers really combine almost at least two, if not three, different kinds of techniques and they use the pharmacological inhibitors as long as molecular approaches to validate everything so it all fits together. In cell lines that's very easy to do. Cell lines are very conducive or very accepting of these kinds of techniques.

So without those kinds of validations it's very difficult to make, you know, conclusions on the specific kinases that might be involved in the pathway.

Q Now, I wanted to move on to a discussion about some of the unpublished data. Now, Dr. Deth in his report cited to a manuscript in preparation, correct?

A Right.

Q I believe, for the record, that's Reference No. 24, which he specifically cites at the bottom of the first paragraph on page 5. Have we ever seen a copy of this? Has a copy ever been provided to you or to the Court, to your knowledge?

A No.

Q But Dr. Deth does discuss this unpublished work in his expert report?

A Yes.

Q Setting aside for a moment the fact that it is unpublished, do the numbers discussed in his report raise questions when compared to other research involving thimerosal, just, again, generally in your review?

A Right. Again, the doses that are presented in the report are implying that there are low nanomolar doses that are doing the, generating the effects that Dr. Deth is saying, and in almost all of the other literature in fact even in other people using the same cells that Dr. Deth uses, they're showing, you know, effects in the micromolar range.

My comment on that I think is that, you know, a cell line is used for a number of reasons. In this case the SH-SY5Y cell line was used, but this cell line is available commercially from a called ATCC

who banks these cells.

So one of the nice things about a cell line, if there are 10 investigators across the country, and they all order the cell line form this company and they grow it in their incubators, then the theory is, or at least, you know, what we find is that there can be consistency between the different labs.

So if you treat the cells the same way with drugs or whatever you see relatively consistent results between the different groups and the different labs that are doing this. You know, there can be some variation between groups, you know, whether the cells are in California or in New York. They could have different, you know, environmental exposures or air.

You know, they have different air concentrations and stuff like that. In general, you know, we could maybe attribute that at very small different, you know, changes. Maybe four or five, maybe 10 fold at the most differentials in sensitivity.

Two or three orders of magnitude, I just can't understand or I can't understand how that would happen outside of just some technical kind of differences or if the cells are really, really sick.

Q So the reported results are substantially

lower, 100 to 1,000 times?

A Yes, and it's the only report that shows that.

Q Any indication that this has been peer-reviewed or tested?

A No.

Q Now, Dr. Deth also discussed quite a bit of unpublished data in his presentation last week. You've just sort of talked about some of the general issues you have with, you know, some of the unpublished data we've seen as it related to Deth Reference No. 24.

My next questions sort of relate generally to that and also what he presented in his testimony. The data that he presented last week as well, in addition to Reference 24, has any indication that that's ever been peer-reviewed?

A No.

Q Not published?

A No.

Q Tested?

A No.

Q We just don't know?

A No. We just don't know.

Q Now, were you present during the testimony?

A Yes, I was.

Q You've had an opportunity to review the slide presentation. Obviously you've incorporated some here already.

A Yes.

Q And now I know you wanted comment on Slide 17, which is Slide No. 7 in your presentation.

A Yes.

Q Why did Dr. Deth discuss this slide?

A Well, I think what Dr. Deth was trying to say here was that the levels of cystathionine and his pathway is on the next page, and I'll show you that briefly, were markedly higher in the human cortex than in other species. So, you know, this slide looks at the human brain and a variety of other animals, including --

Q You've switched quickly to Slide 8.

A -- duck. So what he used this data to do was to justify this statement here that the increase --

SPECIAL MASTER HASTINGS: Which statement?

THE WITNESS: The statement in the middle that's highlighted in the light green.

MS. BABCOCK: Or blue.

THE WITNESS: Or blue, or whatever. I'm not

colorblind but I just don't know colors. My daughter would be able to tell me that's aqua something, something, you know?

MS. BABCOCK: I'm sure we could come up with at least five names of what that color should be but I'll call it light blue.

THE WITNESS: So he's using that to justify this statement that the conversion of cystathionine to cystine is compromised, and, again, here's the word "neuronal cells." That's not true. This is SH-SY5Y cell. Basically, that's impossible to conclude because there's no measurement of cystine or there's no measurement of glutathione.

Maybe the human brain just has more of the cystathionine, but they actually maybe have more of the cystine and the glutathione as well, and without knowing that you can't make any conclusion that there's a partial dysfunction in that pathway.

In fact, if you look at human tissues, and you look at mouse tissues and you look at these other tissues, except for duck, I've never looked at duck, the glutathione levels in the brains of these animals are not different or they, well, they range, but they're certainly within a very close window of concentration. And the duck thing, I kept thinking

about the duck.

BY MS. BABCOCK:

Q Let me get you there. Was there a citation to the source of this data?

A No. Actually, Dr. Deth had no citation.

Q Slide 9.

A When I walked away from this I assumed that he had generated this data himself. Like I said, the duck kept bothering me. I kept thinking about this, and so what I did in this day and age is we Google. So I Googled duck and cystathionine and the top hit was a paper published in 1958 by Harris, et al. who actually measured cystathionine levels in duck.

What I was really surprised to see was that the data that Dr. Deth presented was basically the same table that was published in 1958 but he didn't reference it.

Q Scientifically, do you have a problem with this?

A I have a major problem with this because as scientists, you know, if we're using somebody else's work, we reference it. This brings into question, I mean, you know, the scientific integrity of somebody that's going to be, you know, taking somebody else's data and using it but not actually giving the

reference of where they received it, where they got it from.

Q Now, did you also review Dr. Deth's Slides 24 and 28, also, the unpublished data?

A Yeah, this was also the unpublished data. Yes, I did.

Q What would your concerns be as someone who looked over this, moving on to Slide 10?

A Well, again, you know, I looked at these slides when they were presented, this Slide 24 and Slide 28, and the image that's up here from Slide 28 is a partial, is just looking at the glutathione component, which is a big part. Half of that whole slide.

One of the things that jumped out at me right away when I saw these slides is the concentration of glutathione. So the basal level of glutathione that he shows in the panel on the left, Slide 24, just at the zero point on the curve, is 700 nanomoles per milligram protein in the SH-SY5Y cells. Then in Slide 28 it's the same cells. Here, the nanomole per milligram protein of glutathione is over 1,500.

I thought this was extremely high based on my experience, and so I went into the literature and I

actually looked at about 10 papers that used SH-SY5Y cells and measured glutathione in the same units, nanomoles per milligram protein. And it turns out that all 10 of those papers, the basal glutathione in the SH-SY5Y cells is between 12 and 30 nanomoles per milligram protein.

So, again, this could be a calculation error. It's not an intentional misrepresentation, but it goes to the point that this data was not carefully evaluated as far as the units and the numbers that were used.

Again, it just has a careless nature about it because if you know the literature and you understand the glutathione concentrations in the cells, you would have noticed that these numbers are extremely high and far off base.

Q Now, moving on to Slide 11, I know you had a comment about, you know, glutathione measurement and time.

A Yeah. This, again, is another instance of, you know, basically picking the time that you want to show your effect. In all of Dr. Deth's studies in the previous slides the time after the treatment was one hour. So he's looking at a very, very acute depletion of glutathione in these cells.

We have experience and a number of other labs have shown that if you actually deplete glutathione in a cell, like in a cell line like this, and you get a significantly, like maybe down to 70 or 80 percent, what happens is the cell senses that the glutathione is reduced and the cell compensates so it changes.

What it does is it actually makes the proteins and the enzymes that synthesize glutathione. If you look at the same cells that were 80 percent depleted at four hours, as shown in this slide on the lower right hypothetical data set, if you look at those same cells at 24 and 48 hours, you may actually have two or three times the level of glutathione in those cells.

Those cells would actually be more resistent to toxicity if you actually at that point put a toxin on. So it's a dose and it's a time issue. You really have to run the full spectrum I think of both dose and time to make the right kinds of interpretations about this data. So what this really is showing here or describing is what we call almost like a preconditioning response.

So a little bit of stress is good because a little bit of stress causes you to have this

compensatory mechanism that then makes you more resistant to more stress, okay? So this is a very important concept in toxicology in preconditioning response and the way that cells respond to these kinds of issues.

Q Now, what is carbon 14 labeled THF?

A Carbon 14 labeled tetrahydrofolate.

Q Slide 12.

A So I don't want to make this complicated. This is more of a question I think. I didn't quite understand this, but I wanted to understand the methionine synthase assay. You know, Dr. Deth spent a lot of time talking about methionine synthase. So methionine synthase is this enzyme he says that's inhibited, and he has an assay that he uses to measure this.

I want you to understand this assay, okay? So I'm not going to try to be very complicated with this, I'm going to be very straight. So if you look at this slide, look at the red, okay? So the red in the upper right-hand, the CH3, okay, that's the radio label.

SPECIAL MASTER HASTINGS: That's the what?

THE WITNESS: Radio label. Radioactive. So in the end this is what you measure. So that thing,

that red CH3, has to be on the methionine at that point so that you can actually put it into a machine and the machine tells you how much radioactivity is there, okay?

SPECIAL MASTER VOWELL: And the amount of radioactivity would tell you how much --

THE WITNESS: How much activity the enzyme has, right.

SPECIAL MASTER VOWELL: Okay.

THE WITNESS: And so the traditional, I went back and found the methods in enzymology paper that actually uses this assay. The way that this is done is you have this methyl group that's radioactive, so you could measure the enzyme, but what happens is this tetrahydrofolate donates this to the hydroxy-B12, so then what happens is this B12 carries that label, okay?

Then that label is transferred to methionine by methionine synthase. So in the end of this experiment what you have is you have this radio labeled red thing that moves through this assay and ends up on what you want to measure and gives you some measure of enzymatic activity.

Now, when you go to the next slide, and we'll go to the next slide and want to come back, but

when you go to the next slide what you're seeing here is, and this is *Detn* Slide 28 and it's my Slide 13, is you'll see that there's hydroxo-B12, which is referred to in the red lines of all these graphs, and then we have the methyl B12, which is the blue lines in all these graphs.

Now, the question I have is if you're adding methyl B12 -- so if you can go back to the other slide -- if you're adding this to the assay, which goes right to here with this, but the methyl B12 doesn't have the radioactivity on it that you put into the assay, how can you measure enzymatic activity?

You can't because this is going to be sitting there, you're going to have tons of this around that doesn't have the label on there and all this unlabeled stuff is going to be going into the assay.

SPECIAL MASTER VOWELL: Doctor, I'm going to ask you to run back through that again. Instead of saying this and pointing, tell us what it is.

THE WITNESS: Okay. So the methyl B12 that's shown here that's radioactive.

SPECIAL MASTER VOWELL: Okay. And when you say here, you're referring to the CH3 and B12 on the left side?

THE WITNESS: B12, right. The CH3 B12 in this assay, in this case, when you're looking at this diagram, the way the assay is run is what ends up transferring that radio label to the CH3 methionine. You can measure that.

But if you're adding CH3 B12 where the CH3 is not radioactive, you basically can't measure the methionine synthase activity because you get a CH3 methionine that doesn't have any radio label on it. This is why peer review is important because we don't know what he did. We don't know what the assay was, we don't know how this works.

Then, if you flip to the next slide, it shows that all of the assays where you use this methyl B12, they're all higher than they are with the conventional assay. So I just can't figure it out. This, again, the reason I do this is because I want to point out this is the kind of thing that a peer reviewer would actually want addressed.

They'd want to know how this was done. We'd need to see the methods and so on. So I don't want to do this to confuse you, but I'm trying to just make the point that these are the kinds of issues with unpublished data and a lack of peer review. I spent a lot of time looking at this and it got me really

frustrated.

BY MS. BABCOCK:

Q Now, Slide 34, it was more unpublished data involving an experiment with PCR.

A Right.

Q Is PCR a technique your laboratory uses?

A Yes. We do quantitative PCR all the time.

Q As I think the Court indicated yesterday and I'm also acutely aware, we're somewhat informed on the issue of PCR already so we're not going to go into a big description of that, but just generally, looking at a PCR assay like this, what sort of concerns or questions would you need to have answered in order to place any scientific weight on this?

A Well, first off, I mean, there's no indication of how many samples were analyzed, we don't now how the assay was run. I mean, was there equal amount of RNAs in the assay? How is the assay standardized? Conventionally when we standardize a PCR reaction we actually do a housekeeping gene or another gene that's not going to change, so you can actually control for that to make sure --

SPECIAL MASTER HASTINGS: Let me stop you ad go back because I'm not following on this at all.

THE WITNESS: Yeah, that's fine.

SPECIAL MASTER HASTINGS: First of all, we're on your Slide 14.

THE WITNESS: Yeah, and we're talking about *Detn* Slide 24.

SPECIAL MASTER HASTINGS: Yes.

MS. BABCOCK: Thirty-four.

THE WITNESS: Thirty-four. Sorry. My fault.

SPECIAL MASTER HASTINGS: We're talking about the Deth Slide 34, and *Detn* Slide 34, refresh my memory. That is another one of his slides describing his original research over the last year.

THE WITNESS: Right. Yes.

SPECIAL MASTER HASTINGS: That had not yet been published.

THE WITNESS: Right.

SPECIAL MASTER HASTINGS: And Slide 34 describes what? Tell us what it described before you --

THE WITNESS: So what Slide 34 describes is it's describing the loss of methionine synthase expression or the loss of RNA in autistic patients versus control patients.

SPECIAL MASTER HASTINGS: It describes a test that he did.

THE WITNESS: Yes. It shows the results of a test that he did that in autistic patients this enzyme, this methionine synthase enzyme expression, is significantly lower in autistic patients than in control patients. The problem with the data is that there's no indication of how the data was standardized. The RNA quality is a major problem.

I mean, if there's any RNA breakdown in the samples before you run this assay it can completely mess up what you're trying to interpret. The reason I brought this up is because we have tried for a long time to get reliable RNA to do PCR from Down syndrome patients, postmortem kind of tissue, and we have had an impossible time to get a good yield of high-quality RNA to the point that we feel comfortable actually running the PCR at all.

We haven't even been able to get RNA that we think we're able to run an assay. So without having RNA gels and RNA analysis to find out if the RNA is good to begin with you can't even run these assays.

So the reason I thought this was important is this is a finding that needs to be, you need to see, we need to see in a peer review situation that the RNA preparations and that all the stuff that you need to get before you even do this is of good

quality. None of that is indicated in here. So that's why I wanted to make sure that I pointed this out.

SPECIAL MASTER HASTINGS: All right. Go ahead.

BY MS. BABCOCK:

Q Now, as we've already heard this week a good scientific method involves hypothesis generation and then an effort to try and disprove a hypothesis via testing to see if you can replicate or validate the results, correct?

A Correct.

Q Is there any indication that Dr. Deth's unpublished data has undergone this process?

A No. In fact, it would appear more likely that what Dr. Deth's preliminary data is doing is trying to prove his hypothesis. We had a nice talk from Dr. Mailman about this. I'll tell you, the first thing I do when I get a new grad student or postdoc in my lab and they come into my office and they sit down in front of me and I say what's your hypothesis? What are you trying to test?

They'll say, well, I think this is the hypothesis and this is the result, you know, this is the results that I think we should get. I look right

at them and I said what I'd want you to do is I want you to think about this. I want you to think about the hypothesis.

I know there are 100 experiments that you could do that you could try to prove this, but what I want you to do is I want you to think of the easiest, the most straightforward and the simplest experiment that if it didn't work would refute your hypothesis and defeat it, okay. And do that one first because you could spend four years of your graduate school, you know, working to try to prove your hypothesis and then do that experiment at the end and the whole thing goes up in smoke.

We don't want that. What we want is we want this to be critical from the very beginning, and for the exact same reasons that Dr. Mailman explained, this is a critical, critical experiment, even to the point if the data is so important we'll actually ask other people in other labs to try to validate that data even before we publish it.

Q And there's no indication that other labs have been able to validate this, correct?

A No.

Q Do we have any information about the known or potential rate of error with this research?

A I mean, it could be huge. I mean, we don't know.

Q And based on your knowledge of this area and the scientific literature, is there any indication that this hypothesis is accepted by the general medical or scientific community?

A No.

Q And the idea that TCVs could have a role in autism isn't a new or novel one, is it?

A No. It's been around since 2000.

Q Now, were you here for the testimony of Dr. Kinsbourne last week?

A I was here, yeah, for the direct and part of the cross.

Q And as a neurotoxicologist, does some of your laboratory work involve the same mechanisms in the brain that Dr. Kinsbourne discusses?

A Yes.

Q Now, as I understand it, Dr. Kinsbourne posits a theory that in part relies on astrocytes dying or malfunctioning, unable to properly fulfill their role in the brain.

A Yes, that's in general what he was saying.

Q Now, did you understand Dr. Kinsbourne to be saying that his model of causation was resulting in

neuronal death?

A No.

Q And can activation of microglia actually be beneficial?

A Absolutely. Yeah. There are a number of situations where microglial activation can be beneficial. This, again, is one of these areas where, you know, sitting in the Court and listening to these conversations has been a bit frustrating because I think it's been an oversimplified process. What I'd like to do, if I could, would be to step back.

So when I teach I try to involve my class in doing things, and so there's really three cells involved here. It's the astrocyte, the neuron and the microglia. At least that's what we've been talking about. So, Special Master Campbell-Smith, you will be an astrocyte; Special Master Hastings, you will be a neuron; and you get to be the microglia.

SPECIAL MASTER VOWELL: Macrophages.

THE WITNESS: So in a normal state, you know, the three of you are existing in perfect harmony, and, you know, you're functioning fine, and actually your role is to be kind of this barrier between Special Master Vowell and Special Master Hastings, at least between the microglia and the

neuron.

So in my context the astrocyte is actually a very positive component in the brain that maintains kind of this dynamic. It communicates with both the microglia and the neuron and tries to maintain this homeostatic state.

So in the context of what we've been talking about with the bad effects of microglia, what happens is, for example, the astrocyte in this situation would fall asleep during my testimony, which I hope doesn't happen, be dysfunctional and that would allow the microglia to jump on and kill the neuron.

Not that that would happen either in the courtroom, but, you know, we're trying. So in that case it would be a negative effect. That's kind of what we've been talking about this whole time. Now, on the other situation, there are times and examples, both in vivo, where you, as a neuron, would be damaged.

The ability to repair your damage is not dependent upon the astrocytes but is actually dependent upon the microglia. So the microglia and activation of the microglia in that damage situation is actually very important for you to come back to a normally functioning neuron.

We need to understand that neuroinflammation as a term is not astroglia and microglia. It's really a combination of the microglia and the astroglia and how they communicate with each other and how you are suppressing the negative effects, and you're able to, as a microglia, have positive effects in the brain, too.

So microglia are not always negative, they're positive. They can be negative in certain kinds of contexts as well. It's a very dynamic system and you can't just generalize by saying, you know, specific things. Go ahead.

BY MS. BABCOCK:

Q I'm sorry. Did you want to finish?

A No, that's fine. That's okay.

Q Do microglia release both proinflammatory and antiinflammatory agents?

A Yes.

Q Now, moving on to proliferation of astrocytes, is this what's known as gliosis?

A Yes, it's gliosis.

Q Now, Dr. Kinsbourne mentioned that glial scars are formed from astrocytes dying. Do you agree?

A No, I do not agree with that. Glial scarring actually is a result of activated astrocytes

moving into the region of damage and actually secreting proteins that lay down a matrices that form the scar. Astrocytes in and of themselves aren't dying to form the scar. That's, again, I think a misrepresentation.

I talked to colleagues about this that do this work and they completely agree with that is that the astrocytes in a glial scar are not dying to form the glial scar. What they're doing is they're secreting factors, and proteins and matrices that lay down that form the scar.

Q So gliosis does not result in astrocytic death? Is that a fair statement?

A No. Actually, gliosis results in increased astrocytes.

Q Okay. And can death itself affect what you see in astrocytes?

A Death itself?

Q The process of dying. If, God forbid, I walk out after today's testimony, and I'm crossing 15th Street and I'm hit by a bus, is the fact that I'm hit by a bus and then I unfortunately die, could that affect what you see regarding astrocytes?

A Absolutely. I mean, if you're hit by the bus, you fall and your head hits the pavement, I mean,

that's going to cause dramatic trauma to the brain.

Again, depending on if you're not killed, I mean, I don't want to kill you, but say you're killed instantly and they take your brain out within a matter of an hour, though I don't know why they would take your brain out but let's just say that for example, you may see, you know, inflammatory responses occurring.

Say they put you on a respirator or say that you were in the hospital after this head trauma for three days before you died. If they took your brain out at that time you would see massive probably microglial activation and astrogliosis as well associated with the damage.

So the time between an insult and the cell death and the time between when you're looking at the brain is very, very critical I think, yes.

Q And in animal studies, can the manner in which the animal is sacrificed affect what you see in the brain?

A Yeah. Again, very much so. You have to be very careful in how you handle and how you harvest tissues in animals to make sure that you're not causing effects in the brains of animals or whatever you're studying that are due to the way that you knock

the animals out, and harvest the tissues and prepare the tissues because you don't want to generate an effect which is something that you're looking for but that's due to, you know, an artifact of your preparation.

Q Now, if astrocytes are failing to reuptake glutamate, what are the short-term and long-term effects?

A Well, the short-term effects of not reuptaking glutamate are probably you're going to have an excitatory amino acid. Your glutamate is going to increase in the synapse, it's going to be binding to the receptors and the neuron that's on the postsynaptic side is going to be hyperactive.

In the long term, what's going to happen with the increased glutamate excitation is the neurons are going to die. In fact, in vitro and in vivo there are very well-established models using glutamate and glutamate agonists to basically kill neurons.

Q Now, if you see a significant long-term decrease in astrocytes, what would you expect to happen to neurons?

A They would die.

Q And what if instead of dying astrocytes were dysfunctional? What would you expect to happen to

neurons?

A They would die.

Q And if this was chronic dysfunction?

A They would die.

Q And in your study of a neurodegenerative diseases, once you see symptoms, what happens?

A Well, once you see physical symptoms of somebody having neurodegenerative disease the disease progresses and you get neuronal cell death like, for example, Alzheimer's or whatever, in the region where the cells are stressed that propagates and expands to include the whole brain and eventually kills all this, it kills the cells and you die.

Q And is there ever a plateau?

A No.

Q So does it make sense for Dr. Kinsbourne to say that there is chronic steady cell destruction via the astrocytes without meaning progressive disease and cell death?

A No.

Q So overall, based on your clinical experience, and your research and your knowledge of the reports and the literature relating to your area of expertise, what is your conclusion regarding Dr. Deth's hypothesis in this case?

A It's not valid.

Q And what is your opinion about Dr. Kinsbourne's hypothesis involving the astrocytes and neuroinflammation?

A Well, the astrocytes and neuroinflammation, but, I mean, how that affects, it's not valid with regard to autism.

Q Now, you hold these opinions to a reasonable degree of scientific certainty?

A Yes.

MS. BABCOCK: I have nothing further.

SPECIAL MASTER HASTINGS: Mr. Williams, do you have any questions for this witness?

MR. WILLIAMS: I do.

CROSS-EXAMINATION

BY MR. WILLIAMS:

Q I want to find out first, are you disagreeing with the folks at Johns Hopkins and elsewhere that think that at least some autistic people have neuroinflammation as the primary underlying disease process?

MR. MATANOSKI: I object to that characterization of the work. That is not what the Vargas article, if that's what you're referring to, states.

MR. WILLIAMS: I'd like to hear the witness.

SPECIAL MASTER HASTINGS: Well, did you understand the question?

THE WITNESS: He needs to restate it I think for me.

BY MR. WILLIAMS:

Q What is your understanding of the role of neuroinflammation in autism?

A I don't think neuroinflammation contributes to autism.

Q You don't?

A No.

SPECIAL MASTER VOWELL: Did you say triggers or produces?

THE WITNESS: Doesn't contribute to it.

SPECIAL MASTER VOWELL: Doesn't contribute. Okay.

THE WITNESS: I think it doesn't. That's just my opinion.

//

BY MR. WILLIAMS:

Q And have you reviewed the autopsy studies on the autistic brains?

A Which ones are you referring to?

Q Lopez-Hurtado, for example.

A I looked at it. I didn't review it.

Q By the way, in the autopsy studies, as well as in the adult monkeys studies and the infant monkey studies, they had controls who were also sacrificed, or killed by drowning, or trauma, right?

A I mean, I just made a general comment about harvesting tissues. I wasn't specifically referring to any study.

Q So you're not criticizing the autopsy studies that have found differences in the brains of autistics versus controls, let's stop there, are you?

A I mean, I've never done autistic versus controlled comparisons with postmortem tissues. I mean, I can look at the papers, but I really can't, I mean, I really don't, it's really not what I do, so, I mean, my focus is mainly neurodegenerative diseases, and so we really don't look at autism, per se.

So all I'm saying is that the hypothesized neuroinflammation, the neuroinflammation in neurodegenerative diseases is clearly leads to pathogenic processes that progress cell death and eventual patient death.

Q And I'll get to that in a minute but what I'm trying to find out now is just when you're talking about the trauma of death leading to brain damage that

could, you know, make it more difficult to see what was going on, isn't using a controls a way to handle that problem?

A Absolutely. Yeah. You have to age matched controls, and controls that have the same postmortem time and things like that, sure.

Q And that's also true of the monkey studies, right?

A Absolutely. Yeah. Any study has to have controls. If you don't have controls, it's not a study.

Q Now, one question about the monkey studies. I want to throw them up here because we spent a lot of time the other day when Dr. Brent was testifying, and that may have been yesterday. We saw levels of inorganic mercury in the brain of the adult monkeys as low as 100 parts per billion, 100 nanograms per milliliter.

A I don't work on mercury stuff, so, I mean, I don't think my --

Q Well, but you were saying that you thought that --

A Let me just clarify. The only reason I looked at those papers was to get at what they said in those papers was the dose of mercury in the brains or

the level of mercury in the brains so that I could compare it to the in vitro studies. That's all I looked at that for. I didn't look at it to interpret other data.

Q But you expressed your surprise that Dr. Deth could get results in the nanomolar range when you thought everybody else was only getting results in the micromolar range.

A That's all in vitro studies, yes. I mean, to have that kind of a differential in an in vitro study, as I've already stated, when people are using the exact same cell lines, I mean, I've never seen that big of a differential before, so that's all I was saying. It had nothing to do with the mercury in the primates.

Q But the in vivo studies that we looked at, these adult monkey studies, they were getting activated microglia and astrocyte death in the nanomolar range, weren't they?

A No, I disagree. I mean, we just talked about this astrocyte death. Astrocytic death is probably not occurring in this process.

Q It's not occurring in the adult monkey studies?

A I don't recall. I mean, what adult monkeys

studies are you referring to?

Q I'm referring to the five studies that came out of the mid-1990s.

A You know, again, those seemed irrelevant to me as far as what we're talking about today, so I really didn't focus on analyzing that data.

Q Let's look at one of the autopsy studies on autistics, if we could. This will be Petitioners' Master Reference List 446. I've got a copy to give you. Here's a copy for you. Is that one of the papers that you looked at?

A I glanced at it I think while it was being discussed in Court.

MR. WILLIAMS: We've blown up the title there. It's called *A Microscopic Study of Language-Related Cortex in Autism*. Then I just briefly show you the abstracts here. If you can pull that up. They were looking at three different brain areas here that they had hypothesized might be related to some of the symptoms of autism.

Without worrying about that problem, what I wanted to show you is where they talk about the density of glial cells. It's in about the middle of the abstract. It says the mean density of glial cells. Yeah, that's right, Scott.

THE WITNESS: Yes.

BY MR. WILLIAMS:

Q The density of glial cells was greater, the density of neurons was lesser in autism Area 22 and another area of the brain, and then they also found greater numbers of lipofuscin-containing cells. Now, do you know what lipofuscin is?

A It's some kind of deposits in neurons I think. It occurs with aging.

Q And they say the results are consistent with accelerated neuronal death in association with gliosis. They go on to say that production of lipofuscin is accelerated, which is a matrix of oxidized lipid and cross-linked protein more commonly associated with neurodegenerative disease, and that's accelerated under conditions of oxidative stress.

Now, if there's chronic neuroinflammation in the brains of autistic patients, wouldn't that lead to oxidative stress in their brains?

A Well, I mean, you're asking me a question, I haven't looked at the paper. I mean, I, as a scientist, don't read an abstract and make conclusions based on data in a paper.

SPECIAL MASTER HASTINGS: Can you say that again?

THE WITNESS: I said I cannot just look at a statement in an abstract of a paper and make any conclusions based on what's being done. I mean, I would have to read the whole paper and come back with a critical evaluation of the paper. I'm not going to comment on some highlighted section of the text where I'm asked to make an opinion on that and I have no background for it. I just don't do that.

BY MR. WILLIAMS:

Q Well, I understand that, but you didn't read this before you came in even though you were going to discuss neurodegeneration in the brains of autistics.

A No, I wasn't discussing neurodegeneration in the brains of autistics. I was talking neurodegeneration in what we study. I was just saying that the autistics patients don't have the end result where in chronic neuroinflammation, in chronic astrocytic microglial activation you have progression, neuronal cell death and eventual death of the patients.

I mean, I've seen the word plateau used in almost all the time in autistic patients. Plateau. That doesn't happen. In a chronic neuroinflammatory disease or a neurodegenerative disease that I study, all the time you see chronic inflammation, or you see

astrocytic activation, microglial activation, neuronal cell death, progresses to death of the patient.

Q Is the lifespan of autistic people the same as normal people?

A I have no idea.

Q You have no idea?

A Well, I mean, how long do autistic patients live?

Q Yeah, that's my question.

A I don't know.

Q You never looked at that?

A No. I have no reason. I don't study autism.

Q Are you aware that the Johns Hopkins group that has published some of these papers we've looked at, Dr. Vargas, and Dr. Zimmerman and others, that they've actually applied for grants to find therapies to treat neuroinflammation as a way to try to treat autism?

A No.

Q Do you think that's a silly idea because neuroinflammation can't explain autism?

A I mean, I really can't comment on that. I have to read the review of the grant and see what their preliminary data say. As we talked about this

earlier, as a study section member, what you do is you look at the hypothesis, you critically evaluate the preliminary data and see if the experimental design justifies, you know, the funding of the grant.

So, again, I mean, you're asking me to comment on things when I don't have the background or the understanding of what their proposal is actually trying to do.

Q Okay. Let me ask you one question about the Berman mouse study.

A Sure.

Q One difference that I understood they used in their methods compared to what Dr. Hornig's group had done is that they randomized the mice to treatment or nontreatment with thimerosal within a litter as opposed to having, as Hornig did, have the entire litter treated or having the entire litter untreated.

A Right.

Q And they knew they were taking a risk of cross-contamination because they said in the paper they had done a pilot study to see if there was a problem with cross-contamination. Do you know why they decided to take that risk?

A Explain what you mean by risk of cross-contamination.

Q Don't mice eat each other's feces when they're young?

A You need to explain that to me a little bit better because it's very standard practice to avoid bias by randomizing pups from different litters for your studies.

SPECIAL MASTER HASTINGS: We lost you in the middle of that sentence.

THE WITNESS: It's very common practice to randomize your litters so that you avoid litter bias in your experiments.

So what I mean by that is if I'm doing an experiment in my lab what we would do is if we're going to treat with two different things, let's say, like vehicle and something else, if we need 10 animals, we'll take one animal from each litter, different litters, and make that one group and then take an individual animal, another animal, from the 10 litters and make that a different group.

So the randomization is actually a way to avoid bias in experimental design. That's, actually, I think a very positive thing.

BY MR. WILLIAMS:

Q We've had testimony that thimerosal, ethyl mercury, is primarily excreted in the feces not the

urine, and that's true in the animals. If that's true, and if it's also true that young mice eat each other's feces, isn't that a risk of cross-contamination in a thimerosal experiment?

A Well, I mean, my understanding of litter randomization is that you randomize the litters and then you do the dosing, so you don't mix the groups together like in one cage that have been treated with one thing or the other for one thing. You don't do that. My understanding of what they did is they randomized the litters for treatment.

They didn't mix the litters together. I think you're misinterpreting what's being said in the paper. I really do. That's being cautious.

So the problem, and let's go back to the Hornig paper, if what you said is true, is where they take all of one litter and they treat that with something and they take all of another litter and they treat that with something, then what you could have is you could have a differential between the two litters that are litter bias that has nothing to do with your treatment.

So that's why you do the litter randomization. It makes perfect scientific sense to do litter randomization so that you don't bias

yourself in interpretation data. If there's something wrong with the one litter, say the mom's not nursing as much, I mean, you're going to cause stress in the animal.

That could bias your litter so that that whole litter is biased because they're not nursing as well. It's very standard scientific protocol to randomize your litters. So I think you should probably look at that

Q Randomize within litters, you mean?

A No, randomize a cross-litters. Yeah, because, no--

Q Let's just make sure we're understanding each other because what I understood the Berman paper to do was that they say there were eight mice, eight pups, in the litter and they injected four of those pups with thimerosal and four not, right?

A Right.

Q But they knew they were taking a risk of cross-contamination because they went to do this pilot study to check.

A No. I think that what they're controlling there for is litter bias, and I think that's much more important than what you're trying to infer from this. So if you're trying to control for difference

responses in different litters by doing it that way. I agree with that 100 percent.

Q It's not that expensive to do mice. Why couldn't they do the experiment both ways and see if it makes a difference?

A Well, I mean, I can't tell you why they didn't do that, but I'm just saying that the way that it was done, at least the way that you're describing it to me, sounds like pretty good standard design experiments.

Q I wanted to ask you the question about inorganic mercury in neurons. Is that good, bad or ugly?

A Good, bad or ugly if inorganic mercury is in neurons?

Q Yes.

A Like I said, I mean, I really don't feel comfortable commenting a lot on mercury because we really don't work with mercury. I'm more interested in the oxidative stress components and the pathologic process that we see in the disease. From a toxicology standpoint, I would say it probably isn't good. It probably isn't good having inorganic mercury anywhere.

MR. WILLIAMS: That's all I have. Thanks.

MS. BABCOCK: I have one quick followup.

SPECIAL MASTER HASTINGS: Please go ahead.

REDIRECT EXAMINATION

BY MS. BABCOCK:

Q Now, on your cross, they put up the Lopez-Hurtado paper and I'm not going to ask you to comment specifically on the results. We know it's not something you're comfortable doing. We did note that what was highlighted there noted neuronal loss.

A Right.

Q And you saw that line that they highlighted?

A Right.

Q Assuming that these results are correct, is that consistent with what Dr. Kinsbourne has hypothesized about a steady state of astrocyte death and dysfunction without progressive disease process?

A No.

MS. BABCOCK: Nothing further.

SPECIAL MASTER HASTINGS: Any questions for this witness?

(No response.)

SPECIAL MASTER HASTINGS: Mr. Williams, anything further for this witness?

MR. WILLIAMS: Nothing further.

SPECIAL MASTER HASTINGS: All right. Dr. Johnson, we're done with you. Thank you very much.

THE WITNESS: Thank you.

(Witness excused.)

SPECIAL MASTER HASTINGS: Well, counsel, it's unexpectedly early in the day. Do you have any more witnesses you've planned to call today for the government?

MR. MATANOSKI: No, sir.

SPECIAL MASTER HASTINGS: All right. Let me raise an issue then while we're here. We had talked last week about Respondent's Exhibit LL, the Pardo.

MR. MATANOSKI: Yes, sir.

SPECIAL MASTER HASTINGS: And we had talked about whether we needed to talk further about that exhibit.

MR. MATANOSKI: That's correct. I can tell you right now that Dr. Pardo, my last information was that he is not in the country until the 21st. So I have not been able to inquire of him any further about whether he'd be available to answer written questions, whether he'd be available to come and testify.

I know that my last conversation with him he was not willing to testify, and I haven't been able to have a conversation with him since then since he's been out of the country.

I know we've discussed some other matters

perhaps that might help with respect to further seeing his views, and I, frankly, would be anxious, if it was possible, if he will be willing to, to have those further views in front of the Court because I think they expand upon --

SPECIAL MASTER HASTINGS: Speak up.

MR. MATANOSKI: I'm sorry. I think they expand upon and in fact make even more apparent the limitations he believes that can be placed upon his work, at least for the interpretations or extrapolations that are being drawn from it by the Petitioners right now.

I have not been able to speak to him since actually before the trial before I even received the letter from him, and I understand that he's not back in this country until I believe the 21st.

SPECIAL MASTER VOWELL: Of this month?

MR. MATANOSKI: Of this month, which is tomorrow.

SPECIAL MASTER HASTINGS: Tomorrow. So you hope to have a chance to talk with him tomorrow at some point?

MR. MATANOSKI: I'm not sure when he gets back in the country tomorrow. That was the date I was given. Yes, as soon as he came back we were hoping to

get in touch with him to discuss whether he'd be available in some fashion to elaborate on the comments that he had in his letter.

SPECIAL MASTER HASTINGS: And if I recall correctly, the Petitioners, when the Respondent filed Exhibit LL, Mr. Powers, your response to it was that we would like to cross-examine him?

MR. POWERS: Yes, Special Master. The letter actually turned out to be something a little bit different than what I had expected to see. My understanding initially was that this was something that was being given by Dr. Pardo to Respondent, and when one looks at the letter, it's correspondence between Dr. Kemper and Dr. Pardo.

So I was thinking that it would be something akin to an expert report or a commentary that would resemble an expert report that would be given to Respondent and be filed in, but it's correspondence between nonparties here. So to that extent, we're prepared to use that letter in cross-examination of witnesses since it's been filed in the case to explore the areas that are raised in there.

So we're prepared to use it in cross-examination. On the other hand, if there's any -- and it sounds as if what Mr. Matanoski's further

characterization of what Dr. Pardo might believe, if this is going to be a surrogate for an expert report that they're relying on, then we certainly would want to cross-examine.

We completely understand that under the program an expert report, for example, can be submitted and no cross-examination is had, but what we see in this case is nothing that even resembles an expert report at this point.

SPECIAL MASTER VOWELL: Well, we frequently consider letters, do we not? For example, a letter from one expert physician to the primary care physician saying I think *X* and this is why I think it. You submit those as exhibits all the time.

MR. POWERS: Correct, and that's why I'm saying that if this is correspondence, then, yeah, you take it at face value, cross-examine to explore the underlying basis for what's being said and the context from which its emerged, and we're totally prepared to do that.

All I'm saying is that if this letter is something that is eventually going to be reduced to a form like an expert report stating an opinion being filed on behalf of the Respondent in support of their case, then we would want an opportunity to cross-

examine Dr. Pardo.

At this point it looks like the letter is like any other correspondence that might come in, and we're prepared to use it in cross-examination at this point.

MR. MATANOSKI: The letter obviously was written to one of our witnesses, and, as such, I agree with Mr. Powers, he can cross-examine the witness as to those matters. The letter gives him a little bit of a heads up of what Dr. Pardo has relayed to Dr. Kemper. So I expect we are going to hear some things from Dr. Kemper about what Dr. Pardo and his colleagues have found in those autopsy studies.

The letter is not submitted as an expert report. Dr. Pardo is explaining what his study -- he's almost like a fact witness, if you will. His study has been looked at and relied on heavily by Petitioners at this point. He's seen that it's been relied on by them and he's trying to explain the limits that one can place on his work, at least as far as the interpretations or extrapolations therefrom.

We saw something similar in the last trial when Dr. Oldstone's work was being characterized in certain fashion by witnesses, and Dr. Oldstone came in and said these are the limits of what I think you can

take from my work. I think that's what Dr. Pardo, up to this point at least with respect to the correspondence, has done in this case.

SPECIAL MASTER HASTINGS: With that understanding of what the government's purpose in introducing the letter, are you comfortable, Mr. Powers, with its admittance?

MR. POWERS: Certainly, Special Master. As I said, just on its face right now we're comfortable with it being in the record, and we're completely prepared to use it in cross-examination and explore the issues that are raised in that letter.

SPECIAL MASTER HASTINGS: Let's take a five-minute break here. I want to confer with my colleagues about scheduling very briefly and then we'll get back and presumably we'll be done for the day and we'll make a plan for tomorrow. Let's take a five minute break here.

(Whereupon, a short recess was taken.)

SPECIAL MASTER HASTINGS: During our recess, Mr. Powers noted to us off the record that he would like to make a brief statement of explanation of something, so go ahead, Mr. Powers, and address that.

MR. POWERS: Thank you, Special Masters. I appreciate the opportunity just to take a quick moment

to clarify, particularly for the sake of people who may be listening in outside of the courtroom, relating to Dr. Mumper's testimony. Dr. Mumper described two young adults in her testimony.

One of them is a young man who lives in Maryland and was involved in litigation, and she was involved in that litigation. That was Mr. Blackwell. There was another young man that was a patient of hers, and she was treating him and he died while in her care.

There apparently has been confusion among some people that these are the same person. Dr. Mumper has requested, and the families of those folks have requested, that we just made clear that Mr. Blackwell fortunately is alive and well and is not involved in Dr. Mumper's care, and that the young man who died in, while he was treating with Dr. Mumper that she testified about is a different person.

So these were two different young men, and the young man who died is not involved in this litigation. That's the young man that she was describing the family has made his brain tissue available for research via autopsy and allowing scientific researchers to work with it.

So she was just requesting that we make it

clear that those are two very different young men. I appreciate the opportunity to do that.

SPECIAL MASTER HASTINGS: All right. Thank you, Mr. Powers. The other matter is we were surprised this morning that the testimony of the two witnesses scheduled today was concluded so quickly. Particularly, the direct turned out to be under an hour for each of them.

We just want to make the point that, you know, we want to make the full use of the time we have. If you're in a situation in the future where you realize, you know, we've got two witnesses scheduled but we only expect our direct of each of them is going to be less than two hours that you would let us know and we would perhaps consider the possibility of bringing in a third witness.

With that in mind, I don't know if you have other witnesses today, if you might consider taking a lunch break and doing another witness today? Mr. Matanoski?

MR. MATANOSKI: Unfortunately, sir, I don't believe that we'd be ready with any other witnesses today. I will look ahead at the upcoming witnesses and see if this situation might develop in the future with our trial schedule. If it looks like it will,

then I'll try to move witnesses around in some fashion that makes sense.

Most of them are not local, so with the schedules, their travel schedules as well, there may have to be some movement on that so that we would use the full day and then have as much flexibility and time towards the end of the trial as possible, or if that frees up a day in the middle of it, at least it's a full day and we can go about our other tasks without coming in here and having, you know, just a half a day.

Unfortunately today, I apologize. It did surprise us a little bit. I mean, it became a little bit more apparent as we were running up to today that our direct would be shorter than we had anticipated at first.

SPECIAL MASTER HASTINGS: Then, Mr. Powers, did you want to say something?

MR. POWERS: Yes. I have just two things, both related to scheduling. One, if there's going to be a change in the schedule we certainly would want to know really early because it's one thing to prepare for direct, it's another thing to prepare for cross. They're two different animals.

So the farthest in advance notice we could

get of a change would be appreciated. I also wanted to let the Special Masters, and, again, we've talked about this earlier with Respondent, know about what we anticipate for scheduling so far with rebuttal next week.

SPECIAL MASTER HASTINGS: All right.

MR. POWERS: We have confirmed Dr. Deth's availability on Thursday. I guess that's the 29th.

SPECIAL MASTER HASTINGS: All right.

MR. POWERS: Dr. Greenland is going to be available, and he would likely be available by telephone. He's in Los Angeles. Particularly for how brief we would anticipate his rebuttal, flying him back and forth across the country, seemed better to do it by phone.

We've discussed the scheduling with Dr. Mumper. She has a full day of patient care that she cannot reschedule on Thursday, but she is available Friday. So we anticipate Dr. Mumper being here Friday. Dr. Kinsbourne, it sounds as if at least with one of the witnesses today is raising issues related to Dr. Kinsbourne's testimony.

So if he is in the mix for rebuttal, we will work on his availability for one of those two days, but that's not determined yet. That's where we stand

right now with Dr. Deth confirmed for Thursday, Dr. Mumper confirmed for Friday, Sandra Greenland available by phone. Do you know what day?

MR. WILLIAMS: I don't know which day.

SPECIAL MASTER HASTINGS: Okay.

MR. POWERS: And then Dr. Kinsbourne possibly on either one of those days.

SPECIAL MASTER HASTINGS: All right. Thank you.

SPECIAL MASTER VOWELL: We've just been informed by our technological people that we can do phone testimony but that would preclude people listening in on that date because we only have one phone line. However, because we have the digital audio files that we can post, people can listen to the testimony, it just won't be a live listening.

So I think it would be more important to avoid having Dr. Greenland fly across country for what may be just a few moments of testimony.

MR. POWERS: And Petitioners absolutely agree with that. That makes sense.

SPECIAL MASTER HASTINGS: All right. Any other scheduling matters we should talk about?

MR. MATANOSKI: No, sir.

SPECIAL MASTER HASTINGS: If not, then we're

done for the day, and we're going to start at 10:00 a.m. Eastern time tomorrow with Dr. Rust's testimony.

MR. MATANOSKI: Yes, sir.

SPECIAL MASTER HASTINGS: All right. Thank you very much. We'll see you tomorrow. We're adjourned.

(Whereupon, at 11:43 a.m., the hearing in the above-entitled matter was adjourned, to reconvene at 10:00 a.m. on Wednesday, May 21, 2008.)

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REPORTER'S CERTIFICATE

DOCKET NO.: 03-584V and 03-215V

CASE TITLE: Claims for Vaccine Injuries

HEARING DATE: May 20, 2008

LOCATION: Washington, D.C.

I hereby certify that the proceedings and evidence are contained fully and accurately on the tapes and notes reported by me at the hearing in the above case before the United States Court of Federal Claims.

Date: May 20, 2008

Christina Chesley
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