The National Vaccine Advisory Committee Sponsored Workshop on Thimerosal Vaccines, August 11, 1999, Lister Hill Auditorium

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Abstract

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Proceedings
8:3A.M.

DR. MYERS: Good morning, and welcome to the National Vaccine Advisory Committee Sponsored Workshop on Thimerosal in Vaccines. By starting exactly on time, I hope we'll stay on time, which may be the challenge for the moderators. I'm Martin Myers. I'm the Deputy Director of the National Vaccine Program Office, and I appreciate the willingness of so many people to participate in the middle of the summer and on such short notice on this very important and timely topic.

I have a number of housekeeping and a number of specific things, from a format point of view, to say. The first and most important thing, and someone told me this morning that the only real important job of the person who welcomes, is to say that the restrooms are outside by the elevators. There is a cafeteria downstairs, which is very small. We'll use that for our breaks. We'd suggest that for the lunch hour that people go to the Natcher Auditorium, which is out the front door and the building straight ahead of you, across the street, which has a much larger cafeteria than is in this building.

Thimerosal has been used as an additive to a number of biologics since the 1930s, including some vaccines routinely recommended for use in young children. Because of multiple doses of vaccine, it is possible that some children could be exposed to a cumulative level of mercury that exceeds guidelines for methylmercury.

Nationally and internationally, manufacturers and regulatory agencies are working to replace or reduce Thimerosal-containing vaccines. The purpose of this workshop is to review the pertinent data on Thimerosal: its use; its potential for toxicity; and steps that can be taken to increase the margin of safety, especially during the period of transition to greater availability of vaccines without Thimerosal or with reduced Thimerosal.

It's important to discuss, as we discuss these issues, to balance these with the very real risks of disease resurgence if we have a reduction in vaccine utilization or a loss of confidence in vaccines.

We're a very diverse group of people here today, but let me say that the primary audience to whom this information is directed, the members of the Federal Advisory Committees that relate to vaccines. These include the National Advisory – National Vaccine Advisory Committee that is sponsoring the workshop, the Advisory Committee on Immunization Practices, the Vaccines and Related Biologic Products Advisory Committee, and the Advisory Commission on Childhood Vaccines.

The workshop is convened specifically for the exchange of information. It is not a policy meeting nor is it designed to provide advice. I'd like to say a little bit about the format of what we're trying to do today. The first is, we're going to talk about

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Thimerosal, why we have preservatives in vaccines and some of the issues that surround the inclusion and experience of now over sixty years with Thimerosal. Then we're going to talk about organomercurials, both Thimerosal, as an organomercurial-containing additive, as well as organomercurials in general.

We're going to end the afternoon talking about potential disease impact of the vaccines that would be primarily affected during a transition to a reduced Thimerosal vaccine supply.

Tomorrow we're going to talk about the transition to a greater supply of Thimerosal-free vaccines in reduced Thimerosal-containing vaccines. We're going to talk about issues that relate to the manufacturer and regulatory activities, the European initiative, and then we're going to talk about the transitional vaccine options, the flexibility within the recommended schedule.

At that time, we have a number of groups and individuals who would like to participate by giving their perspectives on these options. We have allowed time in that session for others who would like to give their perspective on this, as well. We didn't know how much time to allow. We have limited time. We have a very full agenda for the next couple of days. So if there are individuals or groups that would like to give a perspective on this, if they'd put together a one- or two-sentence summary, we've asked Dr. Modlin, who is going to be our moderator tomorrow, to triage these and work that last minute changes on the agenda.

And then, finally, many of us feel that the -- one of the most important parts of this meeting will occur at the end, which is a discussion of knowledge gaps that exist.

We've tried to ensure a discussion time after each presentation, and speakers have been asked to limit their talks to allow five or ten minutes of discussion.

To use the microphones, the individual microphones at your seats -- I've got to read this here, and it's tough with bifocals -- you need to depress the "Request to Talk" button, and red and green lights will come on, and that means that the microphone is on, and then you depress it again to turn it off, and both lights will go off.

We'll ask our moderators to triage the questions and also to keep us focused and on time. Dr. Georges Peter, who is Chair of the National Vaccine Advisory Committee, asked me to extend his sincere regrets at his inability to be here today and to express his appreciation to Dr. Klein for serving as both a convener and rapateur.

Dr. Harry Greenberg will be our moderator today. Dr. Greenberg is the Chair of the VERPAC. Dr. John Modlin will be our moderator tomorrow, and he is the Chair of the ACIP. Again, they're going to make every effort to keep us on time. We are going to develop proceedings from this meeting. Therefore, even though everybody knows you in the room, if that's the case, please tell us who you are and your affiliation, so our transcriber will be able to put that together.

So, with no further ado, I will ask Dr. Klein to convene the meeting.

DR. KLEIN: Thank you, Dr. Myers. It's a privilege to be a participant in what I anticipate will be a very informative experience for all of us. I think we start out with a relatively limited base of information about organomercurials and, particularly, about concerns for these products in vaccines. The specific issue of Thimerosal is one that has a history of about sixty years. Its use as preservative in biologics and pharmacologic preparations goes back to the 1930s, and it is present, or has been present, not only in vaccines, but in various cosmetics, contact lens solutions. So its use as a preservative goes beyond the specific area of vaccines.

Thimerosal is an ethylmercury salt, and it's important to keep the distinction about the disasters that have occurred with mercury with which we are familiar, from the paucity of information about any harmful effects of ethylmercury, but we'll hear more about that.

Thimerosal is present in some, but not all vaccines. Most of the viral vaccines do not have Thimerosal. Both the oral and inactivated polio vaccines do not. Measles/mumps/rubella does not. Varicella vaccine does not. Rotavirus, hepatitis A, and Lyme disease vaccines all do not [have] preservatives. They don't have Thimerosal.

Thimerosal is present in some but not all DTP and DTaP preparations. Some of the amphophilous influenza B, polysaccharide conjugate vaccine, the benignococcal and pneumococcal polysaccharide vaccines, as well as hepatitis B. And there will be more discussion about the focus of changes for hepatitis B vaccine.

This product is antibacterial and prevents, as well as may treat, infectious agents that are present in these various products. The antibacterial activity is related to release of ethylmercury after spontaneous or enzymatic breakdown of Thimerosal into ethylmercury and thiosalicylate. It is bactericidal at acidic pH. It is bacteriostatic and fungistatic at alkaline or neutral pH.

The most frequent adverse events that have been identified with Thimerosal are those of a hypersensitivity reaction, papular or vesicular disruptions. Some of the solutions for contact lenses have caused eye irritations.

It is methyl, not ethyl, toxicity that has been associated with the well-known events in Minamata, Japan, resulting from the contamination of fishing waters in the area and the severe consequences for people in that area.

Use of methylmercury has been as a fungicide, and the mistaken use in preparation of homemade bread rather than grain for planting in Iraq led to severe morbidity and mortality.

In contrast then, Thimerosal is ethylmercury; and to underline, there is no evidence of harm from the amounts of mercury administered to infants and children in vaccines.

I think what we'll learn from this experience in the next two days I've categorized in six areas. One, the use of preservatives in vaccines, are they necessary? Are they necessary for specific products? Are there substitutes that can be made if they are necessary for the Thimerosal that is now used?

Two, we'll talk specifically about mercury and the pharmacokinetics and toxicology in animals as well as some human data.

Three, the impact, and there will be considerable discussion later today on any issues that arise that may limit public confidence in vaccines and alter our current success in immunization program.

Four, what are the current plans to reduce or eliminate Thimerosal in vaccines?

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Five, the pragmatic issues about what to do during the transition from the current roster of vaccines that do contain Thimerosal to a Thimerosal-free vaccine period.

And then finally, a review of appropriate priorities for research in these areas.

So I anticipate an educational experience for all of us. To begin this morning's program, I'd like to introduce the moderator for the morning session, Dr. Harry Greenberg, who is Senior Associate Dean for Research at Stanford University and Chief of Staff of Research at the Palo Alto VA. Dr. Greenberg.

DR. GREENBERG: Thank you, Dr. Klein, and thank you all for coming. I see my role as sort of the heavyweight, or bad guy, and I've been advised that I have the privilege of yanking anybody I want off the stage if they talk too long. I will tell all the speakers that there's an incredible little button up here that will eject you if you go beyond twenty-five minutes. And if it doesn't function, I will eject you.

The purpose, I think Dr. Myers really hit the nail on the head when he said the main purpose of this meeting as to get all of us on the same page as far as our database as to what the issues are here, and I look forward to a very, very informative meeting. We're ahead of time, and maybe we'll be able to keep ahead of time during the meeting, but if, by chance, that doesn't occur, like it never does, I may have to cut off some of you who I am sure have the most important question to ask. It is nothing personal, but I will use my prerogative to keep the meeting on time. And so, trying to keep on schedule, I'd like to introduce the first speaker, who is Dr. William Egan, Acting Director, Office of Vaccine Research and Review at CBER, FDA, and he's going to start off that first session that we're talking about: Where Are We Now: A Review of the Data—Thimerosal in Vaccines. His perspective is from the FDA.

Bill? First, I'm starting his time. Instruction is on your time.

DR. EGAN: Okay. Thank you very much. We'd like to thank you, Dr. Myers, for the opportunity to come here and say a few words about preservatives in a FDA perspective.

Let me begin by relating one incident that's described in Sir Graham Wilson's classic book, *The Hazards of Immunization*. It goes: "In January, 1928, in the early stages of an immunization campaign against diphtheria, Dr. Ewing George Thomson, Medical Officer of Health at Bundaburg, in Australia, began the injection of children with toxin-antitoxin mixture. The material was taken from an India rubber-capped bottle containing mL of the toxin-antitoxin mixture. On the 17th, 20th, 21st, and 24th of January, Dr. Thomson injected subcutaneously a total of twenty-one children without ill effect.

On the 27th, a further twenty-one children were injected. Of these children, eleven died on the 28th and one on the 29th."

The death of these twelve children was investigated by the Royal Commission, and the final sentence in the summary of their findings reads as following: "The consideration of all possible evidence concerning the deaths at Bundaburg points to the injection of living staphylococci as the cause of the fatalities." As Sir Graham Wilson also notes in his book, staph toxin was very likely also present in the bottle, thus accounting for the rapid deaths of the children.

Obviously, the bottle became contaminated on the 24th of January, the bacteria multiplied, toxin was produced, and the bacteria then injected into the children on the 27th.

Among the recommendations of the Royal Commission is a very important one, that biological products in which the growth of a pathogenic organism is possible should not be issued in containers for repeated use unless there is a sufficient concentration of antiseptic to inhibit bacterial growth.

The number of similar examples of bacterial contamination, either during manufacturing or during product use, are detailed in Sir Graham Wilson's book, *The Hazard of Immunization*. And, sadly, many additional examples of the consequences of bacterial contamination have been revealed since the publication of that book. However, from these disasters, these and similar disasters, have arisen the regulations that require preservatives in multi-dose, multi-entry containers of biological products.

Indeed, if I may offer a general comment, many of the requirements that now exist for biological products have arisen not from foresight, but from mishaps.

The U.S. Code of Federal Regulation contains a requirement for preservatives in multi-dose containers. This requirement was placed into the Code of Federal Regulations in January of 1968, although biological products had contained preservatives, including Thimerosal, prior to this date. Indeed, Eli Lilly had Thimerosal in their diphtheria toxoid vaccines in the 1930s.

Specifically, the CFR states that: "Products in multi1 3-dose containers shall contain a preservative, except that a preservative need not be added to Yellow Fever Vaccine; Polio-Virus Vaccine, live oral; viral vaccine labeled for use with the jet injector; dried vaccines when the accompanying diluent contains a preservative; or to an allergenic product in 50% or more in volume of glycerine."

The CFR also requires that a preservative that is used shall be sufficiently nontoxic so that the amount present in the recommended dose of the product will not be toxic to the recipient, and in combination uses it shall not denature the specific substance in the product to result in a decrease below the minimal acceptable potency within the dating period when stored at the recommended temperature.

The CFR does not specifically address the use of preservatives in single-dose containers. Currently, some single-dose presentations contain preservatives. Some do not. In the past, it was thought that single dose containers, like multi-dose containers, should contain preservatives, the rationale being that the addition of a preservative during the manufacturing process or during the filling operation served to help ensure that the product was free of microbial agents and their toxins.

Indeed, at the International Symposium on Preservatives in Biological Products held twenty-five years ago, in San Francisco—this was under the auspices of the IABS—Dr. Edward Seligman, Jr., at that time the Director of the Bureau of Biologics Division of Product Quality Control, had the following comment: "Because of the numerous complex processing stages in the manufacture of biological products, good manufacturing procedures include the addition of preservatives early in the manufacture of many types of products to aid in preventing contamination during production. Even if products are sterilized by filtration prior to filling into final containers, contamination during earlier stages can result in soluble products that alter the purity of the product, increase toxicity, and result in pyrogens,

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all of which cannot be removed without alteration of the product itself."

Now, today, GMPs are viewed differently, and it would be argued that a well-controlled process does not require the addition of a preservative to ensure sterility. However, I think at this point, it's worthwhile noting that sterility is not an absolute term. Sterility does not mean zero microbial organisms in 100% of the containers.

Let me show some data that was presented by Koerner and Kindt from Germany at this symposium twenty-five years ago. Well, this is filling data, so number of lots that were filled and the percentage of non-sterile filling lots. And with no preservatives in ampules, 5.6% of the lots were found to be non-sterile. This is using the test that's in the CFR. For multi-dose containers, somewhat better, 2.2%. And even when preservatives were used, if we look at the ampules, the number of lots that were rejected went from 5.to 4.with phenol, to 2.1 with an organomercurial. In the multi-dose containers, it went from 2.down to 0.with phenol and 0.with the organomercurial.

While formaldehyde was in there, they rejected 17% of the lot. This was not statistically different than the 5.6, the small numbers. The numbers in parentheses refer to the number of lots rejected over the total number of lots that were examined.

And even with no preservatives, with the multi-dose containers with some residual formaldehyde, it was the same as no preservative. Formaldehyde does nothing.

The reason I show these data is simply to point out that even with the preservatives, there was still a number of lots that were rejected because of issues of stability.

Now, today, these numbers are significantly lower, and if manufacturers would, you know, would do media fills to test the -- you know, the filling, and we're looking at numbers like one in ten to the three or one in ten to the four containers that might have microbial growth.

However, I point this out simply to say that the numbers will not be zero and the risk of no preservative will be slightly greater than with the preservative. No matter how small they are, the numbers are not zero. There may be some discussion later on this point.

Now, I've spoken for the past nearly five, ten minutes about preservatives, but have yet to say what a preservative is and what precisely we expect a preservative to do. If I may come back and quote Dr. Seligman again, he mentioned that the sole reason for adding a preservative is to protect the recipient. Thus, a preservative must be able to protect the recipient from the consequences of inadvertent microbial contamination while at the same time being nontoxic to the recipient and not denaturing the product.

Sodium azide is a good preservative, but it's use in (inaudible) would not be allowed because of toxicity. Thimerosal is a good preservative, but not for IPV. It inactivates the vaccine. Hence, we have the regulations that I showed before, that a preservative must be nontoxic and must not denature the particular substance.

But what needs a preservative to do? Obviously, as I've said, a preservative must prevent the consequences of inadvertent contamination by microorganisms introduced during use of the product. However, does this mean that a preservative must be bactericidal or fungicidal, or is it sufficient that the preservative assure microbial stasis? And whether a preservative should be cidal or simply ensure stasis, we need to ask as well, against what organisms, at what levels, and if a preservative must be cidal, how rapidly. These issues are not addressed in the Code of Federal Regulations.

Now, under proper conditions of storage, usually refrigerated, and with good medical practice, the extent of potential inadvertent contamination should be minimal. The number of the types of potentially contaminating organisms is quite large, and there are long lists in various texts on preservative and stabilities. And there could be and there has been considerable argument regarding which organisms a preservative should be able to exclude. However, if we look at past examples, past tragedies, that list would certainly include the staphylococci and streptococci.

Now, preservatives are also discussed in the United States Pharmacopeia, and the USP regards antimicrobial preservatives as substances added to dosage forms to protect them from microbial contamination. They are used mainly in multi-dose containers to inhibit the growth of microorganisms that may be introduced inadvertently during or subsequent to the manufacturing process.

The USP further states that any antimicrobial agent may exhibit the protective properties of a preservative. However, all useful antimicrobial agents are toxic substances. For maximum protection to the consumer, the concentration of the preservative should be considerably below the concentrations of the preservative that may be toxic to human beings.

These discussions of a preservative that are in the USP are thus quite similar to those in the CFR. The USP, however, does provide a functional definition of preservative, whereas the CFR does not.

I should add also that the USP tests a preservative only in the original unopened container in which the product was distributed by the manufacturer. So it's not a preservative, per se, as an entity, but only that entity in a specific product.

Now, an ample number of examples may be found in literature wherein a substance at a particular concentration functions as a preservative, per the USP definition, for one biological product but fails in another. For example, a material at a particular concentration may be a good preservative for a vaccine, but in a blood product or in serum does not function, does not meet the USP requirements.

Now, let me outline briefly the USP definition of "preservative." It's a functional definition wherein a specified amount of the product is challenged with a known quantity—actually 0.1 milliliters of approximately 10to 10per ml of the following organisms, or spores: candida albicans, aspergillus niger, escherichia coli, staphylococcus aureus, and pseudomonas aeruginosa, and it specifies the strains from the American-type culture collection. The test sample is incubated at to degrees, and the number of viable organisms determined on days 7, 14, 21, and 28. And a preservative is then acceptable if bacteria are reduced to less than 0.1% of the challenge dose by day 14; yeast and mold remain at or below the initial inoculum on day 14, and the number of organisms—this should be on day 28—are the same or below that on the day level.

Now, for bacteria, the USP definition is a bactericidal one. For yeast and mold, the definition is one of stasis. Although the

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choice of challenge organisms might be argued, most people would agree that the USP challenge assay is quite stringent in that the challenge doses are much greater than might ordinarily be expected to occur through inadvertent contamination during use. Thus, a preservative, as defined by the USP, provides a large margin of safety.

Now, the question may be raised whether the term "preservative" as used in the CFR is defined as per the USP. In other words, must we take the USP definition? The preservative that is in the CFR is a preservative as defined in the USP. The simple answer to this question is no. A material that does not meet the USP requirements may still be deemed by CBER to satisfy the CFR requirements for a preservative. Although a material satisfying the USP definition will certainly be acceptable as a preservative, other definitions are possible. However, if a different set of requirements are to be met—different organisms, different concentrations, different times to kill, etc.—then the rationale for their use must be presented to CBER for approval in the products.

Now, we're at the workshop today to discuss Thimerosal and its reduction and removal—well, removal from existing products. This will entail switching to single-dose vials without preservatives or using single-dose and multi-dose vials with different preservatives. Such changes may constitute a change in formulation of the product. Dr. Baylor, in his talk tomorrow, will discuss how CBER will handle these product formulation changes from a regulatory point of view.

A little later in this talk, in this session, Dr. Ball from FDA will be discussing the vaccines that contain Thimerosal, the content of Thimerosal in those vaccines, and the guidelines that are now existing regarding mercury intake, and I believe that Dr. Plotkin will be following me and presenting some data on alternative preservatives.

Okay. Nineteen minutes, Harry. You got one extra minute.

DR. GREENBERG: Thank you, Bill. Stay up here because we have some time for some questions. I'd like to thank you for an excellent talk.

Can I ask the first question? I assume that Thimerosal or Thiomersal --

DR. EGAN: Actually, one is the term used in Europe, the other is the term used in the U.S. They're the same chemical.

DR. GREENBERG: Good.

DR. EGAN: Next question.

DR. GREENBERG: I assume that that fits under the USP definition.

DR. EGAN: Yes.

DR. GREENBERG: Okay. Do we have any questions for Dr. Egan? You have a little mic in front of you that you're supposed to—yes, you're on. Neal, you're Number 8-A.

DR. HALSEY: Two questions, the first one is, does that USP --

DR. GREENBERG: Could you stand up and identify yourself to the audience?

DR. HALSEY: Neal Halsey, John Hopkins University.

DR. GREENBERG: Then you can sit down. I'm learning as we go along here.

DR. HALSEY: All right. Two questions. The first one is: Does the USP test, the pharmacopeia test, require the product to be used—that preservative to be tested in the final product, and is this being…

DR. EGAN: Yes.

DR. HALSEY: If you might address the issue of the contamination of DTP with Group A strep, and Group A strep is not one of the organisms which you mentioned back there, but the basis for why that doesn't work as perfectly as we would like to, because there are multiple reports of clusters of those cases, and I have always assumed it was because of the particular matter that was in DTP that may have played a role in helping protect it.

The second question has to deal with the definition under the USP and whether it's your understanding in terms of the safety, and I don't have the words in my head exactly, but the toxicity for the recipient must be considerably below that that might be toxic, is the sort of language that you used. Is your interpretation of that definition with regard to Thimerosal, does the current concentrations fall within that safety guideline or they exceed that safety guideline?

DR. EGAN: Okay. Let me try the first question first. That related to the USP definition about whether it corresponds to the preservative in the material, and the answer to that question is yes. So, in other words, they take the final dosage formulation and then it's challenged with those five organisms.

Your second question was –

DR. GREENBERG: Bill, I –

DR. EGAN: Yes?

DR. GREENBERG: Neal, it seems to me that your second question is the purpose of this meeting. So rather than, in the first speaker, trying to—I think maybe you'd be wise to ask that question at the end of the meeting.

Now, any other questions?

DR. McINNUS: Pamela McInnus, NIAID.

I'd like some clarification following this first talk: Are we moving forward with this workshop on the basis that available data do support the decision to reduce and eliminate Thimerosal? Is that up for discussion at all, or is that decision made and is non-retractable?

DR. EGAN: Okay.

DR. EGAN: Well, let me speak for myself personally, and I believe that you know, we, i.e., FDA, have made that decision whenever possible, to eliminate Thimerosal from products. We have asked manufacturers and sponsors in the development of their products to develop them without Thimerosal; and if they're not able to do that, to specifically explain why.

So the use of Thimerosal as a preservative is no longer the default option. And, you know, we did send out a letter this summer again asking manufacturers and sponsors for their plans to reduce or eliminate Thimerosal in their products. So I think that's where we're heading. I'm not sure where this workshop will be headed.

DR. GREENBERG: Pam, I would like to say, also I think your question, at least for me, who is less well informed than many of you, that part of the purpose of this meeting is to get a database in front of all of us at the same time and then potentially to re-evaluate decisions that were made, but at least to have a very broad and deepening airing of available information so that your question can be answered in a scientific way.

Any other questions? In the back?

DR. CORDERI: José Corderi, CDC.

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Bill, what preservatives are now available, other than Thimerosal, that would meet the USP definition for preservative?

DR. EGAN: For the common childhood vaccines, the only one that I'm aware of in the product formulations that is used is 2-phenoxyethanol.

DR. CORDERI: Any others?

DR. EGAN: Not that I'm aware of in the childhood vaccines. In anthrax, for example, there's benzalkonium chloride, which is an ammonium salt. I don't think we have phenol in any of the vaccines anymore, but I would have to go back and check that specifically for all of them.

DR. GREENBERG: Other questions?

(NO RESPONSE WAS HEARD)

If not, I'd like to thank you, Bill. And I'm going to get all of you home early.

The next speaker is Dr. Stanley Plotkin, who is now the Medical and Scientific Advisor to Pasteur Mérieux Connaught, and he is going to be talking to us about preservatives, the manufacturer's perspective.

DR. PLOTKIN: Well, Harry, first of all, let me stress that this talk does not represent the view of the entire manufacturing industry. I have not canvassed manufacturers' views and I would not presume to speak for them. This is my view, reflecting experience both in academic vaccine development and as a consultant to one manufacturer. Indeed, after I am done speaking, manufacturers in general, and Pasteur Mérieux Connaught, in particular, may choose to disavow what I have to say.

DR. PLOTKIN: Vaccine manufacture is, as it should be, a highly regulated industry, designed to produce safe and effective vaccines. Like many of you, I first became aware of a perceived crisis with respect to Thimerosal at the time of the ACIP meeting late in June through communications concerning a meeting held at the FDA.

Subsequently, there was an urgent meeting called by the American Academy of Pediatrics on June the 30th, at which it was announced that there was an emergency based on concerns about the presence of Thimerosal in pediatric vaccines.

This was puzzling, as Thimerosal has been used for at least fifty years, and, therefore, I expected to hear new data concerning its effects. At the end of the AAP meeting, I was largely disappointed. Nevertheless, there were some salient points that emerged from that meeting.

First, that the FDA and the EPA were apparently not in agreement with each other in regard to the guidelines for mercury exposure.

Second, that if the EPA guidelines were assumed to be preferable, some infants might receive a combination of vaccines with sufficient mercury to exceed those guidelines.

Third, that a small uncontrolled study, published only in abstract, showed significant blood levels after neonatal hepatitis B vaccination.

Thus, three changes had taken place with respect to the use of Thimerosal. First, the perception of danger, experience with methylmercury exposures, and increasing environmental concerns led the EPA to issue strict guidelines with respect to mercury exposure. These guidelines were designed to provide a margin of safety based on the available data concerning toxicity of methylmercury.

As various guidelines had been proposed, one could calculate differently the allowable mercury ingestion, and Leslie Ball, I believe, will later give these different calculations. So here we have a situation of apparent disagreement between agencies and where industry may have been following a guideline that could be abandoned or altered.

It is important to understand, as I learned, what is meant by a guideline. The statement on this slide is from the recent EPA report which explains how the guideline was chosen. Now, I don't know that I should read this, but the point is that calculations were based on a hair concentration conversion to blood levels, and these were a blood level of 4micrograms per liter of blood; hair concentration you can read; and then an uncertainty factor of was used to derive the acceptable dose, which was thought to be safe. It was stressed that this reference dose is likely to be without appreciable risk of deleterious effects during a lifetime. Exceedence does not mean that risk will be present.

There is an impression of a certain arbitrariness in the choice, but, of course, a choice must be made. All of us would like more data. And as science advances, we must be prepared to change the regulations in recognition of new data. I trust that we shall see these new data later in this meeting.

The second change is the increasing number of licensed vaccines recommended for infants. While some of us perceive that as a good thing, the concern is that this development may be associated with an accompanying increase and exposure to Thimerosal. I would point out, however, that Thimerosal containing DTaPs have the same concentration of Thimerosal as whole cell DPTs, so there was no change there.

In single-dose presentations, HIB vaccines do not contain Thimerosal, and IPV does not contain Thimerosal. So the only significant addition is hepatitis B vaccine.

The third change, indeed, involves the hepatitis B vaccine, which we all know is recommended in infancy as the best way of preventing later infection, cirrhosis and liver cancer, as has been amply proved in other countries. The birth dose was recommended as a way of reducing the number of injections in two- four-, and six-month-old children, which is itself caused by the problems that few combination vaccines have been licensed in this country, and that some of others may not have been screened for hepatitis B infection during pregnancy.

Well, however, routine neonatal vaccination of premature infants was never recommended. The Redbook recommendation here is that infants be allowed to reach two kilograms of weight before being vaccinated against hepatitis B, unless their mothers are hepatitis B carriers.

Let me now touch briefly on the data that formed the basis of concern regarding Thimerosal. I must start with a disclaimer that I am certainly not a toxicologist and would never presume to give an opinion concerning acceptable levels of mercury. However, I do have a fair amount of experience in evaluating scientific evidence.

Well, first of all, there are apparently no data to show that ethylmercury in the concentrations normally used in vaccines is harmful to infants. The available data concern methylmercury, and we are asked to extrapolate the metabolism and toxicity of the former from the latter, which, on the face of it, introduces a scientific uncertainty.

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Second, with respect to methylmercury, it appears that there are only two large epidemiologic studies concerning methylmercury exposure, both occurring after eating fish, and they are in disagreement. The study in the Seychelles was reassuring in that chronic exposure of mothers to more mercury than is present in vaccines was not followed by abnormalities in children. Whereas, in the Faroe Islands, perhaps because of binge eating of pilot whales or because of concomitant ingestion of PCBs, subtle effects in learning correlated with blood levels of mercury. The blood levels, just to remind you, were on the order of micrograms per liter, with an interquartile range to 41. The mean was 22, as I said, and 75% of infants had cord blood levels over micrograms. Also noteworthy is, it appeared to me, that the hair mercury levels in the mothers were similar to those in the Seychelle study.

So no data have been produced to suggest that vaccinated children have suffered from Thimerosal toxicity aside from the allergic reactions already mentioned.

Admittedly, the effects found in the Faroe Islands exposure to methylmercury are subtle and might be missed by passive reporting. At least, however, one epidemiologic study done in the United Kingdom comparing scholastic achievement in pertussis vaccinated children versus unvaccinated children, as quoted in the IOM report on adverse reactions to pertussis vaccine, show that vaccinated children were doing better in school, an effect that was attributed to their parents being smarter.

DR. PLOTKIN: I mentioned – It's true. I mentioned previously the study reported in abstract for memory in which blood levels of mercury were measured before and after neonatal hepatitis B vaccination in five full term infants and fifteen premature infants. The post vaccination blood levels averaged micrograms in very low birth weight infants, compared to to micrograms in full-term infants. The mean gestational age of the premature infants is given in the abstract as weeks. This would mean the infants were mostly below a thousand grams in weight and should not have received the vaccine in the first place.

However that may be, a few percent of those prematures had peak blood levels in the range of cord bloods associated with learning defects in the Faroe Islands study. No pharmacokinetics follow-up was done, but the Emory data would seem to reinforce the earlier recommendation, not to vaccinate premature infants of very low birth weight.

Plus, there seems to be a paucity of data in the literature to show that infants receiving ethylmercury accumulate mercury in excess of infants who are simply exposed to mercury in the environment.

Now, what are the responses of the manufacturers to this situation? First, well, it should be recalled. And Dr. Egan has already well covered this – why Thimerosal was introduced into vaccines in the first place. I don't think I need to repeat that—and it was chosen indeed because it is the best preservative available.

Many chemicals have been tested, and on the next slide we see a short list of the favorite ones: 2-phenoxyethanol, benzyl alcohol, phenol, cresol. Each preservative must pass tests prescribed by the U.S. or European Pharmacopeia, as Bill Egan has already stressed. And he already pointed out that, although in real life situations, the preservative simply has to keep organisms from growing. When tested for regulatory approval they must show an ability to decrease the number of viable bacteria.

Now, I just wanted to show a few slides on comparisons. Here we see a study that was done in the U.S. in 1981 in which we see that Thimerosal actually in this test failed against staph aureus, failed against the USP criterion. 2-phenoxyethanol also failed against e. coli. In this particular test, phenol was the best. Two more recent studies done in Europe gave the following results. On these slides, "A" means fulfilling the Pharmacopeia's requirement, "B" means a slower killing effect than is stated in the Pharmacopeia, and "C" means stasis. "Inc" is incomplete.

So we see here in this comparison that Thimerosal was the best, 2-phenoxyethanol mixed with formol [sic; formal{in}] was next, and let's say phenol and 2-PE were more or less the same. And another comparison done by another manufacturer again shows Thimerosal to be the better of the three, the best of the three, when you look at the As, Bs, and Cs.

Undoubtedly, new preservatives, or combinations of preservatives, are under study, but any sudden decision to eliminate Thimerosal would create a number of potential problems. The first concern is that, at least temporarily, vaccine available would be disturbed and vaccination delayed or omitted. If physicians or state public health authorities insist on immediate access to Thimerosal-free vaccines, chaos will ensue. This is not a commercial issue. Each manufacturer will have gains and losses in terms of marketshare. The overall loss is to vaccination programs.

Second, there is the risk that substitute preservatives will not be as compatible with the vaccines or have less antimicrobial activity and, therefore, lead to an increased possibility of accidents.

In the absence of preservatives, filling of vaccine vials must depend more on aseptic filling. Although the technology for aseptic filling grows more and more sophisticated, as illustrated on this slide, which shows a filling apparatus in which the operator operates in a sterile atmosphere through these portholes—although, as I say, this technology gets more and more sophisticated, it must be admitted that the absence of a preservative deprives us of a safety net to maintain sterility in later use.

Fourth, as Thimerosal participates in the inactivation and detoxification of Bordetella pertussis in whole cell DTP, elimination of Thimerosal would require reformulation and re-evaluation of the product.

Fifth, as influenza vaccine requires rapid production of large amounts of vaccine, elimination of a preservative will shift filling to single-dose vials and may slow or reduce influenza vaccine production.

Finally, if manufacturers must choose between preparing single-dose vaccines without preservatives and multi dose vaccines with preservatives, Thimerosal or other, in general, they are likely to privilege single doses and therefore reduce the availability of multi-dose vaccines. The effect on vaccination in the developing world may be dramatic, as I am sure John Clements will discuss.

In the United States, we should not forget the effects of loss of multi-dose preservatives and multi-dose forms on the function of public health clinics and on the cost of vaccines. The

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immediate response of manufacturers to this crisis atmosphere will be the usual one. They will respond as fast as possible to a perceived public health and consumer demand. In this case, for Thimerosal-free vaccine, as I understand the situation, HIB single dose and IPV vaccines are already free of Thimerosal, and hepatitis B vaccines free of Thimerosal will soon be brought to the FDA for approval. DTaP is a mixed bag, but the manufacturers who use Thimerosal will seek to bring single-dose preparations without preservatives to the FDA within months. Much will depend on the attitude of the FDA regarding evaluation of existing data. For example, if removal of a preservative is considered to potentially alter stability, there will be delays while real-time stability studies are undertaken by manufacturers and then the results reviewed by the FDA. And, of course, we're looking forward to what Norm Baylor has to say tomorrow.

It is interesting that European regulatory authorities met to discuss this issue in April of this year, as many of their vaccines also contain Thimerosal. A working group on Thimerosal formed by the European Medicines Agency issued documents on the subject. Two of their statements are excerpted on the next slides. As you can read: "For vaccination in infants, the use of vaccines without Thimerosal should be encouraged. However, in order not to jeopardize vaccine supplies and immunization programs, it is advisable to introduce requirements for the elimination of organomercurials in vaccines on a gradual basis."

And another excerpt, the group concluded that Thimerosal should not be banned from medicinal products; however, taking into account the identified and theoretical risks, precautionary measures should be considered. And the most desirable alternative they mention is preservative-free formulations.

It is important to stress that until now European countries that also used neonatal hepatitis B vaccination, such as France, Germany, and Italy, have not changed their recommendations. That includes Spain, which, like the U.S., recommends universal neonatal hepatitis B vaccination.

So, in summary, what is the manufacturers' view, in quotes, of the situation as interpreted by me. Frankly, and I think it is important to be frank early in this meeting to promote a useful discussion, I think that FDA did not give manufacturers sufficient warning that Thimerosal is no longer acceptable, that panic entered into the deliberations of the AAP, and that CDC was partly handcuffed by regulations that prevented adequate consultation with the ACIP.

The published evidence that the Thimerosal contained in vaccines is dangerous is unconvincing. Nevertheless, manufacturers, like everyone else, would prefer to have a less controversial preservative. Many vaccines currently sold do not contain Thimerosal. And even in the absence of any regulatory changes, new vaccines will not be manufactured with it. Yet, it remains the most active preservative and no equivalent substitute is available. Political concerns aside, it may be justified to keep in some vaccine formulations, particularly those in multi-dose preparations. Beyond the factual scientific issues, the process of decision in this matter has been flawed. This meeting should have taken place before a public health decision or a public announcement was made. There should have been adequate consultation and discussion. This point of view probably gives offense to some, and I'm sorry that this should be the case as my remarks are not directed against any person in particular.

Reasonable people may disagree on all of these points, and I, for one, am prepared to modify my opinion based on data displayed later in this meeting. However, so far, manufacturers have seen no evidence for a clear and present danger, but, rather, a rush to judgment.

At the earlier private meeting called by the AAP, I tried to recommend to the participants a bit of what the French call "Sang-Froid." I found it difficult to give an adequate English translation of the term, but, recently, I came across the French definition given by Denis Diderot in the 18th century. He wrote: "Sang-froid, that quality so necessary to those who govern, without which one would rarely apply justly the means to the circumstances, without which one would lack presence, presence of mind; sang-froid which submits the activity of the soul to reason and which preserves one, in every event, from fear, from frenzy, and from precipitation." I believe we could all benefit from such dispassionate reflection. Thank you.
(APPLAUSE)

DR. GREENBERG: Thank you, Stan. That was an interesting talk. We now can take some questions.

DR. ENGLER: Dr. Engler from Walter Reed.

I was wondering if in those discussions there was any consideration of the hundreds of children and adults who between the 1960s and until 1981, when intravenous gamma globulin became available, received weekly or every two weeks, 10, 15, 30 cc's of intramuscular gamma globulin, and in my calculation there's probably a significant cluster of a couple hundred patients or more who have received 10,000 ml of gamma globulin, which is probably more than three logfolds, if not four, more than what are given in standard childhood immunizations, and that does contain Thimerosal. As far as I'm aware, there's only two cases, and these are patients who had received this in excess of twenty years in these kinds of doses who developed some cerebelli ataxia secondary to accumulated mercury toxicity. Now, the incident is a separate issue, certainly, in regards to also the difference in the immune system of the infant from older children or adults, but in other age groups separate from infants, that seems to be overwhelming data in terms of the safety to support some of what you're suggesting.

DR. PLOTKIN: Yes, thank you. I would agree that in looking over the literature, as far as I've seen, the only instances of acute Thimerosal toxicity have been where a gross error was made, I think, in the use of chloramphenicol and, otherwise, the literature show conspicuous absence of acute toxicity. But to be fair, as you pointed out, of course the issue here has focused on the very young infant and the effects on the central nervous system of the very young infant.

DR. GREENBERG: In the back? Could you identify yourself?

INAUDIBLE SPEAKER: Stan (inaudible) from Merck. You covered the other chemical, but did you run across any studies using radiation as a preservative?

DR. PLOTKIN: The question that Stan is asking is the use of radiation as a preservative. That's a good question. I must admit ignorance. I have not seen those studies. I imagine that under some circumstances it might be possible, although, with particulate matter in vaccines, I think there could be some issues

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about sterilization and, of course, the effects of radiation on the active product. So the short answer to your question is "No."

DR. BAYLOR: I just wanted to add what the real issues --

DR. GREENBERG: Identify yourself, please?

DR. BAYLOR: Oh, I'm sorry. I'm Norman Baylor. I'm with the CBER Office of Vaccines.

The real issue is going in and out of that vial. To produce the vial, a final fill, that's sterile, that's not really a problem. But going in and out of that vial, that wouldn't address that problem.

DR. GREENBERG: Any other questions?

(NO RESPONSE WAS HEARD)

DR. GREENBERG: Well, Dr. Plotkin had a pretty controversial talk there. You folks aren't rising to the bait.

(LAUGHTER)

DR. PLOTKIN: I'm glad to be able to get off the podium and still in one piece.

DR. GREENBERG: The last speaker before the coffee break is Dr. C. John Clements, from the Expanded Program on Immunization, Vaccines, and Other Biologics at the WHO, and the title of his talk will be *Preservatives in Vaccines: The Global Perspective.* So he will encompass everything.

DR. CLEMENTS: Good morning, ladies and gentlemen. First of all, I want to thank the organizers for inviting me to come and speak. It's a great privilege to be here in Washington. Before I actually start the presentation, I want to acknowledge that in assembling some of the materials for this I was helped by a colleague of mine, Gary Schatz, who is a consultant that has been working with us from CDC and who tragically was killed in a road traffic accident last Monday. I just want to acknowledge his contribution to this.

As I speak to you this morning, I want you to think of me both as somebody speaking from a global perspective from WHO, but also as an advocate for a hundred million such children as this every year. This young gentleman is sitting in a cardboard box with a hole cut for his legs and he is very interested in what we're going to say this morning.

As you can see from this molecular description of Thimerosal, it's the mercury which is the pride and the downfall of this gentleman, and we can all agree, I think, right away, that the mercury here is not what we want in preservatives. There's ample evidence that it is an undesirable molecule, which is taken in by the human through food and drink and pharmaceuticals and vaccines. In general terms, we're without hesitation in saying we don't want it, and that is a strong basis for further action.

However, I think we need to examine the issues a little bit more. And I must say that I'm delighted being third in a row of three, and I hope you'll find that what I have to say is very synoptic with the previous two speakers. I make no apologies for covering similar ground, although I hope you'll remember my friend from Africa as we speak. And I keep pressing the wrong key. Never mind. Okay. The United States has gone through its due process to identify a problem and take action to remedy it. However, there is a knock-on effect, which the rest of the world must bear as a consequence. And what I want to do is to draw out in the next few minutes some of these consequences for you and examine the knock-on effect. And I want to really say how privileged I am to be here, and I feel that I'm looking over your shoulders as you go through this discussion and make some of these decisions. But also, I'm looking over your shoulder anxiously because there is an knock-on effect, and I want to be really sure that each one of you involved in these decisions understands fully some of the implications of those knock-on effects.

Like Stan, I'm concerned with the scientific process which has gone on to date. There is a lack of agreement about the safe cutoff levels for mercury and there's a variance between the control bodies in the United States, and certainly between WHO, as to what those levels should be. And the infant maximum intake level has been extrapolated only.

As far as toxic effects go, it's not clear what levels of exposure to mercury in the fetus, the neonate, and the infant are harmful. We know that there are harmful levels, but we certainly don't know at what point we have to be concerned.

Now, what does WHO say about this? Well, if we look at the most authoritative voice that I can find, the 33rd Report of the Joint FAO/WHO Expert Committee on Food Additives, JFOA, pronounced on this in 1989. The committee confirmed the previously recommended provisional tolerable weekly intake of 200 micrograms of methylmercury. That is equivalent to 3.micrograms per kilo of bodyweight for the general population, but noted that pregnant women and nursing mothers are likely to be at greater risk from adverse effects of methylmercury. And I should point out that the discussions which have gone over the last two or three months really suggest that possibly we should be looking at a five-fold lower cutoff point for pregnant women and nursing mothers in order to protect the fetal brain.

And even though the JFCA committee that met in Rome in June was aware of the issues regarding Thimerosal, they were not in a position to offer any stronger guidelines regarding cutoff levels for pregnant women and didn't even trespass into the dark waters of recommending levels for infants.

So the figures that I've been able to get hold of, then, are for WHO 3.3, for FDA 2.8, and for EPA 0.micrograms per kilo bodyweight. But I do stress that WHO recommendations are based on the adult level and make no special concessions for pregnant women or infants.

A question already asked: Do we need preservatives in vaccines? And the way that things are going in the United States, there's the clear possibility that as you move to monitor those preparations then there may be a possibility that they are not needed. However, this is not the case for the majority of the world. And in tests that we've undertaken recently in vaccines, it is clear that the lack of preservatives pose a serious threat to the integrity of multi-dose vials, which have already been opened and penetrated by at least one needle through the cap.

These lists vary a little bit depending on who's presenting, but I think we're fairly consistent in identifying some alternatives to Thimerosal. 2-phenoxyethanol looks like the forerunner, but we have limited information on comparative effectiveness. Formaldehyde, cresol, possibly others. Phenol, I should draw your attention to, in the WHO regulations, is not permitted any longer.

If Thimerosal is not available, what alternative strategies are there for developing countries? Well, we can move to a monodose vial without preservatives or we can seek a replacement to

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the preservatives. But as is already pointed out by Stan, there are serious consequences for both options. The product must be reformulated, new clinical data must be presented, and new submission for license must be made, and for vaccine supplied through UNICEF, then a special WHO/UNICEF approval must be processed. All in all, a long time interval before availability of either of these alternatives.

You've heard already, and you'll hear I know in a lot more detail, how the regulatory bodies in the United States go through their debates. In terms of WHO, we have an Expert Committee on Biological Standardizations which meets regularly, which is composed of outside experts. Although it is hosted by WHO, it is not an internal committee, it is an external committee, and it results in WHO producing WHO technical report series, which I've already quoted from once.

Expert Committee on Biological Standardizations, ECBS, what does that say about DPT and Thimerosal? "If the vaccine is to be dispensed into multi-dose containers, a suitable antimicrobial preservative shall be added. The amount of preservative in the final bulk shall have been shown to have no deleterious effect"—Never put that on a slide if you have the say it in public. -- (LAUGHTER) "on the toxoid or on any other vaccine components with which the toxoid may be combined, and to cause no unexpected adverse reactions in humans. The preservative in its concentration shall be approved by the national control authority and don't include phenol."

The other vaccine that we're particularly concerned about is hepatitis B, and the ECBS says about that: "Each final bulk or final lot shall be tested for the presence of preservative. The method used and the permitted concentration shall be approved by the national control authority. The most common preservative used for hepatitis B is Thimerosal," and then it goes on to describe the analytical methods.

So, in summary, through the expert committee at WHO is saying that the task that the preservative is designated for—in other words, to be antimicrobial—must be defined and fulfilled.

Again, as Stan has already pointed out, it must not damage the vaccine in any way, like Thimerosal and IPV, and it must not damage the human recipients, although that is not spelled out how. The level is set not by WHO but by the national control authorities.

Now, what implications has all this to do for the global supply of vaccines? Since Stan has begun to open up this discussion, I need to just clarify for some of you who may not be familiar with it, the majority of the world, particularly developing countries, looks to three main sources to get their supply of vaccines.

The first is the local producer, and that may surprise some of you who are not familiar with this subject; secondly, UNICEF-supplied vaccines; and thirdly, they may go directly to the manufacturer and buy directly through them.

And if you look at this graph, the red at the top is the local production. I'm sorry I don't have more up-to-date information to show you, but the trend has continued where a large proportion of the world's vaccines are produced in country and consumed in country.

If you look at this description of DPT sources by WHO region, you can see that in the Eastern/Western Pacific Region and the Southeast Asia Region, a vast proportion of the vaccine is made locally and consumed locally. We'll discuss the implications in a moment.

And for hepatitis B, many countries in the developing world have HBV transmission by the neonatal route. In other words, the first week, first two weeks of life are crucial in protecting the infant; and if there is no birth dose of hepatitis B given, then there is likely to be transmission of the virus. And this means that without a birth dose in China, between and 15% of all births are likely to result in chronic infection.

What immediate impact on developing countries would there be if Thimerosal were removed from vaccines? As Stan has already said, existing suppliers would be unable to supply such vaccines and supplies would rapidly dry up. Locally-produced vaccines, and remember I've identified them as being a major source in developing countries, would be unable to substitute for this preservative. Local production would either stop—or I'm not sure whether it's worse or about the same level of significance—but they might turn to producing without the preservative.

We've mentioned another strategy of moving to mono-dose vial preparations, but at the moment, basically all vaccines in developing countries are drawn from multi-dose vials. The cold chain could not cope with a five- to twenty-fold increase in volume which would be resulting from this. It would double the cost of the cold chain, and result in a cold chain costing around half a billion dollars a year. There would be a six- to ten-fold increase in vaccine prices for these countries, which could not be borne by them. Even if there was a switch to mono-dose, those products still need relicensing.

The one hope in the dark tunnel at this moment in this scenario is that we are watching the development of a pouch-and-needle hepatitis B delivery system in its field trials, and there is at least the possibility that that will fill a niche as being a disposable single-dose delivery system.

What happens—the alternatives open to developing countries. They could obtain vaccine through their regular UNICEF supply with a new preservative if a new preservative became available. They could purchase directly from industrialized countries. They could use locally produced vaccine, or they could use vaccine which is imported in bulk and filled locally, or they could switch to mono-dose with no preservatives.

And what about the time and the impact of these decisions they would make? If they waited for a preservative to be introduced into UNICEF vaccines, that is going to be a long wait. If they purchase directly from industrialized countries, not only do they have the wait, but they will certainly have an increased cost. If they rely on locally produced vaccines, they have to try and obtain the new preservative, perhaps under license, again a long wait and an increased cost. If they go for local filling from bulk purchased overseas and the license, there's a long wait and an increased cost. And if they switch to mono-dose, it may be relatively quick, but it will be far too expensive, both in terms of purchasing the vaccine and in managing the cold chain.

Now, there may be some discrepancy in the time sequence that I put up here. It's the best we could come up with in WHO on a sort of Gallup Poll basis, and this isn't something that you should take as finite, but it gives you some feel. To find a new preservative—if a new preservative is found, there's no guarantee, but between one and five years. Clinical trials, another two

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years, licensing, a year if it's put on fast track. To reformulate an existing vaccine to a mono-dose would probably take around one year.

In summary, then, my Executive Director, Michael Scholtz put out a press release a few weeks ago: WHO will continue to recommend Thimerosal-containing vaccines. We see no reason for changing that given the present amount of information and the scientific debate. Mono-dose hepatitis B vaccine will continue to be administered in the birth dose and all the other doses from multi-dose vials. At this point, there is no option about using mono-dose. Although, as I said, a light in the end of the tunnel is the patch-and-needle device. And as I indicated already that mercury is a highly undesirable chemical to have in biological products anyway, and we are determined to work with industry and regulate the authorities to eliminate Thimerosal.

One thing I've observed doing this over the last few months is a concern, and I asked the question: Instead of the onus being on the scientist to demonstrate there is a problem, has the onus now shifted to the pro vaccine community to show that there isn't a problem? And remembering my patron sitting there in Africa, what does it all mean for him or her? Well, there is balancing scales out there, and there is a theoretical risk from Thimerosal that we are all aware of and have been discussing. On the other hand, there is the known risk from vaccine-preventable diseases if we stop immunization and if we're no longer able to use the vaccines that we have at the moment and which have been used successfully for fifty to sixty years. And there is the known risk from contamination of vaccines. I put it to you that it is not a nearly equal balance. It is a balance, which is, without hesitation, in favor of continued use on a global scale of vaccines, which now contain Thimerosal. Thank you.
(APPLAUSE)

DR. GREENBERG: Thank you, Dr. Clements. Do we have any questions?

DR. GELLEN: Bruce Gellen from the Infectious Disease Society.

John, has the decision that's been made here and some of the recommendations, has this trickled into developing country programs and has there been some discussion to date at local levels?

DR. CLEMENTS: When the United States generated this interest and it went public on the Internet and in the journals, then WHO put out a press release and distributed information and backup information to all EPI managers throughout the world and to WHO regional offices and country representatives. And to my delight and amazement, I had only one e-mail query of clarification following that. So at this point the world is quiet, and I'm very happy to say that. So it doesn't seem to have had any impact at all, Bruce.

DR. HALSEY: John, the cost of --

DR. GREENBERG: Identify yourself, Neal.

DR. HALSEY: Neal Halsey.

The cost that you put in for the potential use of single-dose or mono-dose vials and so forth, because of the increase in space requirements, you estimated it would increase to five hundred million per year, but you didn't give us what the current cost is and whether that increase in cost is a single time or whether that's recurring year after year after year. I recognize that more refrigerators would need to be purchased at multiple points in the cold chain, but once those are purchased, then is that a one-time cost and, you know, what is the recurring cost?

DR. CLEMENTS: Okay. There are two parts to that. It's approximately doubling the cost of the cold chain to half a billion, and most of that would be capital investment, not recurring costs.

DR. KATZ: Sam Katz from Duke University and the Infectious Disease Society of America.

John, one of the issues that we have heard repeatedly, and this may not be a fair analogy, but that is what the United States policy determines regarding vaccine use has effects on the WHO program. That came up with smallpox vaccine when we discontinued use six years before WHO. More recently, concerns switching to IPV and rejecting OPV as the vaccine of choice in this country. And one side, of course, is your pragmatic issue: Do Thimerosal-containing vaccines remain available?

The other is, perhaps, related to what Bruce Gellen was asking, which is its influence on policymakers in other countries, particularly the developing nations. Do you see this as an issue?

DR. CLEMENTS: It's potentially an issue. I think a lot of countries use whatever the FDA does as a benchmark, and in my own country, New Zealand does the same. It looks to FDA, and if it passes a vaccine, that in itself is crucial in the vaccine being accepted in that country. Do they accept it without process? No. And I think our job has been in this last few weeks to be the moderator of the information coming out of the United States and to say that has been deliberated in the United States and it has relevance to that country, but it needs to be processed and seen in the light, in this particular light, for the rest of the world. So, yes, it has a powerful influence, but countries make their judgments. The end call is that they make their own judgments.

DR. SNIDER: Dixie Snider, CDC.

John, How do you see moving forward on this from a global perspective? I mean, it seems to me, as you've indicated, it's going to be a long process, and I'm very concerned about the trends, as you pointed out, were to use local producers, and there are a lot of reasons for that, which you may want to elaborate on. But there seems to be, by doing that, an increased need for a preservative if you're going to rely on a variety of local producers, unless somehow GMP, Good Manufacturing Practices, can be upgraded in many of these countries. And so I wonder, realistically, how do you see this playing out to achieve the goal of maintaining the availability of these necessary vaccines while at the same time getting the mercury out?

DR. CLEMENTS: I think we have perhaps a different perspective on the urgency. I think the United States is faced with a different set of pressures from some other countries and it must respond to them. But I think our job in WHO is to guide in as wise a way—I wish I could remember what Stan's quote was—to have the wisdom to guide countries in making decisions in an appropriate time base. And what we'll be doing is working with the Experts Committee on Biological Standardization to come up with something similar to the European vaccine manufacturers in encouraging a gradual shift towards mercury-free preservatives, but it will be something which is measured in due

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time and with due consideration of as many factors as necessary.

So I think that's how I'd answer it. We will definitely be encouraging the process. We will probably be funding research from researchers who wish to investigate the potential for new preservatives. We'll be looking at industry and encouraging them to do the research. We'll be putting out feelers in many directions to try and encourage the development, the rapid development of that preservative, because for us there is no turning back from multi-dose vials and there is no getting away from the fact that due to human error, potential for human error, it is essential that those multi-dose vials have some preservative system in them.

DR. PLOTKIN: Plotkin, PMC.

I'd just like to point out that there's been kind of a subtle fall down the slippery slope here. That is to say, the discussions have started out by talking about limits, tolerable limits, to the amount of mercury, and now we're talking about zero tolerance. So we've now progressed—I'm generalizing here, of course. We've now progressed to the point where no mercury is tolerable at all, whether it meets EPA requirements or not.

Now, in the particular situation of the developing world, John, I mean, could you not envision a situation where there would be an allowable amount of mercury given in the multi-dose vaccines, considering that in the developing world the number of vaccines being used in not the same as in the U.S.?

DR. CLEMENTS: Well, I think, Stan, you made a rhetorical statement there, which I certainly don't agree with, that we're wanting zero dose mercury. That has not been established in any scientific setting. It may be an emotional response which you're talking about on a slippery slope, but mercury ingestion and environmental mercury that we have around us now make it impossible to think that we'll be mercury-free.

What we're talking about is how much mercury is acceptable. That doesn't negate the desire—the desirability of having mercury-free vaccines—but we certainly are not targeting that as—that is not necessarily our immediate goal, although it may be our long-term desirability. Thimerosal has been a fantastic preservative for 50 to 60 years, and it has done a fantastic job.

DR. WANACOTT: I'm not sure whether we have representation—I'm Dave Wanacott from Merck. And I'm not sure if we have representation from the Pharmacopeia decision makers in this meeting, but have you considered at WHO talking to some of the pharmacopeias? Because they have really been a large driver for the higher levels of preservatives to meet the antimicrobial effectiveness testing, and they consider backing off on both levels. Has that consideration been discussed?

DR. CLEMENTS: Yes. I'm speaking from a particular unit in WHO, the Immunization Unit. We work hand-in-hand with Biologicals. So I'm not privy to everything to the Chief, L. Wynn Griffith, has been doing in that area, but I know he has been in contact with them, and absolutely, I think it's a good point.

DR. GREENBERG: Well, we're actually a little bit early. So I'd like to ask whether there are any questions for our last two speakers, after you've heard all three, or whether any of the speakers have anything to say to the other speakers that might be informative or help clarify this issue? Bill?

DR. EGAN: If I could just make a comment. First of all, Thimerosal, or if you want to go on the other side of the Atlantic, Thi0mersal, has not been banned. So we're not talking about that it must come out of all vaccine. So, you know, Thimerosal has not been banned. We are, nonetheless, concerned about the cumulative doses of mercury and we prefer to have mercury-free vaccines and preservative-free vaccines, i.e., single dose presentations in the United States.

We have asked manufacturers for their—you know, for their plans for elimination of Thimerosal and that it'll still be a—you know, if they cannot eliminate it, to justify it and be allowed where justified. So, you know, we haven't gone to that point of saying, you know, as of such and such a date, mercury cannot be in any preservative—in any vaccine.

DR. SNIDER: Dixie Snider. I just wanted to raise one additional point that I think has been implied but really hasn't been made explicit, and that is that I think there is an important issue here around the credibility of immunization programs nationally and globally, and that although it may not be in the best interests of everyone to eliminate mercury entirely because the risk or the price of doing so might be a price we don't want to pay, I think the concern about the integrity of the entire immunization effort, if you will, has been on many people's minds and has been a part of the decision-making process up to this point and will continue to be a part of the consideration here. Not that people do not want to react to scientific information that is available in an appropriate way, but, in addition, when there are choices that can be made to move from a Thimerosal-containing vaccine to one which can be found to be just as safe and effective without that agent, then it's to the immunizations programs' advantage to be seen as not adding to the mercury that people are ingesting all the time, not be adding to mercury burden.

So I think the credibility of all immunization programs is important to maintain, and one aspect of the reason why we have declared concern, if you will, about the amount of mercury that we are delivering.

DR. ZUNE: Kathy Zune, CBER.

I just wanted to make one comment regarding the issue of the timing here, and it was alluded to that this was rather sudden. The issue and concern over Thimerosal has been an ongoing discussion, and I think the discussions with manufacturers looking at the reduction and/or elimination of Thimerosal is not a new issue. I think some of the aspects which triggered some of the current information that has been discussed has been during the FDA Modernization Act of 1997. We were directed at the FDA to do an evaluation of mercurials in all FDA-regulated products. As part of that initiative we worked cooperatively with the manufacturers to get the data, which is what you will be hearing later in the workshop. The issues are then looking at cumulative levels, as was discussed by Dr. Snider, I think became the issue of concern. The vaccines are believed, when looked at, safe and effective, but when you're looking at cumulative doses in small neonate typing, I think the issue and the concern was raised and should be looked into, both from a scientific as well as a public health issue.

My sense is that this workshop is very valuable to the public health service, FDA included, in order to have a very important

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scientific evaluation of the data available and what data we need to get. So, thank you.

DR. GREENBERG: Dr. Plotkin.

DR. PLOTKIN: Well, several points. One, actually, in responding to Dr. Zune, I think there is general agreement that mercury is not going to be used in future vaccines. I think the issue is more whether it needs to be removed immediately from currently licensed vaccines.

In relation to Dixie Snider's comment, I would like to say that if anti-vaccinationists did not have mercury, they would have another issue, and one cannot prevent them from making hay regardless of whether the sun is shining or not. So I don't think that's really a valid reason for making decisions.

Lastly, I don't want to lose sight of the comment by, I think Dr. Wannake from Merck. I am certainly not a vaccine production person, but in looking at the Pharmacopeia regulations, I was struck by their, let's say, apparent excessiveness, and whether one could -- And this is actually be considered in Europe, whether one could adopt different criteria which would allow reduction of the concentration of preservatives in vaccines. In other words, that you would require only stasis rather than cetyl activity against 105 or 106 organisms, as Bill Egan mentioned.

DR. GREENBERG: I know less about this than Dr. Plotkin, but it certainly seems to me that the biologic experiment, there's a lot to be said for that, but it doesn't seem to me that usually contamination should be occurring at quite that level and that you might be able to get exactly the same effect with less than -- If somebody in the audience knows how that criteria -- what the thought process behind it was, that would be an interesting thing to hear about. Bill?

DR. EGAN: I can't comment about, you know, the thought process, and it goes back quite a few years, I think somewhere around 1970, when the USP introduced those requirements, their definition of a preservative, but I would like to add again what I mentioned during my talk, that I did think that, you know, those are very stringent requirements and that in the United States, it is not necessary that a preservative per, you know, the CFR must meet the USP definition. Certainly, you know, that's acceptable, and it has been, but it's not a requirement that it meet the USP to satisfy the CFR. I did run that through our general counsel.

DR. GREENBERG: All the pharma—did the big pharma hear that last statement?

UNIDENTIFIED SPEAKER: Just one comment. Usually when we're manufacturing, we think on the international level, and, particularly, it's the European Pharmacopeia that is the mandatory one, and their requirements are perhaps even more strict than the USP. Therefore, you know, I'm thinking in the international scheme of things, that becomes an issue. Let me give you an example. A few years ago, quite a few years ago, we were working with the Europeans and taking a product that's no longer—a diluent that's no longer on the market that had a preservative in it, and it was a single-dose vial, but there was a very low level of Thimerosal in it which would not pass the European Pharmacopeia. And we said, well, basically this is a single dose, it's there as assurance for misuse after it leaves the manufacturer. And they said, well, no, still got to meet European Pharmacopeia. So I think that needs to be brought into the equation here in looking to evaluate some of these requirements that may not be a requirement in the U.S., but our impact on the international basis.

DR. SNIDER: Dixie Snider again.

I just wanted to respond to Stan by saying that I wasn't speaking—in talking about credibility, I wasn't speaking to try to address issues that antivaccine groups might raise because I do realize that there are incredibly an unending list of complaints or charges that could be made.

I'm more concerned, though, about scientists at the Agency for Toxic Substances and Disease Registry and the National Center for Environmental Health and the Environmental Protection Agency and others who have expressed concerns about the mercury that we are delivering and was only trying to suggest that, in view of concerns of scientific groups, it is reasonable to consider how we can lower or eliminate the mercury that we deliver through vaccines since people will get it through, unavoidably, a series of food supply.

DR. GREENBERG: Dr. Klein?

DR. KLEIN: Jerry Klein, the Boston University.

Stan, as a point of information, could you clarify the many products that do not have Thimerosal? Now, do they have other preservatives, or are they free of any preservatives? And if so, what is the basis for their success and is it just something that is necessary for the manufacturing products in selected vaccines? As example, there's one pneumococcal vaccine that has Thimerosal, as the alternative product does not, and the same thing with *heamophilus* influenza.

DR. PLOTKIN: Well, there are many parts to that question. The best table on the list of vaccines containing Thimerosal is the one published by the AAP, and I refer to it often. But as Bill mentioned, IPV contains 2-phenoxyethanol because Thimerosal will inactivate the polio component. Other than that, I think, but I'm not absolutely certain, that benzyl alcohol may be in some unusual vaccines, but in terms of common vaccines, I think those are the only two.

Now, why is TM, to give a nondenominational name—why is it present? Usually because manufacturers are making multi-dose and single dose and prefer to have one product that they fill from. Now, of course, as I stressed, where single-dose presentations are the only form, you can, in fact, do simple aseptic filling with the risks that Bill mentioned.

So the choice of whether there's TM in it or not depends on largely what forms are being made, whether bulks have to sit around for some time before they're combined for filling, and issues which relate to the perceived production process and the subsequent use—that is, the subsequent use by physicians—whether in the single-dose form or in the multi-dose form, and also capacity of the manufacturer to make one or the other.

I'm not sure that I've answered your question very precisely, but that's about the closest I can come.

UNIDENTIFIED SPEAKER: But there are a number of products that appear to be in multi-dose form that do not have preservatives?

DR. PLOTKIN: No.

UNIDENTIFIED SPEAKER: So any multi-dose form does have a preservative?

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DR. GREENBERG: Well, I think we're almost back exactly on schedule, which is good. You can all take a 33-minute break, so 11:00 o'clock, and be back in your seats then. Thank you.
(RECESS FROM 10:30 A.M. TO 11:00 A.M.)
DR. GREENBERG: If everybody could take their seats, please? In the back, sit down. Okay. We're now going to finish up the morning session. Before we start, I have one question that several people have asked, and I just wondered whether any of the speakers from the morning could answer it, and that was: For multi-dose vials—Measles/Mumps/Rubella is a multi-dose vial and does not have preservative in it—do people know how the problems of preservation are dealt with in that vaccine? That's the question. Does anyone have an answer? A quick answer?
UNIDENTIFIED SPEAKER: (Unable to hear speaker)
DR. GREENBERG: There are no multi-dose vials of Measles/Mumps/Rubella? Somebody over there. Neal?
DR. HALSEY: My mic won't come on.
DR. GREENBERG: Okay. Then, Stan?
(LAUGHTER)
DR. GREENBERG: I'm not responsible. Okay. We're having -- If there's somebody in the back, the lights don't seem to be coming on. I'm going to save that for the end of the session, and people can think about that.

So the next speaker is Dr. Jeffery Englhardt, Senior Research Scientist at Eli Lilly, which is the company that makes Thimerosal, and his talk will be *Toxicology and Metabolism of Thimerosal in Animals*.
DR. ENGLHARDT: Thank you. I appreciate Dr. Myers' invitation to come to this. I am a veterinary pathologist, so I look at things from a slightly different perspective in that I work in the toxicology or drug safety component of Eli Lilly and Company.

When the question came to me about toxicity of Thimerosal, I had to scratch my head and wonder, what the heck is this? This is not a product that I have on my horizon very often, and I had to talk to one of my more senior colleagues who said, "Oh, that's Merthiolate." As I started getting into this particular topic, I had to go back into our corporate literature but also start searching the scientific literature. Though we keep information from a material safety data sheet standpoint, we don't keep an active research program going on this compound, mostly because of its historical perspective. If you'll bear with me a little bit, I'd like to take a few minutes to retread some of the ground that was covered this morning, but it's important to, I think, see where the database has grown on the toxicity of this compound and where are the holes in terms of the toxicity of this compound.

As was mentioned earlier, Thimerosal is an organomercurial. It's ethylmercurithiosalicylate and it's just mercury that's part of the ethylmercury that has apparently become the issue that's being discussed here at this workshop. And just to note from a molecular standpoint, in this complex salt, the mercury composes about 49% of the molecule.

Looking back into the historical literature, Thimerosal had a variety of chemical properties that made it very attractive. And one of the things also, as I was reading this literature, is that not all mercuries are alike, and I'd like to retread that again a little bit later in the talk. Now, Thimerosal is found to be very water soluble. It was created stable solutions and also compatible with a variety of biological materials. As Dr. Klein mentioned earlier, we were one of the first to be using Thimerosal as a preservative in some of our older vaccine days in terms of the diphtheria vaccine. It was also used in some of our other toxoids that were produced back in the '30s and '40s. And as mentioned also, this has been marketed since the '30s, and as I got into our literature, I found that there is very little in terms of toxicology in animals. Most of it is quite old. The primary reference is a 1931 reference in the *American Journal of Hygiene* and it's often in obscure journals or cited as one or two sentences within review articles, and it's very difficult to find very explicit information on Thimerosal.

As has been well described this morning, it's been used as an antiseptic, fungistat, and a preservative for a number of years. The antimicrobial activity has been attributed to the release of this ethyl mercuric ion and thereby acting as an oxidizer for groups leading to changes in intracellular calcium and that is the mechanism that it causes cell death. I also found that it's very interesting that there are as many articles on using Thimerosal as an in vitro reagent to study the calcium fluxes in cells as there are uses for – or publications on use in vaccines.

One thing that I did find is that the ethylmercury and thiosalicylate are the primary metabolites which were described in an article published from Lilly in 1956. In this particular issue, they were looking at the question around the inactivation of IPV with the use of Thimerosal and had discovered that these metabolite ratios can be altered by the presence of copper within either the vials that are being filled or within the production materials and that the copper drives the reaction not to the mercuric ion, but to the mercuric oxide. That is one of the materials that is purported to inactivate the protein in the polio toxoid.

So, so much for the history. What I'd like to do now is talk a little bit about what do we know about the toxicity of this molecule. Again, these data are from some of these older articles. There's been nothing that I've been able to uncover published in about the past 25 years in terms of new animal data on this molecule.

Oral toxicity in rats has a MLD of about 73 mg/kg and, as you can see, when you look at the rodents and the lagamporphs [sic; lagomorphs], there is a disparity in terms of what the bodyweight toxicity is, but the overriding morphological alteration that occurs in these animals is renal necrosis. This is interesting in the fact that this type of toxicity is what has been described most widely with mercuric chloride studies, which is renal necrosis.

One human study—and I should note that I found a couple of human correlates to go along with this during my searches. There was one human accidental or—I can't say if it was accidental. It must have been intentional in this case. An individual consumed some liquid Merthiolate and successfully done himself in. He consumed an estimated 83 mg/kg showing that the oral toxicity in rats is pretty well on, but the presentation that this individual had was, again, very similar to what's been seen with mercuric chloride, that he presented with gastritis, renal failure, and gingivitis. It wasn't until the very late stages before he died of respiratory failure that any type of polyneuropathy was identified.

Also as mentioned earlier, Thimerosal is a very exquisite antigen, not only in people but also in guinea pigs and rabbits,

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and it is also a dermal irritant as was described in some of the earlier literature when Thimerosal was used as a contact lens solution preservative. The ethylmercuric chloride is the purported allergen that's responsible for these phenomena not only in people but also in animals, and one of the disparities from the animal studies that's been identified is that, unlike people that can occasionally have a systemic hypersensitivity reaction, those particular phenomena have not been identified in either the rabbit or the guinea pig studies.

When we start looking at the non-rodent species, the only studies that I had found on toxicity were some in the 1931 publication on toxicity in dogs, where 2 mg/kg was administered every 3 days and then 10 mg once weekly over a 6-week period, and at the end of that the animals were examined and there was no clinical toxicity nor pathologic alterations that were identified.

I was also surprised to find that there was a 2-year carcinogenicity study that had been conducted on vaccine preservatives and Thimerosal was included in that particular study, and the outcome of that was that there were no compound-induced neoplasms. It should also be noted that Thimerosal does cross the blood/brain barrier. It also crosses the placental barrier. However, there has not been any evidence of turadnogenicity that's been shown with the compound in a study that was conducted with one of the contact lens preservatives.

It should also be noted—and this is one of the gaps that I identified and this is part of the concerns that are raised here in looking at the neonatal vaccine issue—is that typically now with the pharmaceutical agents, we do what's called a post-natal development study or a Segment III study, and there's nothing in the literature right now that has anything that looks at *in utero* exposure to Thimerosal and in post-natal development in rodents. So we do not have any data that would indicate either a risk or a lack thereof.

I did find one article that I found very informative and that was an article published in 1975 by Blair *et al*., that was looking at the metabolism and excretion of Thimerosal in adult squirrel monkeys and this was a chronic study, a chronic daily administration study. Thimerosal, at a concentration of 0.002%, and this is, I believe, in the range of what's used as a preservative in the vaccines. I think that's allowed to go up to about 0.01%. This was administered in 2 ranges, either 2.2 or 12 micrograms per monkey per day for 6 months and that the total Thimerosal dose was 418 or 2280 micrograms. If you remember, this has a 49% of mercury, so this means that these animals received roughly 200 micrograms of mercury or 1100 micrograms of mercury. Now, this is a classic tissue distribution study and, unlike what's done with pharmaceutical agents, they had to use atomic absorption to look for the mercury. So the tissues were dissected, analyzed for the presence of mercury and what form was that mercury in and also histologic evaluation of those tissues to see if there were any accompanying morphologic alterations due to the presence of absence of the mercury.

The data from this study showed that there was no evidence of toxicity either seen clinically during that 6-month administration phase or during the pathology evaluations. There was variation in the mercury concentration in individuals. That is, within those given groups, there was a disparity in how much mercury, even though they were given the same dose by the same period of time, on how much mercury was accumulated in different tissues, but what was of note was that the mercury that was present in the blood and tissues was primarily in the inorganic form and also that the distribution within the tissues had kidney as being the primary organ, followed by liver, muscle, brain, and the least of all, in blood.

Now, some of this conversion from the organic to the inorganic may lead to the point that I made earlier, that all mercuries are not alike and that within the organomercurials, there is a difference in the stability of that carbon/mercury bond, and I'm hoping that when Mr. Lucier presents later, talking about ethyl and methylmercury that he will be striking on that. It also should be noted that the ethylmercury compounds, particularly Thimerosal, will also undergo this biotransformation of organic to inorganic in human tissues, and that was described in a report by Suzuki in 1971.

As I mentioned, the kidney had the highest concentration, and you can see we've got over 3000 nanograms. These are the mean values that were presented in this article—and that the predominant form that was present within the kidney tissue was inorganic. And as you go through, you can see that from the kidney, as you move down, there is a quite a disparity between the average values that were present in the brain in terms of inorganic mercury and what was present in the major excretory organ and very little present in the blood.

Again, there was no evidence of toxicity seen clinically or evidenced morphologically that the presence of this mercury was causing any deleterious effect on these animals.

One thing that was brought out in this article is they mentioned that a critical brain level of mercury range from 3 to 9 micrograms per gram in the brain to cause toxic effects. What should be noted is that even though there were differences among all these animals, the highest level in the high-dose animals was only 245 nanograms per gram in the brain and 73.1% of that was organic. Now, what this article did not provide us was elimination data. We do not know how rapidly the mercury that was within the animals was removed. However, one could extrapolate that since this is present primarily in an inorganic form that it would likely follow the types of kinetics that have been described experimentally for inorganic mercury. There was an abstract presented at the 1998 Society of Toxicology meeting looking at a population pharmacokinetic study following mercury vapor exposure in humans that determined that the half-life in the kidney compartment was roughly nine days. So if you start thinking of the amount that is given as part of a preservative relative to the accumulation that was seen over six months daily administration in this study, there may be some disparities in terms of toxicity relevance from what we know in the animal studies. And one of the differences between methyl and ethylmercury, if this—and also the inorganic mercury— is present inorganic form, it should be eliminated more rapidly than what's known for methylmercury. It's known that the inorganic forms are removed more rapidly than methyl. Also with inorganic, about 50% of the material is eliminated in the feces without enterohepatic circulation which known for the methyl form.

In summary, I'd just like to say that the animal studies that have been conducted, even though they are very limited, have

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looked at doses that are greater to or equal than what's present in preservatives. What we did find in terms of the acute lethal dose is that there seems to be some correlation between the one human study—or one human case report that I uncovered and what the animal studies indicate and that the presentation does look very much like what's been described in the literature for the mercuric chloride studies and that renal toxicity is the primary alteration and this occurred only at high doses in all of these animal studies. This particular change may also be consistent with the kidney being the primary organ of accumulation that was seen in this study by Blair. It should also be noted that at no time in any of these animal studies that have been described was there any evidence of neurotoxicity or morphologic alterations anywhere within the brain.

This is a very exquisite dermal irritant and allergen and as I went through the literature, I found a plethora of reports on allergic reactions and this is a very important issue in its own right, not to downplay anything relative to the accumulation of mercury, but the mercury itself is present within blood and tissues and generally as an inorganic. From that standpoint, its particular relevance in terms of cumulative effects and, again, its tissue distribution, I hope are considered as part of the toxicity information when you're deliberating how to look at alternatives and really what the toxicity issues are with Thimerosal.

So that's the end of what I have. Again, it's over old, very limited, and in difficult-to-find places, and I thank of our archivists for having some of these older articles around. If it weren't for them, I probably would not have uncovered some of this information.

DR. GREENBERG: Thank you, Dr. Englhardt.

(APPLAUSE)

Well, working with little data hasn't hurt most of you in the past.

DR. KIM: Dr. Kim, from Los Angeles.

You provided data, I think, primarily in adults. Are there any data available in either experimental animals and inputing rodents and monkeys, primarily looking to the tissue distribution and metabolism in babies, neonates?

DR. ENGLHARDT: No, there's no neonatal data that I've been able to uncover. The last article for an animal study that I found was that 1976 article by Blair. I have not been able to uncover anything in terms of new studies that have been published. We did have one unpublished report on the teratology study, but nothing in terms of postnatal development or exposure in the neonate.

DR. KIM: It seems you indicated that mercury compound crosses the blood/brain barrier and the placenta barrier. I guess at this juncture it is unknown whether the exposure of a single dose or chronic doses may have a deleterious effects on the neurodevelopmental aspects?

DR. ENGLHARDT: That's correct. That's one of the gaps that I identified, the lack of the postnatal development study. That's typically where we would pick these things up. You expose the fetus as you would in the teratology study but allow the delivery to take place and then do the behavioral assessments postnatally. And no data relative to that was present in any of the literature packs. Again, that would get after your question.

UNIDENTIFIED SPEAKER: (inaudible) and Disease Registry.

Is there any data to show how rapidly the ethylmercury that's broken through (inaudible) the Thimerosal?

DR. ENGLHARDT: I did not see any kinetic data other than this biotransformation will occur, not only in circulation but also in tissues. The report by Suzuki was cited in an article by Dr. Clarkson and the original article was in Japanese and I was unable to understand that, but I believe that kinetics were discussed because there were x/vebo (phonetic; in vivo) studies that were also cited. Unfortunately, I can't give you a kinetic number for that. All we know is that there is conversion, but how rapidly that occurs, we don't know.

DR. KILBOURNE: I'm sorry. My name is Ed Kilbourne from NC—from CDC, NCEH.

The acute toxicity studies that you showed, were those LD 50's?

DR. ENGLHARDT: Yeah, those are LD 50 or MLD's.

DR. KILBOURNE: And I'm sorry, but I didn't get the units of the organ-specific concentrations that you showed later on.

DR. ENGLHARDT: Those are nanogram per gram.

DR. KILBOURNE: Okay. Thank you.

DR. ENGLHARDT: So even much less than what was presented earlier from the Faroe Islands study because those were all microgram per gram concentrations.

UNIDENTIFIED SPEAKER: (Inaudible) Is there any evidence or is there anything known whether the compound, the ethylmercury, is covalently bound to proteins?

DR. ENGLHARDT: There is nothing on covalent binding to proteins. We do know that the mercuric ion will react with subhydrol [sic; sulfhydrol] groups. So if you figure the number of cistines [sic; cysteines] that may be present in any given protein, you can have oxidation of that subhydral [sic; sulfhydrol], reading [sic; leading] to a denaturative event, but there's nothing that says that there is covalent binding to that particular protein. Even some of the in vitro studies haven't addressed that question.

DR. GREENBERG: Anymore questions?

(NO RESPONSE WAS HEARD)

The last speaker of the morning is Dr. Leslie Ball, who is the Medical Officer at the Center for Biologics Evaluation, FDA, and she is going to talk on *Thimerosal in Vaccines*.

DR. BALL: I would like to thank Dr. Myers and the other organizers for the opportunity to discuss the findings of our review on the use of Thimerosal in vaccines. Specifically, what I will be reviewing today is the FDA safety assessment of Thimerosal in vaccines. We concentrated our review on vaccines that are used in infants because this is population that is receiving the largest dose of Thimerosal per kilogram and, because the developing brain of infants, may be affected by a mercury-containing compound, including preservatives.

I think much of this has already been covered. We all know that Thimerosal is the most widely used preservative in vaccines. It's present in over 30 licensed U.S. vaccines, in concentrations of 0.003% to 0 .01%. And in the recently collated call-for-data from manufacturers, the manufacturers reported a total of 32 licensed vaccines that contained Thimerosal. It's important to note that list contains products that are currently licensed and in production and distribution. And we know that there are a great deal more vaccines that are no longer in production and distribution but have been licensed with Thimerosal.

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As Dr. Zune mentioned earlier this morning, the FDA has been examining the uses of mercury-containing compounds, specifically intentionally introduced mercury into food and drugs, as a result of the FDA Modernization Act of 1997. This act had three components. The first was to provide Congress with a list and analysis of the food and drugs containing mercury. This is the only component of the FDAMA that had a statutory deadline. The statutory deadline was two years from the date of enactment, or November 18th, 1999. Under this provision, the FDA issued two call-for-data in the Federal Register that was directed at vaccine manufacturers, and this was a voluntary call for information. The first one was published in December of 1998 and the second was published in April of 1999. The latter had a due date of June 1st, 1999. The other two components consisted of the effect of mercury in nasal sprays and, finally, for the FDA to study or contract with the Institute of Medicine to study the health effects of mercury in food and drugs, specifically the adverse effects on the health of children or other sensitive populations. And it was with this latter caveat in mind that we undertook our review. In terms of the relevance of this, well, you know, it's been mentioned that there's been an increase in the number of vaccines recommended for routine use in infants, and there's a potential increase for exposure of infants to mercury in the form of ethylmercury from Thimerosal.

One thing I want to emphasize, you know, I think we've all heard about the lack of data both in humans and in animals regarding Thimerosal. But one thing that we kept in mind is that the absence of data of a harmful effect for a low-level exposure of infants to ethylmercury is not the same as data demonstrating the safety of Thimerosal, particularly the type of effect that we're likely to observe. It's not likely to be clinical toxicity, it may not even be pathological toxicity, but it may be cognitive effects that we are concerned with, such as observed with methylmercury.

I put this slide up to remind us that life was simpler not too long ago. This schedule was taken from the 1988 Red Book—this was when I was a pediatric resident—and it demonstrated that during the first six months of life, infants only received five vaccines and only three of which, the DTP, contained Thimerosal. The HIB vaccine here at this time was recommended at the eighteen-month visit. This slide was adapted from the 1999 ACIP, AAP, and AAFP Routine Childhood Immunization Schedule. As you can see, we have several new vaccines in the infants' schedule, including hepatitis B and HIB vaccine during the first six months of life. Also note the bars here for some of the vaccines that denote the inherent flexibility in when a vaccine can be administered according to the schedule. Depending on the particular brand of vaccine, as well as the schedule that is used, an infant may receive as many as nine vaccines during the first six months of life that contain Thimerosal. I think these—Thimerosal human toxicity has been reviewed in performing our safety assessment review the published literature on the toxicity of Thimerosal, and as I stated, there have been three toxicities identified. Sensitization reaction, specifically delayed type hypersensitivity reactions were described in multiple reports after doses that are found in vaccines. It's important to note that the latter two, neurotoxicity and nephrotoxicity have only been observed in very high doses and also with regard to inadvertent overexposure of Thimerosal. I've put together a summary list of the reports that we had, references for acute toxicity other than a sensitization reactions.

The first report that I could find, well, was really just a summary report, 1941, where it looked at the therapy of bacterial endocarditis, and it reported four cases, one of which had mercury poisoning on autopsy. It was not otherwise specified how that was determined, or where, and which organs were determined.

Secondly, there's a report by Axton in 1972 with chloramphenicol that inadvertently had 1,000 times the dose of Thimerosal added as a preservative.

The next case was 1977, where Fagan reported treatment of omphaloceles in neonates that received this. This is an abdominal wall defect, and they had this Thimerosal coated on, and the infants—this was prompted on the basis of a sudden death of one of the infants, and they went back and reviewed the cases. This is a hospital for sick children in Toronto. And that out of the ten of those died, nine of them had autopsy results, and there were mercury levels in the blood, liver, brain, and kidneys that were established in those cases. However, I would also note that similar to as has been described with the previous animal data, is that pathological changes were not demonstrated.

With regard to Matheson, in 1980, reported a case of—and this may be what Dr. Engler was referring to—gamma globulin, accumulative dose. Rohyans in 1984 reported the use of Thimerosal irrigation of the external ear with tympanotomy tubes. And Lowell, in 1996, reported the use of intravenous HBIG, off label, after a liver transplant, and the final citation was the report that was previously mentioned in the Pfab, 1996, of the Thimerosal suicide attempt, 83 mg/kg was ingested. This patient did survive, but the patient did have some C and S effects that was observed at time that he was maximally ill, as well as developing polyneuropathy and respiratory failure. And to summarize these studies, some of the effects that were seen were local necrosis, acute hemolysis, disseminated intravascularcoagulation, acute renal tubular necrosis, obtundation, coma, and death.

It's also important to note that we found no evidence of data on Thimerosal toxicities at the doses found in vaccines in the published literature. We queried the VAERS database for reports of adverse events attributed to Thimerosal. We found 45 reports from the more than 90,000 total reports that were submitted between 1990 and 1998. It's important to remember that here you see that most of the reports involve local hypersensitivity reactions. The most common vaccine that was identified was hepatitis B. And it's important to realize the limitations of this data. Causality cannot be inferred both because of the passive nature of VAERS and the many antigens present in vaccines in addition to Thimerosal. Because of this lack of data on low-dose Thimerosal toxicity, we made the conservative assumption, and perhaps controversial assumption as we'll hear and we've heard already, that ethylmercury toxicity was analogous to methylmercury toxicity. Since Thimerosal is metabolized to ethylmercury, we looked for evidence of chronic effects of methylmercury to identify risks from chronic low exposure to Thimerosal. Obviously, this assumption will be the point of the next session and the discussion in much of this workshop.

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Based on two types of exposure, the first was poisoning in the Minamata Bay in Japan and, secondly, Iraq pesticide contamination with methylmercury. And the second came from population-based studies, looking at populations eating ethylmercury-contaminated fish in the daily diet, such as the Seychelle and the Faroe Islands. We concluded that one of the possible risks of low-dose Thimerosal exposure may be developmental delay.

On the basis of the studies that I mentioned with regard to methylmercury, several organizations have set safe limits for exposure from methylmercury, primarily from the diet, and these have all been alluded to. EPA has set a limit of 0.1 microgram per kilogram per day; ATSDR has set at 0.3 micrograms per kilogram per day, with the FDA at 0.4 micrograms per 1 kilogram per day. And I think one thing that we noted when we did the review was that the EPA report – sent a report to Congress that was submitted in December of 1997, only made a very tangential reference to mercury in vaccines, and the mercury toxicological profile that was published by the ATSDR also did not look extensively at the issue of ethylmercury from Thimerosal and vaccines. And I think we'll hear in great detail the caveats that must be mentioned when using this kind of analogy.

First, as we mentioned, the assumption was that the methylmercury toxicity is the same as ethylmercury, and this will be discussed and debated. Secondly, we did not take into consideration differences in pharmacokinetics, such as the root of administration. Methylmercury is ingested orally on a usually low-level basis; whereas, the root of administration for Thimerosal is intramuscular, kind of in a bolus-type exposure. Also, there is, as I mentioned, differences in daily schedule and the magnitude of doses and the possible differences in elimination, and we've already heard about some of those differences.

So next what we looked at was what the exposure of infants to methylmercury is from the U.S. Recommended Vaccination Schedule and how it compares to suggested limits for safe intake of methylmercury. As I mentioned, this is the final concentration of Thimerosal in vaccines. If it's present in multi-dose vials, it's often but not always present in single-dose vials. One example of this is Hib vaccine. And as we have heard, Thimerosal is 49.5 mercury by weight in the form of ethylmercury. An example of the calculation of the amount of Thimerosal—I'm sorry, the amount of mercury—can be done this way. Hepatitis B vaccine is 0.005% Thimerosal and is added in the final concentration. It's 15 micrograms of Thimerosal per 1 ml, or 25 micrograms of Thimerosal per half a mL, which would translate into 12.5 micrograms of mercury for a half-a-ml dose. These are the U.S. licensed vaccines containing Thimerosal. We've all seen this in the AAP interim report. There are additional vaccines that contain Thimerosal, I think as was pointed out. Influenza, all of the vaccines contain Thimerosal. In addition, there is one pneumococcal vaccine that contains Thimerosal and one that does not. This list is a list of Thimerosal-free U.S. licensed vaccines that are given routinely in infants and children. This is not an exhaustive list. Obviously, there are more vaccines that do not contain Thimerosal. But you can see DTaP, there is one. Hib, several preparations. There's a combination Hib/hepatitis B. Then there are these additional vaccines. There are no U.S. licensed Thimerosal-free products for these vaccines.

So next what we did was, we calculated the maximum of exposure of Thimerosal from vaccines and infants less than or equal to six months of age. And at six months, according to the recommended schedule, an infant may receive three DTaP vaccines, three Hib vaccines, three hepatitis B if it's given on the schedule in which the last dose is at six months, and in selected populations, influenza vaccine may be given. I didn't include this in the final calculation except in the bracketed form. But as you can see, the total amount—the total maximum exposure from the U.S. schedule would be 187.5 micrograms. My apology to Dr. Bernier in advance for this slide. I think that this can be misinterpreted and over interpreted, but I just wanted to say that the reason why we preformed this exercise is because of the lack of data that we had. And what we did here is, we used the suggested limits for safe intake for methylmercury from the EPA, ATSDR, and FDA that was previously shown, and it calculated the amount of methylmercury for safe intake during the first six months, or first 26 weeks, to look at what the maximal exposure would be in that six months.

And we calculated this for the 5th, 50th, and 95th percentile for female infants, which provides the most conservative estimated limit of intake. As described by these box figures, only EPA guidelines were exceeded using the assumptions listed here. Since these calculations are hypothetical, we looked to find data that mercury levels can be increased at vaccination. This study was found in an abstract in *Clinical Toxicology* last year. A manuscript based on these data has recently been accepted for publication by *General Pediatrics*. This was done at Emory, and I think Dr. Plotkin had already mentioned this, but they looked at 15 pre-term infants. Mean weight was at 748 grams for those infants and five term infants with a mean weight of 3.5 kilograms. These infants received hepatitis B within the first 48 hours of life, as was the practice for all preterm infants in that hospital even though that did not agree with the AAP recommendations. Of note here, as was previously noted, was an increase in mercury levels seen post-vaccination when compared with pre-vaccination, and this change was more noticeable in the pre-term infants. And I think that there can be problems with the methodology of this study, but I think the change here is what is salient.

And we put up this slide to show that there is a minimum exposure of mercury from vaccines given to infants in the U.S. schedule. For instance, less than six months, there can be a total of zero given if you utilize this certain schedule with certain products. Of course, infants with hepatitis B surface-antigen positive mothers or mothers of unknown status would still receive hepatitis B at birth. In conclusion, we found that published reports of Thimerosal toxicity in the form of local hypersensitivity reaction at the doses found in vaccines, that there was evidence of acute nephrotoxicity and neurotoxicity at very high doses. Thimerosal as a preservative in vaccines given in the first six months of life may result in the intake of ethylmercury that exceeds the EPA safe limits of intake for methylmercury, recognizing all the caveats that were previously stated. And, finally, infant exposure to mercury from vaccines may be avoidable by the use of Thimerosal-free products. And I wanted to acknowledge the contributions of Dr. Bolger from Center for

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Food Safety, Dr. Baylor, and Dr. Goldenthal, as well as the other participants in this review, Dr. Ball and Dr. Pratt.
(APPLAUSE)
DR. GREENBERG: Thank you, Dr. Ball. We have some time for some questions. Dr. Plotkin?
DR. PLOTKIN: Yeah. I have a question concerning the calculation, just so that I can understand it. If, let's say, for the 50th percentile, the EPA, you came up with a figure of 95 micrograms. That's based on exposure—I assume that's based on 0.1 micrograms per kilogram per day. Is that correct?
DR. BALL: I'm sorry. Are you talking about the number that we had on the charts?
DR. PLOTKIN: Yes.
DR. BALL: That is based on the -- For each of them we did -- for EPA, ATSDR, and --
DR. PLOTKIN: Yes. And so in the EPA case, it would be 0.1 microgram per kilogram per day?
DR. BALL: Uh-huh (affirmative).
DR. PLOTKIN: And that's based on how many days?
DR. BALL: It's 26 weeks of life, six months.
DR. PLOTKIN: Six months. And the number of vaccines, then, up to the six-month visit were calculated?
DR. BALL: Right.
DR. PLOTKIN: Is that right?
DR. BALL: Right. And that is assuming that at the six-month visit, you know, with the maximum exposure, that they would have received all of the Thimerosal-containing vaccines at that visit.
DR. PLOTKIN: My question basically is: Would it be, in your view, more or less logical to use seven months as the figure, considering that the six-month dose has to be observed, et cetera?
DR. BALL: That's a good point. I think there – that Dr. Bernier and I have had this discussion, and, you know, I think that without getting into the details, seventh-month may be very appropriate, but we were using a maximal exposure, given the fact that infants may receive those vaccines at the six-month visit. I think the main point is that—and I don't have the slide there—is that for both Dr. Bernier's calculations, as well as mine, only the EPA guideline was exceeded, not the others.
DR. GREENBERG: Can I ask for just a clarification for me? Presumably what, Stan, you were getting at is that there's a blip of exceeding at six months, but if you charted month 1, 2, 3, 4, 5, 6, 7, 8, 9, you would only see exceeding the EPA guideline at the six-month calculation, the seventh-month would then be below again, or do we know that?
DR. PLOTKIN: Since it's a multiplication of micrograms per kilogram per day, if you use seven months --
DR. GREENBERG: You have more days.
DR. PLOTKIN: Right, there are more days.
DR. GREENBERG: Well, then if you use eight months, you have more days --
DR. PLOTKIN: Agreed, agreed.
DR. GREENBERG: So what I'm asking is, has somebody calculated this with a graph with each—you know, for each day for a year, and say on how many days of a year you're in excess of EPA guidelines?
DR. BALL: There has been that calculation, and if I can get it, I'll pull it up, but—I don't want to—You know, I hesitate showing—Dr. Barry Rumak (phonetic) did a pharmacokinetic-kind of evaluation. However, you know, he's not here to explain the calculations that were done, but I don't know if this can be projected. Is there a possibility for projecting this?
DR. GREENBERG: Is there somebody back there? Yeah. Thank you.
DR. BALL: This is, you know, a representation of the hypothetical cumulative mercury body burden from vaccines in the first six months of life and looking at the kinetics of it. And, again, this is hypothetical because there aren't good data on elimination, but this is the EPA standard and this is the ATSDR standard . . . if that helps you. I'm sorry, I'm sorry. I reversed that. EPA, ATSDR. If that helps graphically . . .
DR. CLARK: Mr. Chairman?
DR. GREENBERG: Can we have the lights back on? Thank you.
DR. CLARKSON: I'm Tom Clarkson from Rochester.

I talked with Dr. Barrett about his calculations. Do you mind if I just show a transparency? I've done some similar calculations on this topic. Do you have time?
DR. GREENBERG: Sure, if you can move quickly.
(LAUGHTER)
DR. CLARKSON: This is very similar to what's been talked about as to how frequently these infants get the Thimerosal. The assumption is, from my colleague from FDA, that there's a vaccine at birth where they get about 12.5 micrograms. There's a vaccine at two months where they get 62.5, one at four months where they get about 50, and one about six months where they get about 62. I'm indebted to Dr. Halsey, I think, for some of these numbers here.

A calculation based on distribution in the body, with about 5% of the dose—this is using methylmercury statistics, not ethylmercury—with about 5% of the dose going to the body burden and about—the blood volume, which Dr. Halsey gave me, of 8.5% bodyweight, you get blood numbers like this, that there is this saw-tooth effect of a sharp rise, as you might imagine, after each vaccination, and sort of gradually rising to levels of doing 20 and 25 parts per billion in blood. The two lines, one is for the very low bodyweight infants, three standard deviations below the normal, and the other is for the 95 percentile and that's – A key calculation in this is whether or not any excretion took place during this six-month period. There is no information on that with regard to humans. There is information with animals which suggests that they do not excrete methylmercury or inorganic mercury during the suckling period, and this is one of the big questions we have for humans, whether any excretion took place. Here the calculation, just assume there was a dilution due to the growth of the baby, an increase in the volume of distribution of mercury. These levels of 20 parts per billion are about the WHO upper safe limits for the general population. For EPA guidelines, they will be higher than this. I think the EPA guideline would give a blood level of about 5 or 5 parts per billion. So it depends which agency's point of view you take. The toxic effects of ethylmercury on growing infants, as has been pointed out, is unknown, but with methylmercury effects have not been seen in populations at 20 or 25 parts per billion, but may have been seen at levels as low as 40. Thank you.
DR. GREENBERG: Thank you. Do we have other questions?

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DR. GERBER: Michael Gerber, NIAID.

Let's see, I'm a little bit confused about your description of that report from Toronto and the neonates who died after the Thimerosal exposure. You said on postmortem exam there was no pathological evidence of acute mercury toxicity. Did the authors believe that the mercury was the cause of death, or was there some other cause of death?

DR. BALL: It was not mentioned. The index case was one case that died suddenly, and they must have had some reason to examine mercury, because then they looked the previous 13 infants who had omphaloceles treated with Thimerosal, and they came up with nine of them who had necropsies and got tissue mercury levels on those infants.

DR. GREENBERG: Dixie?

DR. SNIDER: Dixie Snider, CDC.

Leslie, a very simple question: In the tables and the graphs I was looking at, I'm not clear on what's being compared. As I recall your calculations—but the micrograms you were coming up with were—in Thimerosal— were micrograms of mercury.

DR. BALL: Exactly.

DR. SNIDER: The EPA, ATSDR, FDA limits, are they methylmercury?

DR. BALL: Methylmercury.

DR. SNIDER: So you're comparing mercury to methylmercury.

DR. BALL: Well, from Thimerosal, it's ethylmercury.

DR. SNIDER: Since it's most --

DR. BALL: Right.

DR. SNIDER: But your calculations were actual micrograms of mercury?

DR. BALL: It's in the form of ethylmercury.

DR. SNIDER: So are you comparing ethylmercury to methylmercury or --

DR. BALL: Yes.

DR. SNIDER: -- ethylmercury to methylmercury?

DR. BALL: Ethylmercury to methylmercury.

DR. SNIDER: In micrograms?

DR. BALL: In micrograms.

DR. SNIDER: Okay. So, ideally, you would do moles --

DR. BALL: Right.

DR. SNIDER: -- but since there's not much molecular weight difference, it's going to be close.

DR. MAHAFFEY: Kate Mahaffey, U.S. EPA.

The references for methylmercury is set assuming there's not a lot of exposure to other sources of mercury. Are the infants exposed to additional sources besides the vaccines? Because we know that those that are breast fed, at least, have an ongoing exposure to mercury from their mothers.

DR. BALL: Yeah, that's an excellent point. In the calculations, we were assuming no other exposures. And, in fact, infants are exposed to mercury from other sources, even infants that aren't eating tuna fish sandwiches, but maybe getting exposed through the breast milk, or, prenatally, have mercury levels, as you saw in the abstract, probably also related to either ingestion of fish in the mother or from dental amalgams.

DR. MAHAFFEY: And is there any effort to look at these additional sources of mercury and incorporate them in the cumulative exposure to mercury that you've described from the vaccine?

DR. BALL: You know, there weren't any references that I was aware of that had good data on the alternative exposures. So I think that would require an effort with the various agencies that do have expertise in looking at those other exposures.

DR. GERBER: Gerber, NIAID.

I just have a question for Dr. Clarkson. When you were talking, you were talking in terms of parts per billion, but your "Y" axis was in micrograms per liter. Are you just assuming those are same thing?

DR. CLARKSON: That's the same, yes. Right.

DR. ROGAN: I'm Walter Rogan from NI Environmental Health Sciences.

As Dr. Plotkin pointed out, the choice of the denominator for time is kind of arbitrary and scientifically, I guess, it would depend on your model for how you think toxicity is occurring. And although I think it could be argued that toxicity is directly related to cumulative exposure, I think that for this class of compound that, you could also make an argument that toxicity is related to peak excursion. So just an argument, it could be made to go in the direction of seven months, or eight months, or nine months. The argument could be made to go in the direction of one day and how high you got on the day of vaccination. So the sixth-month is not a maximum in terms of consideration of toxicity. It's just sort of an intermediate level that they, you know, chose to display.

UNIDENTIFIED SPEAKER: Dr. (inaudible) from CBER.

Just a point of clarification. The EPA numbers are in micrograms per kilograms per day?

DR. BALL: Correct.

UNIDENTIFIED SPEAKER: And in your calculations, I'm not clear on how you looked at the bodyweight of the babies.

DR. BALL: Ours were in total micrograms. And they were total micrograms, and then when we did the calculations, we used the weights for the 5th percentile, 50th percentile, 95th percentile. So we took into consideration the weight of the infant.

UNIDENTIFIED SPEAKER: So one of the percentiles was about 400 micrograms. That was micrograms per kilogram bodyweight?

DR. BALL: That was the maximum -- Are you talking about with the guidelines, the graph on the guidelines?

UNIDENTIFIED SPEAKER: Yes.

DR. BALL: Those calculations were based on the total safe intake that you would calculate for that weight of infants. So if it was, for example, 5th percentile infants, you would use that weight to reach that total maximum level, using the analogous EPA, ATSDR, or FDA standards or guidelines.

UNIDENTIFIED SPEAKER: That wasn't clear in the presentation. Thank you.

DR. GREENBERG: We have a minute left for a quick question.

DR. MYERS: Martin Myers, NVPO.

Leslie, just in your review, what proportion of vaccines in the first six months are actually distributed in multi-dose vials?

DR. BALL: I think that CDC has those data and will be presenting those this afternoon.

DR. GREENBERG: We now have thirty seconds for one more question. Last question.

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DR. FISHER: Yes. Barbara Lowe Fisher with the National Vaccine Information Center.

I'd just like to congratulate the FDA on performing this analysis and for taking the action that it did to ask the manufacturers to take Thimerosal out of the vaccines. I think that the public expects a strong and effective FDA, and that this kind of action, where it may temporarily cause questions about vaccine safety, in the long run, it's going to instill confidence and trust in vaccines and in the system.

I have one question. On your total of 187.5 for the vaccines in the first six months that are given, you used DTaP, three doses for DTaP for American infants. What would the total be if DPT were used, because some infants are still getting DPT?

DR. BALL: It's the same.

DR. FISHER: The same thing?

DR. BALL: The same amount.

DR. GREENBERG: Okay. On that note, I'll call the meeting to an end. I'd like to thank all the speakers who did a great job. Now, you have one hour for lunch, so you have to be back here at 1:00.

(LUNCH RECESS FROM 12:00 NOON TO 1:04 P.M.)

DR. GREENBERG: Well, this afternoon we're moving onto a couple of other important areas, and the first is going to be the organomercurials, and we have two substantial talks. The first is by Dr. George Lucier, who is the Director of the Environmental Toxicology Program at the NIH, and he's going to talk to us about *Ethyl and Methylmercury: Pharmacokinetics and Toxicology*.

DR. LUCIER: Thank you. I think. Actually, this invitation to speak here was accepted by my office staff when I was vacationing and camping in the Adirondacks and not accessible to any phone. So Martin coerced my office staff into me accepting this, but I'm glad they did. I think it's an appropriate activity for me to participate in. I believe the reason that I was asked to give this presentation is that beginning in 1997—I should point out, first of all, that I'm not a mercury researcher, although I did have a couple of papers back in the early 1970s. I have a research group, but it's in receptor-mediated talks against dioxins and estrogens and so forth. But my involvement with methylmercury emerged in 1997 when I was asked to chair an interagency review of EPA's report to Congress, which, of course, was due in the end of 1997. I was assured that this activity would only last two months. But while this was going on, ATSDR released a draft toxic profile. Phillipe Grandjean published his papers in neurobehavioral changes observed in the Faroe Island children exposed prenatally to methylmercury, and a number of other activities emerged that really called for attempts to harmonize across federal agencies what the science was telling us and what it wasn't telling us regarding methylmercury, particularly as it relates to developmental neurotoxicology.

These activities led to a workshop that we had in North Carolina in 1998, the fall of 1998, about 8 or 9 months ago. In that, we addressed in a very rigorous way the major studies that had been used in health assessments for methylmercury toxicity. We had remarkable cooperation from the interagency committee, including EPA, ATSDR, FDA, NOAA, the relevant parts of CDC, and other agencies as well and equally remarkable cooperation from the major investigators who's studies we were reviewing. Tom Clarkson, who's here, and showed one of his overheads this morning, which I thought was particularly insightful, as well as Phillipe Grandjean and Donna Merguler, who is conducting some studies in the Amazon.

That's my name and where I'm from.

My presentation will be, in a sense, two parts. And the first part is a summary of the interagency activities that we've had regarding methylmercury, particularly the areas of agreement and the findings that emerged out of our workshop in 1998. And the second is what we know, and that's written very small, it probably should be written smaller, and don't know about ethylmercury. That'll be a shorter part of the presentation because, as you heard this morning from a number of the speakers, there just isn't too much information out there on ethylmercury. I'll discuss a few issues that perhaps weren't presented this morning.

The purpose of the workshop was to discuss and evaluate the major studies, epidemiologic studies, associating methylmercury exposure with an array of developmental measures in children. It was in response to the requirement that the emerging data from the Seychelles and Faroe Islands undergo a level of scrutiny beyond journal peer review if they are to be used in policy setting.

So, keep in mind, this was an extraordinary rigorous review in such a way that I think is rarely done in terms of individual papers. This workshop involved presentations by the groups who were conducting the studies, really a barrage of questions about what they did, how they did it, how they analyzed the data, information that really isn't found in the published literature, and can't be found, because the journals would never allow publication of that volume of information.

This was really done under the impetus of the White House Science Office, the Office of Science Technology Policy. Fran Sharples (phonetic) there was the point person. It involved a number of different agencies shown here. I hope you can read it okay. A number of institutes, agencies within DHHS; the NIEHS, which is where I'm from, Bill Raub's Office of the Assistant Secretary for Planning and Evaluation, and, of course, he'll give the next presentation and share the panel discussion; parts of CDC; ATSDR; FDA; again EPA; NOAA; OSTP; and also the Office of Management and Budget who was involved in this. So you should keep in mind, as I go through what I'm going to say, in terms of the points that I make, they're really not my points. It's really the points of this interagency activity that basically was approved by all these various agencies and, in a sense, also approved by the major investigators whose studies we were reviewing, and generated by the reports, subreports, that were prepared by each of the panels, and I'll get to those later.

First of all, a number of key issues that we kept in mind as we went through the interagency deliberations. I think it's important to point out here that we hear a lot about interagency differences, particularly in regards to the methylmercury issue. It is clear that we do differ. Agencies do differ in some respects, but there are much more areas of agreement than there are areas of disagreement, and let me go through some of these issues that we are cognizant of before the workshop began.

One, methylmercury is a developmental neurotoxin in people. There's multiple publications, from Minamata, Iraq, and

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others to document that. The developing fetus is roughly ten times more sensitive than adults. This is a rough estimate, but probably not too bad of one. I think Tom Clarkson made that original estimate, and from my read of literature it can't be too far off. The relative sensitivity of infants to methylmercury is unknown, but they are likely more sensitive than adults. We really don't have information in infants. We have to keep in mind that the central nervous system and the brain is still undergoing assembly and it's likely it would be sensitive to toxic insult, but we really have very little information, nothing near the extent that we have for prenatal exposures of the developing fetus and also for adults. We just don't have much for infants.

Effects: This is a no-brainer. Effects at low-level exposures are difficult to evaluate. Methylmercury is ubiquitous and nearly everyone has some exposure. Kate Mahaffey brought that point up in the question and answer to the last presentation, that virtually everyone in this room has some degree of methylmercury in their bodies. So any additional exposure that's received—and infants have some as well through lactational exposures and other sources. Anything we receive is really an incremental exposure to what's already there. So we need to be especially cognizant of the issues related to cumulative health assessments from the multiple sources of methylmercury, mercury in vaccines only being one of them.

Finally, initial efforts to establish safe exposure levels acknowledge the need for further studies in populations with low levels of exposures. And that's really what led, back in the 1990s to early 1990s, funding for the studies in the Seychelles and the Faroe Islands, because of a need to have this information after seeing that the developing fetus was really at risk based on the data from Minamata and also from Iraq.

The workshop that we had was structured around the deliberations of five panels, and these are five panels that were basically external to the federal government. I think of the 27 panelists that we had, I think there were only two representatives from the federal government on them. Walter Rogan from the NIHS was one of them, and he's here today and could perhaps help me answer some questions regarding the neurobehaviorial endpoints. But these are the areas that we felt that needed to be addressed in a critical rigorous way regarding those major studies: exposure, neurobehavioral endpoint, confounders and variables, design and statistics, and we also had a group looking at experimental studies, studies in rodents, studies in monkeys, to see whether or not the experimental models gave results similar to what we were seeing in people. If that's the case, then it gives us more confidence in using those experimental studies in public health assessments.

Major studies that we looked at was Iraq, where the consumption of bread prepared from wheat seed treated with methylmercury fungicides; the Seychelles, the consumption of fish as a significant source of dietary protein; and the Faroe Islands, where the consumption of pilot whale meat which contains higher levels of methylmercury than local fish. I'll get back to the importance of some of the consumption habits in a minute or two.

These are the outcomes, and I hope you can read that okay. I recognize that it's somewhat small. In Iraq, affected individuals consume 50 to 400 milligrams of methylmercury over six months. Motor retardation was seen in infants born of mothers with hair levels in the to part per million range. Now, there were effects seen at much higher levels, obviously, but this was as low as the evaluations could get, and maybe Tom Clarkson in his comments could elaborate on that if necessary.

We really spent the bulk of the time in the Seychelles and the Faroes. In the Seychelles, infants were born of mothers with mean hair levels of 6.8 parts per million, the range of 0.5 to 27. No developmental effects were detected using standardized measures of global neurological function in children up to 66 months of age. There is also prior looks at developmental aspects, I think, at 29 months of age as well.

In the Faroe Islands, infants were born of mothers with mean maternal hair levels of 4.parts per million, very similar to what was observed in the Seychelles, in a similar range. They also had mean cord blood concentrations, and I just noticed looking at this that it's not parts per million, that it's parts per billion. So the range of 22 parts per billion, a range of 0.9 to 351. Quite a broad range. The Faroe study assessed the main specific effects, which are different than the global measures in neurological function. Test of memory, attention, and language were negatively associated with methylmercury exposure in children up to 84 months of age. So these kids were 84 months of age and 66 months of age, up to 66 months of age in the Seychelles. It's important to note that the follow-ups continue in both of these studies with Tom Clarkson's group, as well as with Phillipe Grandjean in the Faroe Islands.

Well, why is the Seychelles study negative and the Faroe study positive? That was a big question for the workshop, and I'm going to not present all the information, but I'm going to briefly go over some issues relative to exposure, study design, confounders, and data analysis that could possibly account for the differences.

In regards to exposure, we had quite a bit of discussion about cord blood versus hair levels, but I think the overriding conclusion of the panel was that hair levels are a pretty good marker of methylmercury exposure. Cord blood is a good marker as well. Each of them have their advantages and disadvantages, but there's a wealth of literature now on hair levels of methylmercury as a marker of exposure.

I was just reading in the, flying up here this morning, USA Today, and there was an article about Andrew Jackson and why he died, and some people, I guess, had theorized—I hadn't known that—that he had died of mercury poisoning. But 200 years later, nearly 200 years later, they analyzed his hair and found there's not enough mercury in Andrew Jackson's hair to account for his death. So it has to be a pretty good marker of exposure to be used 200 years later to help ascertain the cause or what was not the cause of death in the case of Andrew Jackson.

The second issue was—and this one I think was particularly important and may be relevant to the vaccine issue—exposure in the Faroes was considered to be more episodic than in the Seychelles. In the Faroes, basically, there's about 1 pilot whale meat meal consumed per month, maybe one to two fish meals consumed per week. In the Seychelles, I think it was something like 10 meals or so of fish that were consumed per week. So it was a much more spiked exposure, if you could look at it that way, in the Faroes as compared to the Seychelles. Many of the panelists in our review groups felt that this is possibly an im-

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portant factor in accounting for the differences in results between the Faroes and the Seychelles, particularly when you consider that we're looking at windows of sensitivity for the developing nervous system.

Third, exposure response relationships were based on surrogate markers and hair or blood concentrations in fetal and children's brains can only be estimated. While this is true, I think for the reasons that I've said before, I think we have a wealth of information about exposure and what it means in terms of hair levels, not that we can't get more, but I think that information was pretty good. It was not considered a major problem or a major reason by the panelists for the different results between the Faroes and the Seychelles.

Now, getting to the study design issues, there was one here actually was left off of the slide that should have been first, and that's the neurobehavioral endpoints. As I had mentioned earlier in the outcome slide, the Seychelles Islanders were monitored for more global measures of neurological function, whereas the Faroes were looked at for more domain-specific effects: memory, attention, language, these sorts of things.

Many of the panelists felt that these were like comparing apples and oranges, and I think everyone on the interagency committee and the scientists themselves agreed that they were really measuring different endpoints of neurobehavioral function. So this could very well explain the differences. It's important to note that in the follow-up studies that are being conducted, there will be great effort made to measure common endpoints in those children, who are, of course, getting older and older, and also to go through some of the same analytical processes that also exhibited some differences between the two studies in terms of analysis of the data sets.

Another one that was discussed in great detail: selection bias. This was a potential concern in the Seychelles studies because some individuals—I think 39 or something of the 79—were excluded because of debilitating conditions. Thorough analysis of that suggests that the selection bias was really not an issue in explaining the results. The panel, I think, felt almost unanimously on that issue. Effects of culture and language were discussed in terms of the questionnaires, usually going back and forth between English, Creole, and French, and Scandinavian in the Faroes study. Again, the panelists felt that this was not a major issue. The age of testing, the panelists, on the other hand, felt that this was potentially an important issue, because at 66 months of age, there's a lot more variation among normal individuals in those parameters that were assessed. In other words, there's a lot of noise in the system and it might be difficult to pick up an effect if one was present. And, again, continuing to follow up these kids at the later ages will help address that issue, but that was an area of potential importance that was earmarked by our review groups.

Order effects and effects of tests administration, as I recall, in the Seychelles study they gave the same order to each of the individuals in terms of the administration of the test. In the Faroes, I think they had four predetermined orders of how the tests were administered, and that wasn't really controlled for or dealt with in the model analyses that evaluated the results. So this was a potential issue of concern that the panelists raised regarding the Faroes data. Confounders and data analysis issues, in the case of the Faroes,

PCB exposures were also occurring. As most of you know, PCBs are also developmental neurotoxicants. They affect some of the same parameters as methylmercury effects regarding the developing nervous system. The PCBs were measured in both the Faroes and the Seychelles. There was significant PCB exposure in the Faroes, essentially none in the Seychelles. So it's a potential confounder for Faroes but not the Seychelles. The neurobehavorial endpoints subgroup of the panel said that they did not feel that the PCBs are really confounding the results that were observed, even though they could have some effect on them.

Selenium, I knew selenium was a messy issue going in, and it still is. Some people think it affects one way, other people think it affects the other way, but everyone agreed that it would be important to use that as part of the analyses of the data, and that wasn't done.

Likewise, a number of dietary nutritional factors, the omega-3 fatty acids, which are beneficial to brain development need to be looked at in subsequent studies, as well as a number of nutritional and dietary data that really weren't collected in the studies that have been published to date.

Genetic difference is potentially important. There may be ethnic differences in responsiveness, but given our lack of information about mechanism of action for developmental neurotoxicity for methylmercury, or PCBs for that matter, we're really not in a good position of pinpointing particular differences in gene activation pathways and so forth, that could possibly account for these differences.

Influence of covariants, in general, the panel felt that the Seychelles tended toward a slight overcontrolling and the Faroes a slight undercontrolling. Some particular issues that were raised were maternal smoking, which even though 40% of the women smoked in the Faroes, this was not controlled for in the analysis.

Birth weight, that was controlled for in the Seychelles study, but birth weight could be associated with a methylmercury exposure in the development effects. So, perhaps, that could have influenced the results and minimized the ability to detect an effect if it was there.

Town versus rural residence wasn't accounted for in the Faroes study. To make a few brief points about the studies in experimental animal models, basically, they were in pretty good concordance, both qualitatively and quantitatively, with what was seen in people. There have been effects of methylmercury and effects of PCBs in the sensory system, motor function, and cognitive deficits, but at this time it's not possible to differentiate the effects of PCBs and neurodevelopment from effects of methylmercury in experimental animals mostly because of the lack of mechanistic information.

We have to keep in mind that in this situation, we have a very rich data set, at least for us who do environmental kind of exposures think it's rich, and it's extraordinarily rich regarding exposure and extraordinarily rich regarding response. What we don't know is what's happening in between in terms of the critical cellular steps that may be involved in producing the neurological effects that may be seen, the migration of critical neurons and so forth, and that's an area of research that would yield great benefit to the public health assessments of both methylmercury and PCBs.

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There are five panel recommendations and findings that emerged out of the workshop, and I'll go through them one by one. Again, this was agreed upon by all the participating agencies, the panel, and also the major study groups out of the Seychelles and the Faroes.

1. Methylmercury is a developmental neurotoxin, but effects -- We still got the same sentence in here—at low does encountered by eating fish are difficult to evaluate. Not too much progress there, but certainly a strengthening of that statement.

2. All the studies reviewed were considered of high scientific quality and the panel recognized that each of the investigators had overcome significant obstacles to produce important scientific information. That was uniformly felt throughout the panels. We felt that a continued funding of these studies is necessary for the full potential to be realized. It's particularly true for the Faroes and Seychelles, which are currently assessing developmental effects of methylmercury in the fish-eating populations, of course. The developmental studies would benefit by evaluation of common endpoints using similar analytical methods.

And we noted that the Amazon study, although positive results were seen, did not look at developmental endpoints. A later study out of Grandjean's group that's just been published has looked at the Amazon studies where methylmercury exposure occurred through gold mining, and those results were positive as well in terms of visual-evoked potentials and some other measures of neurological function, following prenatal as well as post-natal exposure.

3. Results from the Faroes and Seychelles studies are credible and provide valuable insights into the potential health effects of methylmercury.

4. Some differences are clearly present in the results of the studies, but the panel was unable to clearly identify the sources of these differences. Among possible sources are the different effects of --

Again, coming back to this one—episodic versus continuous exposure, ethnic differences, a lack of common endpoints in the Faroes and Seychelles studies—a very important one, of course. And several other confounders or modifying factors such as those found in the diet, lifestyle, as well as chemicals present in seafood, which is a source of methylmercury to these populations. The other chemical constituents that may be explanatory include those that may be beneficial to fetal development, like the omega-3 fatty acids, and those that may be harmful to fetal neurodevelopment, such as the PCBs.

5. These studies have provided valuable new information on the potential health effects of methylmercury, but significant uncertainties remain because of issues related to exposure, neurobehavioral endpoints, confounders and statistics, and design.

There has been a few publications I mentioned that have come out since we've had the report, and maybe Tom Clarkson will give us an update of what's going on with his group as well in terms of recent publications. These are mostly from the Grandjean group and they involve the one shown here in terms of the Amazon study, which I mentioned; another paper from the Faroe Islands on the delayed evoked potentials in children exposed to methylmercury from seafood; a paper with Murata as the first author and Grandjean the last, evoked potentials in Faroese children prenatally exposed to methylmercury; and another one that examined hypertension, a reported increase in hypertension in the kids exposed to methylmercury, also in the Faroe Islands. This paper, I believe, now is in press. It was presented at that Rio De Janeiro meeting in May of this year.

Ethylmercury or Thiomersal? You'll notice I'm using the European spelling, because it was in the reprints I had, so I used that spelling. Now, I'll make a few points here that I think most of them have already been made, maybe some of them haven't, regarding ethylmercury and possible comparisons with methylmercury.

Exposure: Depending on the vaccination schedule and bodyweights, a two-month-old infant receives a bolus injection of 3 to 18 micrograms per kilogram. This was information I got by Bill Raub via Neal Halsey, and I assume that those calculations are correct. They seem similar to what was presented later on this morning, so I believe they're roughly correct. This dose of mercury on vaccination day is much higher than daily exposure in the Seychelles and the Faroes, although the total dose received from vaccines is less than the mean exposures in the Faroes and Seychelles.

Infant mercury intake per day from dietary sources is estimated to average 0.05 micrograms per kilogram per day in a chronic exposure, and this would be primarily through lactation as well as some other sources. And there's a few pieces of information in the scientific literature that support that estimate of infant uptake of methylmercury, exposure to methylmercury.

Biological half-life, similar to methylmercury. This is a little bit different than what was said this morning. For methylmercury, it's 40 to 150 days, and this was based on a number of different studies that have been presented. I think different agencies use slightly different numbers, but I think the average —Chris, would it be right, it's about 70, 60 or 70, in that range? The one study I got a hold of regarding Thimerosal, or ethylmercury, came from a suicide attempt. This was published three years ago actually, in *Clinical Toxicology*, and this one lived. He also got about 80 milligrams per kilogram of Thimerosal, and the half-life—and Chris (inaudible) had sent me this reprint on Friday. It was estimated that the half-life, the second phase of the half-life, which is the one we need to look at here, was roughly 40 days in this one individual who survived that episode. Of course, we don't know what a near-death experience does in terms of the physiological factors that govern half-life, so I wouldn't guarantee that that's the half-life.

The information that we have in total suggests that it might be slightly shorter than methylmercury. And there is really no definitive information on potential differences that I could uncover between infants, children, or adults regarding biological half-life. I don't know, Katie, if you have some more information on that.

Metabolism: And I think this was brought out in the presentations this morning—that demethylation of methylmercury appears to occur more slowly than deethylation of ethylmercury. I think there's a growing body of knowledge that suggests that that is, in fact, true, and it's significantly different. In other words, the demethylation occurs much more slowly than deethylation in terms of the conversion to inorganic mercury.

What about the toxicity of ethylmercury or Thimerosal? Again, we talked about the adult squirrel monkey study today, —this was adults again and not a developmental study. Again, significant conversion to inorganic mercury; high levels in the

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kidney, as was presented this morning; lower levels in the brain; and no evidence of toxicity. And the doses that were given were equivalent to 1 or 6 micrograms per kilogram per day.

A second study, which was not discussed this morning, is that adult male and female rats were administered 5 daily doses of equimolar concentrations of ethyl or methylmercury by gavage and tissue distribution, neurotoxicity, and nephrotoxicity assessed. This was a Magos study in 1985 in the *Archives of Toxicology*. And the key points of that paper were: neurotoxicity of methyl and ethylmercury were similar, although higher levels of inorganic mercury were seen in the brains of ethylmercury-treated rats consistent with what we'd said about metabolism; and likewise, because of that, the renal damage was greater in the ethylmercury treated rats. Unfortunately, neither time-course nor dose response was attempted in these studies, nor was any developmental studies attempted.

And after having said that, there are a number of critical toxicology studies that could be conducted to address some of the uncertainties and you probably all know about and we talked about this morning. Unfortunately, all of these take time and, you know, clearly, if we embarked upon these studies now, we're not going to have results until long after some of the initial and significant decisions have to be made regarding the vaccine program. I think we have to acknowledge the paucity of data and move forward with the decision-making process, but I think it's good to think about what knowledge gaps do exist that really limit our ability to make those assessments in a way that we would like to make them.

Developmental neurotoxicity, we need to assess those response and age dependent responses in appropriate systems. We need to, for the reasons I discussed earlier regarding the PCBs and methylmercury, look at mechanistic studies, and we need to focus on critical changes in gene function and cellular pathways. In all the toxicology studies we do in the national toxicology program, and we do 30 or 40 of these a year as part of that interagency program, we're starting to take increasing advantage of the human genome project and what that allows us to do in terms of looking at patterns of gene expression following exposure to various toxicants to compare potency of different agents and also mechanism of action, as one agent going through a similar mechanism of action as another agent. That might be particularly relevant to the issues at hand for the ethyl/methyl issue.

Evaluation of possible sensitive subpopulations based on either genetic predisposition, diet, or cumulative risk. Again, we're exposed to other developmental neurotoxicants. Are they additive? Are they synergistic? Are they antagonistic towards each other? Do they block each other's effect? And biomarkers of exposure, including hair, need to be evaluated. There are no studies in developmental toxicity that I was able to find in experimental models or people, and because of this, in my opinion, health assessments for ethylmercury at this time must assume that ethylmercury is producing the same effects at the same doses as methylmercury.

I couldn't help but to show a couple of slides here. One of the things that I do in my own laboratory is work with biomathematicians to develop physiologically-based pharmacokinetic models, and this is a model that might be applied to a prenatal methylmercury study. When you have various kinds of compartments in the maternal system and also the fetal system, looking at placental transfer. Of course, excretion in the maternal system, either through the urine or the feces. Blood levels, relationship to hair levels, secretion in the milk, of course, when you're looking at lactational exposure postnatally. And once you have some information regarding all these parameters, and it has to be done in an iterative way with generation of laboratory data, you can develop mathematical models that predict the movement of the chemicals throughout these various compartments. And once you can do that with your existing database, it gives you a great deal of confidence in extrapolating that model to expose your circumstances for which maybe you don't have data.

So I think these kinds of models are always very helpful in health assessments. And I know agencies such as EPA, ATSDR, and FDA use them extensively in the health assessments that they make. But in the case of the vaccine issue, we really have to look at it in terms of the infants and children issue, which we've discussed already, and I think the point has been made that we have information in adults, we have information in effects on prenatal development, and we have very little information about the relative sensitivity of infants, either to adults or to the developing fetus.

So we need to develop that type of physiological-based pharmacokinetic model, to look particularly at the issue of infants and children and how tissue concentrations might be related to the potential for adverse health effects. I also pointed out that in the case of the biologically-based modeling, this is an iterative process. You don't just get yourself a mathematician friend and say, "Do this model." They usually come up with some sort of model that is filled with flaws, and then you go back, and through additional experiments, start refining the model. So you collect the data, refine the model, compare it to the existing knowledge base. You start circling through this thing a few times. By the time you get through it a few times, you're then in a position to use it in dose response assessment and other aspects of quantitative risk assessment, but, again, these things take time. We're not going to both generate the data and generate these types of models, you know, within the next six months. It's going to take some time to do that.

And finally, I usually show this slide when I want to offend people. It's not that I want to offend anyone, but I show it when I give talks about risk assessment for environmental agents, and because we deal with a lot of different types of folks in terms of evaluating what we should do and shouldn't do in risk assessment. And these are meant to be caricatures. They certainly don't reflect anyone in this room, I'm sure. (LAUGHTER)

DR. LUCIER: But, you know, some of my favorite, of course, are molecular biologists, you know, you're stupid, I'm smart. I actually know a lot of molecular biologists that aren't smart. (LAUGHTER)

And of course you have mathematicians that think an equation like this can give us truth. And it helps, but certainly not by itself. Regulatory official, that's definitely not true in this room. I tell you, the interagency group that I worked with in this was absolutely terrific. But one caricature would be, "Don't trouble me with science." Industry, "Positive results are meaningless."

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And environmental activists, "If it's chemical, it's bad." Lawyer, do we have any lawyers here?
(LAUGHTER)

I heard a joke about lawyers the other day, that 99% of the lawyers give the other 1% a bad name.
(LAUGHTER)

And as a result of all this, frequently the public health decisions that come out of the federal government, because of these various caricatures, really aren't believed and the public doesn't trust us. So I feel very good about this workshop because, I think, as was stated in the original goals that the purpose, to get all the information out on the table, what we know and what we don't know, do it in an open context where people can comment, add to it, subtract from it, and so forth, I really think is the way to go about this. So I appreciate the invitation and the opportunity to participate. Thank you.
(APPLAUSE)

DR. GREENBERG: Thank you, George. We have some time for some questions. Too much data for you, huh? Dixie?

DR. SNIDER: Dixie Snider, CDC.

You indicated that the mechanism by which methylmercury might be exerting its neurotoxic effects is unknown. Are there any reasonable hypotheses in your mind? And how would that relate to ethylmercury and methylmercury with regard to mechanism?

DR. LUCIER: You know, there's some information available. And, again, I'm not a neurochemist or a neurotoxicologist, so maybe some of the other folks who have looked at this on the panel could add to my answer. But there have been effects shown on various constituents that are involved in their own migration and other aspects of neurodevelopment. I don't think there's anything that people would say, "Aha, I think I understand what that critical event is that's producing the toxicity."

You don't have to know all the steps that are involved, but what you really want to know is what the key critical event is or the mode of action is, and once you have that information, you're on much better footing in which to compare and predict responses that might be occurring across the chemical class.

Say, for example, it was done with the environmental estrogens or the dioxins where we knew the mode of action was receptor mediated. Let me talk about something I know something about—we're then able to take classes of chemicals and see how well they interacted with that system and produced a specter of deemed changes that are associated with it and use that information in regulatory decision-making in terms of determining which of these dioxin analogues or which of these environmental estrogens are the ones we need to be worried about. And if we had the same sort of analogy with the methylmercury and PCBs, we would be able to go much further in that type of comparison.

DR. GREENBERG: Gina, did you have a question?

DR. RABINOVICH: You stated—and I'm questioning this because I'm not sure I understand it or if anybody else in the room does also. You stated that the demethylation of methylmercury appears to occur more slowly than the deethylation of ethylmercury. Can you expand on the implications of that? Is that good or is that bad?

DR. LUCIER: Well, you know, I'd like to say I knew, but I've heard that it's good and I've heard that it's bad.
(LAUGHTER)

DR. LUCIER: I've heard that it's good because this is a detoxication step in some respect. Say, in terms of the kidney, it's a way of, you know, getting the mercury out of the body. And I've also heard—but since we don't know how methylmercury works, we're at a little bit of a loss to make too much of a definitive statement. I've heard from others that maybe it creates a mechanism for retention of mercury in the brain as the inorganic mercury does not retrograde cross the blood/brain barrier. So it's a mechanism retaining mercury in the brain.

So, I don't know. I think it's a real finding . . . and I think it's an important finding, but I don't know how to quite put it in the context of the comparative toxicity issue. I think it is important to note from the Magos study, in which he directly compared ethyl and methylmercury, that he found essentially the same results in both studies, with the exception that the renal toxicity was greater with ethyl, and I think that was because of the demethylation as a way of concentrating the mercuric chloride or inorganic mercury in the kidney.

DR. RABINOVICH: Okay.

DR. PLOTKIN: Let me try to frame this question intelligently if I can. In analyzing the Faroe Island data, which are the positive set of data, at least in thinking about microbiology, one can usually calculate a 50% dose, that is, to say a dose that caused a reproducible effect 50% of the time. Now, from my reading of the Faroe Island studies, there is no level in those studies that had a 50% effect, but there are mathematical ways of trying to predict the 50% effect.

So my question, if it is a question, is: Can you calculate from the Faroe Island study what is the 50% effective dose, either in terms of hair level or blood level of mercury?

DR. LUCIER: You know, you are in much better shape to do that when you're interpolating within your data set, rather than extrapolating outside of it. The Faroes data doesn't have adequate information within it to define a slope down in that low-dose region. Now, in the absence of that type of data, one can use various types of models to extrapolate to an EC-50 concentration using some of the parameters already looked at. Several assumptions would have to be made, but my guess is any extrapolation of that nature, because of the nature of the data set, would be highly subject to debate and criticism because of the assumptions that would have to be made. But I think the effort itself may be a worthwhile one, and then point out sort of what the uncertainties are with that estimation.

DR. HALSEY: You mentioned that we don't understand --

DR. GREENBERG: Identify yourself?

DR. HALSEY: Neal Halsey. I'm sorry.

You mentioned that we don't understand the mechanism by which the neurotoxicity occurs, and we also don't know what the relative sensitivity of the infant is, which is what we are all concerned about right now. I'm wondering if there's any information that might be applicable or might help educate us with regard to the slope of the curve for other developmental neurotoxins.

There's lead, there are others. I don't think this audience knows what those slopes look like, and whether you think they may be at least informative. You can't necessarily apply them directly to mercury, but it would help to try to get some estimate of what the relative increase in toxicity for an infant is at

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birth, at two months, as compared to at six months or at twelve months.

What is the shape of those curves of change in the neurotoxicity from other products?

DR. LUCIER: Yeah. I think that's a great point, and I'm not a neurotoxicologist again, so I don't have that information at hand. We have analyzed through the NTP a lot of chemicals in our neurotoxicology batteries. So maybe it would be worthwhile for me to go back and ask those folks to look at that particular issue and see what comes out of it.

And many of these, of course, are assumed to have threshold effects, that there will be a dose below which no effect would occur. My guess is—and this is a guess, so take it for what it is—that you'll still get a variety of dose response curves because there are multiple mechanisms of developmental neurotoxicity. I presume that some would drive it very steeply and others would drive it in a more shallow sense, but I don't know that for sure, Neal. Did you have something to add to that, Katie?

DR. MAHAFFEY: Yeah. Speaking for --

DR. GREENBERG: Identify yourself, and why don't you step up here an use the mic.

DR. MAHAFFEY: I'm Kate Mahaffey with EPA.

Looking at inorganic lead, you can get an interesting comparison because the occupational levels that are considered acceptable are more in the range of 40 and 50 micrograms per deciliter, with reproductive effects certainly at lower levels.

There's also a body of literature showing sort of neuropsychological changes at around 25 to maybe 40 micrograms per deciliter as a blood level. For the infant and young child, the levels which effects are found are certainly less than 10 micrograms per deciliter, with some studies finding effects below 10.

These effects are sustained in that when these levels were observed in children and the children followed two decades, or 15 years later, as adolescents, adverse effects of lead were still seen, which sort of argue for infant/young child changes at perhaps the fourth to a fifth, the levels that affect adults, which is not really dissimilar from what some of the people who have studied mercury experimentally and some of the European agencies who have done regulatory evaluations on mercury are suggesting is the ratio between effects in the young child or -- I'm sorry, effects in the fetus and effects in the adult. So I think it's kind of roughly in that range, but it's really the type of effect you're looking at and, certainly, a lot of variability within individuals.

DR. RABINOVICH: I guess to follow-up one question to either of you -- I'm Gina Rabinovich, NIAID.

Is it appropriate at this point in the discussion to be using the word "mercury" versus methyl or ethyl? Do we accept that methyl is the appropriate model for what's going on in the infant? And you were talking about mercury. Is that relevant, you think, to both?

DR. MAHAFFEY: I think George's views, that given our limited information on ethylmercury, that methylmercury appears to be the closest chemical species we have to do that. And so it is a matter of where you want to go with the kind of uncertainty that's there.

DR. LUCIER: My statement was based on assumption, not convincing scientific evidence, because it's not convincing evidence that tells me that they're acting identically. There's some evidence, or similar. My statement on treating ethyl as methyl was based on really the lack of information, and given that lack of information, that's the assumption we would have to make. It might be after we generate more data we're willing to say, "Hey, there's some key differences here," that we need to treat it differently.

DR. RABINOVICH: Given that statement, when you describe an infant mercury intake per day from dietary sources, this is all mercury, all forms, or this is methylmercury? Because you stated that the dietary exposures is estimated to be 0.05 microgram per kilo per day, which maybe present a number that looks like we know, we measured it, we know what's going on.

DR. LUCIER: This was taken out of a review article that was prepared by Tom Clarkson a number of years ago in which these were estimates, and I think he was taking it from another source, but I think you need to keep in mind that, particularly as it relates to infants, it's an estimate, but probably one that is usable in terms of at least framing some of our questions.

DR. RABINOVICH: What is the source of that infant intake? Because you specifically stated infants. Was it formula, or it's in the environment, or is it food as the child becomes from six to twelve months of age?

Because --

DR. LUCIER: My guess, in a nursing infant, it would be primarily from lactational exposures. In a non-nursing infant, it would be from formula and it would be from, you know, other kinds of ubiquitous exposures. I haven't seen anything in where those exposures would have been broken down in terms of relative proportions.

DR. KLEIN: There's a statement in the European --

DR. GREENBERG: We're recording all of this, so we need to --

DR. KLEIN: Jerry Klein, Boston University.

I think you may have answered this question, but there's a statement from the European Agency for the Evaluation of Medicinal Products, of July 8th, that I'd be interested if you concur with. It says: "Data on methylmercury has been used in the assessment of risks associated with ethylmercury as the toxicity profile of the two compounds would appear to be similar."

DR. LUCIER: I wouldn't fully agree. I would say the limited data that's available does not justify anything else but assuming that they're similar. So I basically agree with it, but not fully.

DR. GREENBERG: We have time for one or two more questions.

DR. MYERS: Martin Myers, NVPO.

In these studies that are dietary intake of the mother and evaluation of the child, could you comment on the immunization practices in those communities?

DR. LUCIER: I think maybe -- Tom, did you hear the question? Tom Clarkson, who conducted the Seychelles studies, the lead investigator is here. He's asking whether or not the records that you have regarding immunization practices were kept as a part of your study. I assume they had a fairly active program in the Seychelles.

DR. CLARKSON: No. That's a very good point. I've learned a lot from this meeting, that I don't think any of the epidemiological studies, either now or before, have really taken into ac-

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count the intake of mercury from vaccines. So we're going to have to look again.

DR. MYERS: So the impact we're talking about, then, is the maternal intake superimposed on the infant immunization, which I gather is quite high in that community; is that correct?

DR. CLARKSON: They have an extensive medical program there and it could be substantial. I'll have to check on that. It's an interesting point. Now, bear in mind that the way we measure exposure there, and the way most of these studies measure exposure, is by biological monitoring, you see. We measure the mercury in hair or in blood, so wherever it comes from, you know, we're measuring the total exposure.

So although vaccines could contribute to this, we've been assuming it's mainly coming from fish, it may contribute to this in terms of ethylmercury, we will be measuring the total mercury in blood or total mercury in hair.

Now, some very interesting questions come up. Only methylmercury gets into hair. Inorganic doesn't very well. So whether ethylmercury gets into hair is a very interesting question. It probably does based on the chemistry of the thing. You know, they look very similar in their behavior—but we have not—we will now. We will now check the hair samples to see if there's any ethylmercury in there. So this meeting's going to be useful, at least from my point of view. Thank you.

(LAUGHTER)

DR. LUCIER: That's a good question, Martin, and the answer is, yes, we have to think about the vaccine exposure in addition to the exposures that are already occurring.

DR. GREENBERG: Can I just ask, off the back of your notebook, do you have a rough idea, assuming that ethylmercury gets into hair as efficiently as methylmercury, what proportion of all your Seychelle data would have been vaccine-contributed, assuming that they all got their full compliment of vaccines?

DR. CLARKSON: Is that for me?

DR. GREENBERG: It is.

DR. CLARKSON: Bear in mind that the average level in the Seychelles in hair is about, let's say, 7 parts per million, which roughly corresponds to a blood level of about 30 parts per billion. Okay. That's the average. So the calculations I showed you this morning, which were very extreme calculations assuming a very small bodyweight and assuming they got the full 3 or 4 doses of vaccines, you know, the blood level might get up to 20. But you saw the published figures I think were quoted from the Emory study of about 7, as I remember, 7 parts per billion. So certainly it could make a contribution. There's no doubt it could make a—it wouldn't be an overwhelming one, but it would be a contribution.

DR. GREENBERG: Maybe I misunderstood. I got somewhere between 20% and 60% of blood level from what you just said.

DR. LUCIER: But I think you have to go back and – I think that the age at which these assessments are being done, in the last case, in Dr. Clarkson's study, of 66 months of age, and the Faroes is 84, so there's been a lot of half-lives that have elapsed since the vaccination had occurred.

DR. CLARKSON: The interesting point you raised, though, about -- I mean, you're talking about, of course, post-natal exposure, now, from the vaccines -- Right?

DR. GREENBERG: Yes.

DR. CLARKSON: -- in the first 6 months of life. Although Dr. Lucier pointed out we don't have a lot of information on this, nevertheless, both our studies in the Seychelles and in the Faroes do not find any dramatic effects of post-natal exposure levels. The Faroes is essentially cord blood correlating with adverse effects; whereas, later levels at 12 months and at 7 years, post-natal, do not seem to have much of an effect. So there's not -- There's evidence in the literature. It's really that the post-natal period is not as sensitive as the prenatal, and the numbers you're dealing with from the various agencies are coming from prenatal exposures. That's another big assumption here, that the prenatal is important to this, and it's probably not.

DR. GREENBERG: One last question.

DR. DAUM: I'm Robert Daum from the University of Chicago, and I want to follow up on something that Dr. Rabinovich was asking about.

I presume some babies at both of these sites are breast-fed and some babies are not breast-fed, and I guess I'm wondering about—and this is an immunization practice question—do very young infants eat fish there? Do they eat this whale meat, blubber and things, because they certainly don't eat—very young children don't eat fish in this country very often. So I wonder about the magnitude of the exposure, whether you expect there to be a difference given your proposed route of exposure, breast-fed versus not breast-fed.

DR. LUCIER: I wouldn't expect that they do, but I don't know that for sure. Does anyone -- Can anyone comment on that, regarding the -- particularly the Faroes study? I wouldn't expect that they'd be eating many meals of homogenized pilot whale meat.

DR. GREENBERG: I'm going to have to end this very interesting discussion now because --

(LAUGHTER)

I'm getting sick to my stomach.

The next speaker is Dr. William Raub, who is the Deputy Assistant Secretary for Science and Policy in the Office of the Assistant Secretary for Planning and Evaluation, HHS, and the title of his talk is "Guidelines for Safe Levels of Exposure."

DR. RAUB: Thank you very much, and I appreciate the opportunity to join you this afternoon. The format for the next hour, or a little bit less, is that I will make some introductory remarks around the health guidance values, and then I will be joined by a set of colleagues, including Dr. Clarkson, as a panel discussion, and they have promised to answer every question that I manage to raise. We've heard repeated references or questions to the health guidance values this morning and issues around whether to use them, and if so, when and how to use them. I believe we will be able to do more to raise issues than to give sharp definitive information around some of those questions, but I thought it might be helpful to have some of the background around what these concepts are, what's the philosophy, and the generic approach to them.

All of these guidelines attempt to focus on a concept for which I made up a neutral name, the "Safe Daily Exposure." The emphasis is on long- term. The emphasis is generally on very low levels of exposure. The usual units are the quantity per unit of bodyweight per unit of time. And, for example, for mercury in its various forms, methylmercury, in particular, micrograms per kilogram of bodyweight per day.

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These health guidance values are calculated individually for many different hazards, depending on the regulatory or other mission of the agency that's involved. They are calculated specifically for various primary routes of exposure, ingestion, inhalation, or dermal exposure. In general, they are projected either as a lifetime value or, more conservatively, at the very least, for some substantial indefinite period.

The three most common of these health guidance values are the reference dose, or RfD, of the U.S. Environmental Protection Agency; the minimum of risk level, or MRL, of the Agency for Toxic Substances and Disease Registry of the Department of Health and Human Services; or the acceptable daily intake, or the ADI, employed by the Food and Drug Administration.

Algebraically, these are essentially the same thing. They are used depending on the mission of the various agencies. They may be used as the starting point for health assessments in such situations as evaluating the risks presented by a superfund site. They may be used in a formal risk assessment of a particular hazard, including all of its distributional phenomena and the like. They may be used as a starting point for developing regulatory requirements for emissions in the air or water, for assessing the toxic levels in particular situations, or, in the FDA's case, for the regulation of commercial seafood. But, again, the common factor is the notion that these are starting points for those more specific assessments and applications, and in virtually no case is the guidance value considered the last word. It's usually considered the place to begin in terms of a specific use.

In all of this, there is a driving desire to have science-based values to the extent possible. And in its simplest form, the algebra comes down to the notion of the safe daily exposure being a ratio of an estimated gleaned from real data, either experimental data on animals or epidemiologic observations with humans, divided by one or more uncertainty factors. And what this says is the science-based goal here involves two aspects of science. One is actual data, experimental or observed, and the other are informed judgments at to the utility of that data, the limitations of it, and the ways in which it might be applied, and that's everything from the selection from the particular studies from which to fill the numerator to the judgment about the number and size and the rationale for the uncertainty factors that constitute the denominator.

Certain priorities obtained in general with respect to how one chooses that numerator term. Other things being equal, there's a clear preference for what is called from the direct data, the "no observed adverse effect level," or the NOAEL. If there's dose response information available, and one can indeed identify the level, usually the highest level at which no adverse effect is seen, then this is often an excellent beginning for this calculation.

More often than not, we find ourselves faced not with the "no adverse effect" level but rather observing adverse effects in many different levels and, therefore, being forced to choose the lowest observed adverse effect level. This has a bearing then on what uncertainty factor is chosen, because having seen the lowest observed one, one may have no certain information or no good basis to predict where the level of no effect actually is.

Another priority judgment around the selection of that numerator term is the type of information on which the experimental or observational data are based. Ideally, it's direct information on the most vulnerable human subpopulation, as we believe is the case with the Seychelles and the Faroes studies with respect to methylmercury, but sometimes one must settle for information on the general human population, not being sure at all that the most sensitive subpopulation has, in fact, been measured or that it can be discerned.

Failing that, data from non-human primates are obviously desirable, and failing that, data from other mammals.

In the totality of these types of studies, we find ourselves, more often than not, relying on data from the bottom parts of this list, and, therefore, for all the uncertainties and complexity, as George was indicating, the methylmercury discussions and debates have been a relative pleasure in that we're talking about real data on real humans, in this case, the developing fetus, and a relatively rich source of pertinent information compared to many other areas of toxicology.

Getting to the denominator in that element of informed judgment, uncertainties are very much tailored to the particular situation at hand. When we must extrapolate from information on humans in general to the human vulnerable subpopulation, analysts usually determine that some uncertainty factor is appropriate for that.

The same is true for having the lowest observed adverse effect level, but wanting to estimate where the "no adverse effect" level might be, or at least to take account of that difference. Acute exposures extrapolated to chronic exposures, animal data used where no human information is available.

More often than not, the uncertainty factor chosen for any particular entry is 10, although the richer the data set the more relevant it is. Sometimes individuals doing these calculations choose a smaller value, such as 3 as a half-log unit, or sometimes 1½.

If two or more uncertainty factors are employed, in my experience, more often than not, they're multiplied. But, in certain circumstances, if there is some mechanistic information, one might choose to do an additive of those instead. Again, there may be no right answers with any complete determination, but informed judgments as to how best to weigh the quality and relevance of the information to the task at hand.

And finally, these are some, and only some, of the characteristics that affect these health guidance values. A number of my colleagues who will be speaking to you in a few minutes could give a week-long seminar on the intricacies of the assumptions and the calculations that go into these determinations. But, in general, these focus on chronic exposure, seeking that long-term, potentially lifetime level that is judged to be safe.

Most important, none of these are offered as a bright line between what is safe and what is unsafe. Rather, there's built in a substantial margin of safety, with the realization that the number proffered is almost certain to be a safe level. Values immediately above it are most likely to be safe as well, but the higher one goes above it, the greater the risk becomes.

From my point of view, they are most important the starting point for situation-specific assessments. That is, rather than giving the definitive answer to any generic set of situations, they are the values that raise the flag, they are the values that trigger curiosity or concern, and the values that cause one to look into the specifics of whatever the situation is.

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In this case, I believe it's been quite appropriately applied as a takeoff point, and the challenge of attempting to understand what these estimated safe daily values mean into an exposure scenario that by its very nature is episodic and where there are blips of boluses of exposure.

The safe daily calculations generally assume that there's some modest excursion around that level on a day-to-day basis, but, in general, they do not assume that very large derivations on a daily basis from those are automatically included. And so, therefore, in this particular situation, I think we move ever quickly from using the safe daily level as an indicator for concern to some focus on, in this case, the toxicokinetics of what the nature of these particular kinds of bolus exposures might mean.

Last, I stress the importance of a uniformity of precaution in making these calculations across various hazards. The precautionary principle always applies in doing these calculations in that, depending on the application at hand, one wants to be sure that the level is one that one is not likely to miss a potentially problematic situation. On the other hand, most risk assessors and risk managers are willing to tolerate what I'll call a false positive, as are willing to tolerate the need to do further exploration on a particular situation, only to find that it might be safe, but at least this value is set at a level that provides that degree of protection and extra caution. But if each of the different hazards, say, at a superfund site, were somehow evaluated differently, if the level of precaution were extraordinarily greater or extraordinarily less from one to another, it can compound those situations tremendously, can cause risk managers to invest resources easily in the wrong place, or to be pursuing what is, in fact, the relatively lesser risk and missing a higher risk.

So in all these calculations, a discipline of trying to make the precautionary uses as nearly uniform as possible becomes very important. With that as a backdrop, I'll ask that my colleagues might join me here, and I believe they're prepared to make a few minutes of commentary from the perspective of their individual agencies, the nature of the guidance values and how they apply to the particular exposure situations we find with the vaccines.

I thank you.

And, Dr. Clarkson, if you would like to join us, as well? Before we begin, are there any general questions or comments about the methodology?

(NO RESPONSE WAS HEARD)

DR. RAUB: The table here, beginning on your right, is Dr. Kate Mahaffey of the U.S. Environmental Protection Agency; Dr. Clarkson, the University of Rochester; Chris DeRosa from the Agency for Toxic Substances and Disease Registry; and Mike Bolger from the Food and Drug Administration. Kate, would you like to start us off?

DR. MAHAFFEY: I'd like to do this really with some overheads, because I think it summarizes what you've heard much of this already, so we'll go through it quickly.

This is simply some of the things that were pointed out on the comparative knowledge about susceptibility of the young infant and the fetus. The fetal brain is considered the most sensitive. C and S development continues, of course, postnatally. We have done some PBPK modeling of lactational transfer of methylmercury, and also there are analysis data that support this showing that at the same exposure, the fetal levels are higher than the nursing infant and the nursing infant would be higher than the adult at approximately the same exposures.

The acceptable of mercury, whether they are—and here we're talking about methylmercury—whether it's the RfD or the MRL, are basically set for one chemical species. We don't assume a lot of contribution of either exposure or neurotoxicity from other species of that chemical or other chemicals. So it's a chemical-specific determination to get to that reference dose.

There were questions about the dietary exposure of infants, and I believe George had cited a review article done by Dr. Clarkson, and that was an average value, if I understood what was said, of about 0.05 micrograms per kilogram. Our estimates based on dietary intake in this lactational transfer of methylmercury model suggests that about 7% of women and around 7% of the breast-fed infants have dietary intakes in excess of the reference dose, and this is based on consumption data that's averaged over a month. So it's easily a period that's long enough to be toxicologically relevant. These other numbers are a repeat of something I had shown you previously. The reference dose was developed in 1995, which is prior to the publication of the data from the Seychelles or the Faroes. New recommendations of our Scientific Advisory Board were that with the multiple publications coming forth, that we should sort of await the results of these before attempting to make any revisions of the reference dose. Currently, there is an NAS committee evaluating a lot of the newer data on this topic. The 1995 level, though, is a benchmark dose of about 11 parts per million in maternal hair. WHO had done an evaluation that suggests risk developmental deficits when maternal hair was in the 10- to 20-parts per million range.

Subsequent to these evaluations, there have been publications from the Faroes and the Amazon suggesting the importance of hair mercury levels less than 10 parts per million. There are also certainly the important studies from the Seychelles suggesting that higher levels of mercury exposure in that population did not produce adverse effects with the tests utilized.

The reference dose is considered to be a level that is associated with safety. The way it's developed, it implies its exposure is safe over along period of time. The thing that we really don't know very well is what period of time is relevant for these developmental effects, any more than we really understand what period of exposure during early infancy when infant brain development is underway would be an important exposure period for methylmercury and, certainly, by implication for the vaccine ethylmercury.

And just this one final point, we believe this ongoing exposure through lactation in the young infant, and then as you get some older children, 18-month-olds, 2-year-olds, may have some intake of solid food that, certainly in my experience with children, could include fish sticks, is something that you have to consider as mercury exposure. There may also be additional exposures from other mercury-containing products. So, to me, this is an example of cumulative risk of certainly exposure. The extent to which the toxicities resemble one another is something that, as Dr. Lucier has point out, we are certainly lacking data on, but there is a question of what you do with this uncertainty and the level of prudence you think it's appropriate to adopt.

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That's the extent of my comments.

DR. RABINOVICH: Can I ask a question now, or do you want to hold them to the end?

DR. RAUB: I think it might be best if we go through the panel and then do it all at once. Chris DeRosa?

DR. DeROSA: I think I can dispense with the use of overheads. My comments are really things that will perhaps echo some of the things that have already been stated here, but I think they do merit further discussion.

From our perspective, I think it's important to view health guidance values as something other than thresholds for toxicity, and I think very often when we begin to talk about these different values that we tend to equate them with thresholds at which something is going to begin to happen, when, in point in fact, we have developed these values intentionally with the idea of building in a significant margin of safety.

Our value of 0.3 micrograms per kilogram per day, which you've seen today, we estimate is associated with the margin of safety of at least ten-fold, and possibly two orders of magnitude in totality. And that's fine because of the way we use the health guidance value.

As Dr. Raub pointed out, we use these as a trigger or as a flag to serve as the basis for further evaluation. And we carry those chemicals that are at this level, at way sites forward, for further evaluation in the broader context of biomedical and other technical judgment, what we know about demographics, what we know about other concurrent exposures, and those types of things that would serve to either elevate or diminish our concerns about exposures. But there is a bias here toward ruling out false negatives and a tolerance, as Dr. Raub pointed out, for false positives in the interest of being consistent with this precautionary principle.

I think that one of the things that has been mentioned here on a number of occasions is the issue of the concern about a bolus dose, and one of the things that we would possibly do in evaluating or exercising biomedical judgment as it relates to the bolus dose that is presented by vaccination or any other elevated intermittent exposure would be to see how that comports with the broader database on which our health guidance value is predicated, and that would specifically refer to the peak exposure levels that we saw in the Seychelle Islands. And if we look at the mean of those peak exposures in the highest quintal of exposure in the Seychelles, we see that that mean is marginally above what we would project or what has been projected as being delivered in a series of vaccinations or three vaccinations over the period of—a sequence of—three vaccinations carried out in the first six months of life.

I think the other aspects that we would consider is the fact that we recognize the effects on the developing fetus is the basis for our health guidance value, and that our concern here is for the neonate, and we view the neonate as sensitive to methylmercury but less sensitive than the developing fetus.

We would also look at the point that the average daily dose is associated with the highest quintal of exposure is, again, one that is occurring throughout gestation via exposure through what the mother is ingesting, and that we know that the exposure scenario is continuing postnatally, initially through breast milk and then subsequently, as the child is weaned, through the consumption of fish, which is a very key component in several populations, including those in the Seychelles.

So those are the points that I wanted to just re-emphasize or reinforce in terms of our broader discussion.

DR. BOLGER: I'm just going to make a few points that have already been made by many people before. It sounds like much of this has been discussed throughout the preceding discussions, but in terms of—and this was what I was asked to do—in terms of looking at this particular issue that you're confronted with, the Thimerosal issue, how would this compare in terms of the methylmercury issue that we have to deal with in terms of fish.

I want to pick up on several sorts of key points that were made by Dr. Lucier and Dr. Raub, and in thinking about using methylmercury as a surrogate for Thimerosal, what are the significant areas of uncertainty that you are confronted with. All of this has already been mentioned, but I think you really have to keep this in mind, because at the end of the day you have to make a policy call and you're relying on a safety assessment.

So we have as I see it, the very significant issue of the frequency and duration of exposure issue. You have an acute intermittent type of exposure through the first year of life. Maybe somewhat after that, the time point versus the methylmercury issue, where you have generally steady state exposures that occur on a chronic basis. You have the root of administration differences, the IM versus PO difference, which then leads you to the toxicokinetic differences that Dr. Lucier described in his closing remarks. You also have the target organ differences between ethyl and methyl. I mean, while ethyl and methyl demonstrate remarkable, I think, similarities, there are differences in terms of specific target organs. Methylmercury, C and S, ethyl, C and S and the kidneys. And then you have the dose effect differences. While this doesn't seem to be as significant an area of uncertainty as the preceding four, it is an area of uncertainty.

In regards to the safety assessment paradigm, and this has to be emphasized. I think Dr. DeRosa just emphasized this. This is a first step in an iterative process. Unfortunately, a lot of times my perception is it's perceived to be something more than that, which it's described as being, well, if you exceed the safe level, you are unsafe, or I think the phrase that's commonly heard, "the population is at risk." Well, that implies that the risk has gone up once you've gone over the safe level, when, in fact, the safety assessment paradigm doesn't provide you with any insights into that. I mean, the uncertainties surrounding the safe level as described in the RfD definition is ten-fold. So we don't know how the risk changes as you move about the safe level. You could risk a change not at all until you get to levels considerably above the safe level.

And I think in terms of the safety assessment paradigm, and I think this is the crux of the matter in my mind in terms of this particular issue, was ethylmercury, and one that we have to weigh in with in terms of methylmercury, is that it doesn't really allow you to gauge the level of effort in order to mitigate that risk.

In other words, you're over the safe level, then how quickly do I need to respond if I'm over the safe level? How much effort do I have to do to minimize that source of exposure? And if you try to do that within just the safety assessment paradigm, it doesn't really tell you as you move above the safe level how much risk reduction am I achieving.

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Now, I'm not sure in terms of this particular issue with ethylmercury, because the amount of data that you have in terms of dose response with ethyl is -- my perception is fairly meager. So then you would have to use methylmercury as a surrogate, and there is a plausible way, I believe, in looking at dose response using methylmercury. That is the next step in the safety risk assessment paradigm that hasn't been done.

I mean, in the RfD/MRL/ADI paradigm, dose response is not part of that consideration. You identify it, a particular study, you identify a particular dose level, you apply your uncertainty factors, but you are not taking into account dose response, which I think is a critical issue if you're trying to get a handle on risk above the safe level so that you can then figure out, "Well, how fast do I have to move and how much effort do I have to put into reducing this level of exposure that I'm concerned about?"

So those are the points I wanted to make in terms of the kinds of considerations that we have to deal with in terms of methylmercury in fish, which I think there's so much analogous to this situation.

DR. RAUB: Thank you, Mike.

We'll wrap up with Dr. Clarkson. As many of you heard by the repeated references this morning, much of what we know about methylmercury and its toxicity comes from the studies in Iraq and the Seychelles, and for that we're thankful to Dr. Clarkson and his colleagues.

DR. CLARKSON: Thank you, Mr. Chairman. You're more than generous. We've contributed a little bit, but not that much. I don't have an agenda or anything. You know, I'm not representing a government agency, but this university that lives in the tundra north, in New York State, and the only bias I have is to get as much research money as possible. (LAUGHTER)

Naturally, that tends to make things look as dangerous as possible, so that I can get more research money, but, unfortunately, in the Seychelles study we did the opposite. So we're probably going to be bankrupt before long. (LAUGHTER)

So I can make comments, Mr. Chairman, about—or we could postpone them until there's a general discussion. I don't know.

DR. RAUB: Whatever you'd like.

DR. CLARKSON: Why don't we postpone them until --

DR. RAUB: In that case, we have a substantial block of time for questions or comments. Yes?

DR. RABINOVICH: This is Gina Rabinovich, NIAID.

The question is generated by a comment from Dr. Mahaffey, but it probably could be commented upon by many other members of the panel.

In discussions leading towards this meeting, it was my understanding, and I seek clarification, that in evaluating the neurological deficits that these indeed were not overt, clinically overt, that it actually took the detailed neurocognitive evaluation to define them. And you talked about clinically overt neurological deficits that maternal hair was greater than 20 parts per million.

We've been talking—using that term as though it meant something. I realize I no longer know what it means. So what are we talking about, really, in terms of neurological deficits?

DR. MAHAFFEY: Well, I can tell you what we did with respect to the reference dose, and probably Dr. Clarkson can comment some, because the reference dose was based on findings from the Iraqi study. And in that, that was a poisoning episode of about six months duration. And while it's been called an acute exposure, it was certainly one that was long enough to produce fetal effects. Approximately two years later, two of their neurologists were in Iraq and evaluated as many of the children they could find who were born from mothers who were exposed during that epidemic, and, ultimately, I believe there were 81maternal-child pairs who were assessed.

The reported paper from Marsh *et al.*, in 1987 talks about endpoints such as delays in walking, increased neurological scores on a standardized neurological assessment, seizures, delays in talking, and there may have been another endpoint or two in there.

Where the data turned difficult is that the culture in Iraq and the nomadic living conditions in these villages made it hard to find these people, as well as hard to get certain types of information from them. So there is a level of uncertainty in this data, which we readily acknowledge, but in terms of clinically significant endpoints, that's what we're speaking of.

DR. RAUB: Dr. Clarkson?

DR. CLARKSON: One of the advantages of prenatal studies versus studies in adults is you have a much better recapitulation of the dose. You have to make it over a 9-month period, and so the studies that have gone on prenatally, like the Faroe studies and the Seychelles and Iraq, really are a fairly good measure of what exposure was.

The problem with adult studies is that you don't. The people in the fish-eating populations who are adults have been exposed all their lives, and you only have a measure going back a year or two. So it makes interpretation of a lot of the adults quite difficult. So that there is a tendency, quite understandably, number one, for risk assessment to be based on prenatal exposures because of the better measure of dose, a more clear cut situation, and because the evidence seems to be the developing prenatal brain is more sensitive to methylmercury. It's a big question that affects this whole debate, which is, how sensitive the situation is after birth.

DR. MAHAFFEY: If I could follow up slightly, the indications that the fetus is more sensitive than the adult, in part, comes from the Japanese epidemics, in which mothers, who themselves had very limited evidence of neurological problems, gave birth to infants that had damage, clinically overt damage.

DR. CLARKSON: Yeah. The other evidence is also that in Iraq, when we examined adults… Now, the advantage of Iraq, with all its disadvantages, is it was a sort of a short-term, 6-month, or whatever, exposure, to 3 months to 6 months. So we did know, even in adults in Iraq, what the exposure was, you see, and what the maximum exposure was, which you don't know in a fish-eating population. It goes all of their lives. So even with adults in Iraq, you could get their maximum levels with some, you know, calculations and some assumptions, but you could come up with something that at least approximated their actual exposure, and knowing that this was a one-shot incident, there probably wasn't much exposure earlier in life. Now, in that case we got, you might call, I'm an old fashioned toxicologist—a threshold value, say, of about 100 parts per million in hair with the adults; whereas, with the kids, our lowest estimate was as low as 7 parts per million. Now, there's an error

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on that, but it's the lower end of our estimate. So from a quantitative point of view, Iraq also supported the fact that the prenatal life was more --

Now, the Iraqi thing, too, raised some very interesting questions about post-natal exposure. Dr. Amanzaki (phonetic), who was head of pediatrics in Baghdad, examined a number of children, along with their staff, who had been exposed postnatally to mercury in milk. Of course, all feeding of infants there is from human milk until they can take solid stuff, which, of course, would be bread. And these infants, some of them were totally breast fed, and some a little older had some of the contaminated bread. Some of these infants developed—5 of them developed blood levels of 1000 parts per billion. And at least from the pediatrician's point of view, there's nothing wrong with it. Well, we weren't measuring a 5-point drop in an intelligence score. But from a point of view of a pediatrician, a pretty competent, experienced pediatrician, these kids looked normal.

And there was 1 child—I think there was a group of altogether. I'll have to look up the paper, but it was about 15 altogether we did. All of them were above 200 in their blood levels and one of them was 1500. It was heroic. And this raises, first of all, a question about the actual sensitivity of the post-natal period.

I'm not sure I totally agree with my colleague, Dr. Mahaffey, that you can extrapolate from lead to mercury. She has been a lead worker after all. I think the two metals are very different in their biochemistry and in their mechanism of action, but it does raise a question about the sensitivity of this post-natal period.

Both the Seychelles and the Faroes, which disagree in terms of results of prenatal exposures, have not found any dramatic effects due to post-natal exposures, either in the Faroes or in the Seychelles, which also tends to give credence to the idea that the post-natal period ain't all the sensitive.

In fact, one of the most interesting to me of the Faroe publication, which hasn't been mentioned so far, is that they looked at children at 12 months of age and found that the higher the mercury levels in the hair of these kiddies at 12 months, the better off they were. They achieved their developmental milestones more rapidly if their mercury was higher. That is kind of an interesting result. The authors attributed this to a confounder. The confounder was breast-feeding, because the longer the breast-feeding period, the more mercury they got from the milk and, therefore, the higher their mercury levels were. They showed that in the study, that the length of breast-feeding actually resulted in higher mercury levels. And their conclusion was, you know, breast feeding is good for you, it's beneficial, and that was the confounder in this study. It may have a lot to do with Iraq, too, that human milk is good for you. And it raises the other issue that when we look at these numbers, whether coming from Iraq, from the Seychelles -- The media in which methylmercury is presented is very important. It might make a difference to the toxicological outcome. Certainly, the Faroes group suggested that it was the sort of protective and beneficial effects of human milk that outweighed any possible potential effects of methylmercury. Something clearly was happening in Iraq to allow these very high levels.

Now, with Thimerosal, I mean, it's a different thing altogether. It's being injected. And so you're comparing quite a different media of injection here, which might not be good news for you. I mean, you're not giving it in human milk, so you might not get the protection that you would see there.

DR. RAUB: Dr. Bolger?

DR. BOLGER: I just wanted to comment on two things. One is, bear in mind that these estimates of relative sensitivity based on the Iraqi study are fairly uncertain. I mean, we only [had] 81 subjects in there, and, in fact, the bulk of those children's mothers had body burdens well above 50 parts per million hair levels. So you only had several subjects in the low-dose range, of course, which is the dose range of concern for methylmercury in terms of fish-eating populations.

And then, in terms of the indices of development that were measured in Iraq, delayed walking and delayed talking, when Dr. Clarkson's group looked at those endpoints in the Seychelles, they did not see that kind of corresponding correlation. So, bear that in mind, that there are still some significant uncertainties in terms of how you measure development and what you're looking at.

DR. RAUB: Yes? You're up again.

DR. RABINOVICH: I'm not sure if everyone is still in the nap time. I'm just trying to understand the many issues that you're raising. I think I've heard it at other meetings, but perhaps it should be stated here. What do we know about breastfeeding and intake through oral and exposure to a breast-feeding infant for methylmercury, ethylmercury, whatever you found?

DR. CLARKSON: The breast milk contains a fairly proportion of inorganic mercury. People exposed to methylmercury, certainly in Iraq and in fish-eating populations, breast milk is in both the methyl and inorganic. A great deal of attention has been played to the methyl and very little to the inorganic that's coming in breast milk. This may have some reverence, this Thimerosal, really, because it also breaks down to an inorganic mercury. To the best of my knowledge, it has never been looked at very much from a health risk point of view, but inorganic mercury in breast milk is probably well absorbed. In adults, the absorption of inorganic mercury averages around 7%. There's a range, but it averages about 7. Probably in suckling infants it's much higher, of the order of maybe 50%. The most divalent ions are absorbed to a much higher extent in the intestines of the immature infant.

So one has to worry, too -- This hasn't been looked at as to how the absorption of the inorganic might have an impact, for example, on kidney function. So to the best of my knowledge, it has not been looked at in any detail, not even with methylmercury.

DR. RABINOVICH: The environmental health people, if you could summarize briefly how you think differently about organic metallic, like methyl or ethyl mercury, and inorganic mercury in terms of health impact.

DR. MAHAFFEY: Well, our understanding of this, based on Swedish data and modeling a PDPK model that was done at EPA, is that both methylmercury and inorganic mercury can enter the mother's milk, and it depends, in part, on what her own exposures are. If she has comparatively high seafood intake, she can be expected to have comparatively more methylmercury in the milk.

It's known, too, that dental amalgams can contribute to the inorganic mercury level in the mother's milk. I was interested

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in Dr. Clarkson's comments about Dr. Amanzaki's work, which are found in the *American Journal of Diseases of Children*, Volume 130, October, 1976, and I guess there must have been more infants than were written up, because this one only describes one infant who did remain well, but she was only evaluated for a short period of time, and they make specific reference to concern over what her longer-term effects might be. So, I mean, you have to -- This is Amanzaki in the *American Journal of Disease of Children*, '76.

DR. CLARKSON: Well, we're in a better journal. We have one in the *Journal of Pediatrics*. Okay? So this is -- this has 15.

DR. MAHAFFEY: Okay. So there were additional ones.

DR. DeROSA: I just wanted to return to the comment about the exposure through breast milk, and there have been some studies done, the Swedish study, in particular, that suggested a 50% distribution between the inorganic and the organic forms of mercury, that when they looked at the kids who were nursing that the relative proportion was 75% organic to 25% inorganic because of the greater bio availability, greater uptake of the organic form vis-à-vis the inorganic.

DR. RAUB: Dr. Plotkin?

DR. PLOTKIN: Well, since everybody's been extrapolating, I thought I might take a shot at it and ask the panel what they think of this. The only data we have, and, obviously, they're insufficient, are the 5 term infants from the Emory study who had a blood level averaging 2.3 micrograms. Assuming that they were 3½-kilo infants, that means they—and there's 12.5 micrograms in hepatitis B, so they received about 4 micrograms per kilo. Now, at two months an infant could conceivably receive 5 times that. That is, 62.5 micrograms. Dr. Bolger seemed to say that there are no dose response data, but assuming what I guess is the worst-case scenario, you can multiply, that suggests that they would have a peak. That is, at two months, they would have a peak of 7 micrograms, assuming, of course, the factor of growth. Now, is that extrapolation—assuming that the Emory data are correct, is that way out of line, or does that, indeed, suggest that they would achieve blood levels of about 7 micrograms, which would translate, if I understood Dr. Clarkson, to about 1 or 2 parts per million in the hair?

DR. CLARKSON: I think it does. Can I show my thing again?

DR. CLARKSON: These are the data I used, which I got from Dr. Halsey, I think, by permission of the American Academy of Pediatrics, so it must be right. And obviously, those bodyweights are rather low. I used 2 of them: the three standard deviation one and the fifth percentile. These were the doses I was given from the vaccines; is that correct? 12.5 at birth and so on and so forth. Now, if you go through the arithmetic on this, it's simple enough even for me to do it, you assume that 5% of this dose goes to the blood compartment, and that's mimicking methylmercury, I might add. And usually, distribution is complete in about 3 days in humans. Then you assume that the volume of the blood compartment—Dr. Halsey, correct me if I'm wrong. You said 8½% of the bodyweight, correct?

DR. HALSEY: At birth.

DR. CLARKSON: At birth, yeah. Well, I took it for 6 months, as well. Not being a pediatrician, I just did. So if you do that -- Because I felt they're only numbers, you know, you can do the arithmetic better than I can—you come up with blood levels shown on that last column. Can you read that? -- of -- Well, not on that. That's the dose. Now, the blood levels you get are on the next slide, which I showed you this morning, and you can see that it's a small dose at birth. The yellow one is the smallest bodyweight, of course, the three standard deviation one. If you can read the white one, it's the fifth percentile. You can see that after the first vaccination, background levels in blood are about 1 part per billion, depending on fish consumption and all that. Generally speaking, they're down there. You get a modest increase to less than 5. And then this decline here is simply due to the increase in bodyweight. I'm making that key assumption that there's no excretion whatsoever of mercury during this period, and that assumption comes only from animal experiments. We think we know the mechanism of that, but we don't—and it probably should apply to humans, but there's no observations made yet on humans. And I think this discussion of vaccines might help us solve this problem, might be able to get some samples. Don't give me too many fecal samples at once, but we want to be able to get some samples that might solve this problem. And then when you give the larger dose, the 62.5, obviously, there's a rather sharp increase, again a decline due to growth, and so forth. You can see this sort of pattern will eventually get you up into the 20s.

Now, the regulatory guidelines are roughly for EPA around 5, 4 or 5. I think FDA is around 20. It's the classic one we've had for ages and ages. WHO, as well, is around 20, about here. So that we just edge up and sort of go between the various guidelines on that. It's a matter of what arithmetic you want to do, what assumptions you want to make about the bodyweight of the child, and how frequently the vaccines are given, and what's the mercury in the vaccines. And my view is that it's the maximum level that determines the damage. Methylmercury is an irreversible poison. It knocks out the brain cells. So probably, it's not so much the length of exposure, it's the peak exposure that's really going to do the total damage. The Iraq dose response that the EPA used in their risk assessment was based on peak levels, not average levels, but peak levels. And so in this sense, it's the peak levels here I would imagine that are probably important to worry about. And this is obviously a worst-case scenario. These are the lowest possible bodyweights. And I heard this morning that you're not even supposed to give a vaccine to an infant at 1.8 kg, and this is 1.8 kg here.

Okay? Thanks.

DR. RAUB: We just have a few minutes. There's one hand in back and then a couple down front here. We probably have time for about two or three more questions. The gentleman in the back?

DR. BERNIER: My name is Roger Bernier from the National Immunization Program at CDC.

I wonder if we could get some more discussion about the application of these standards, because I think one of the things that characterized the policy-making around this episode was, I think, the perception or the interpretation of these guidelines as in some ways bright lines where there, in fact, was a violation of safe levels. And the insights that I'm getting from hearing you talk about these is very interesting because you're talking about these guidelines as starting points, as screening levels that you would then begin to investigate further. I guess it suggests to me that there's an art to the application of these guidelines. And I wonder if you have ideas about, or from past experience,

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a protocol or a checklist for once you have hit this screening level and you are now beginning your further investigation, what are the things to do. I mean, from other situations where you have experienced violations or things have occurred in excess, is there guidance that you can give in this art of applying these standards so that we can then judge what we are doing in the vaccine area and how we are doing as appliers of these standards?

DR. MAHAFFEY: If I could offer one comment. One of our concerns with our estimates for reference dose and mercury exposure is over what time period of both exposure and, in the case of methylmercury, developmental period these exposures are appropriate for.

When we did the report to Congress, there was a lot of back-and-forth discussion over what time period of exposure we should average mercury intake from fish. We had some daily exposures in there. We had monthly exposures in there, too. Certainly, the day-to-day variability in fish intake will produce a much higher range of exposure if you look at a one-day kind of intake. At that point, we looked at 30-day intakes. In listening to the experimental animal panel talk about the importance of an intermittent high-dose exposure on C and S development, at least in animals, I personally began to wonder if our 30-month period was too long. I don't know what the appropriate period really is, but it has been the topic of a lot of discussion.

The reference doses are intended to be a level that's thought to be safe over a very long period of exposure, and clearly what that relevant period is can be, in part, determined by the what the endpoint is you're trying to look at. If you're looking at carcinogenicity, clearly a longtime period of exposure is the period of greatest interest. With methylmercury, we know that there are developmental windows of importance. I think with this, as others have pointed out, this peak exposure that happens is something that is fundamentally quite different from the usual application of reference doses, and I would think the kinetic information has got to be very important here because it may suggest that the risk is higher than what might be assumed from just applying the reference doses, or the MRL On the other hand, additional kinetic data may show that ethylmercury is a sufficiently different compound in its metabolism that the RfD, or MRL for methylmercury, may not be that relevant, but, in the interim, risk managers will have to make some decisions.

DR. GREENBERG: I think this has been a great discussion, but we should take a break now. You can continue this discussion in the hallways, and we'll be back here at 3:30 for the last session.

(RECESS FROM 3:00 P.M. TO 3:34 P.M.)

(END VOLUME I - DAY ONE)

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