

AT

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

**ANESTHETIC AND LIFE SUPPORT DRUGS  
ADVISORY COMMITTEE MEETING**

Thursday, March 29, 2007

8:00 a.m.

Doubletree/Hilton Hotel  
and Executive Meeting Center  
1750 Rockville Pike  
Rockville, Maryland

## MEETING ROSTER

Steven L. Shafer, M.D., Acting Chair  
LCDR Cathy Groupe, MPH, Executive Secretary

ANESTHETIC AND LIFE SUPPORT DRUGS ADVISORY  
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James C. Eisenach, M.D.  
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Sulpicio de Guzman Soriano III, M.D.  
David J. Wlody, M.D.

ANESTHETIC AND LIFE SUPPORT DRUGS ADVISORY  
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Charles McLeskey, M.D. (Industry  
Representative)

## TEMPORARY MEMBERS (Voting)

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Jayant K. Deshpande, M.D., MPH  
Vesna Jevtovic-Todorovic, M.D., Ph.D.  
Jeffrey R. Kirsch, M.D.  
Donald R. Mattison, M.D.  
Julia E. Pollock, M.D.  
Wayne R. Snodgrass, M.D., Ph.D.  
Daniel Zelterman, Ph.D.  
Athena F. Zuppa, M.D.

GUEST SPEAKER (Non-SGE, Non-Voting, Presenting)  
John W. Olney, M.D.

## FDA PARTICIPANTS

Robert J. Meyer, M.D.  
Bob A. Rappaport, M.D.  
William Slikker, Jr., Ph.D.  
R. Daniel Mellon, Ph.D.  
Arthur F. Simone, M.D., Ph.D.

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P R O C E E D I N G S

**Call to Order and Introductions**

DR. SHAFER: I would like to call to order the meeting of the Anesthetic and Life Support Drugs Advisory Committee. Our very first order of business is to go around the table and introduce yourselves.

Dr. Rappaport, if you want to start.

DR. RAPPAPORT: Good morning. I am Bob Rappaport. I am the Director of the Division of Anesthesia, Analgesia and Rheumatology Products. Let me just note that Dr. Meyer, who is the Director of the Office of Drug Evaluation II, will be here later this morning, and Dr. Slikker, who is the Director of the National Center for Toxicological Research, will also be here later this morning.

DR. MELLON: My name is Dan Mellon. I am the Pharmacology/Toxicology supervisor for the Division of Anesthesia, Analgesia and Rheumatology Products.

DR. SIMONE: I am Arthur Simone. I am a

Medical Officer for the Division of Anesthesia, Analgesia and Rheumatology Products.

DR. RAJA: I am Srinivasa Raja. I am the Director of the Division of Pain Medicine with the Department of Anesthesiology at Johns Hopkins University.

DR. HENTHORN: I am Tom Henthorn. I am the Professor and Chair of Anesthesiology at the University of Colorado.

DR. EISENACH: I am Jim Eisenach. I am an anesthesiologist at Wake Forest University and Editor-in-Chief of Anesthesiology.

DR. JEVTOVIC-TODOROVIC: Good morning. I am Vesna Todorovic, one of the anesthesiologists at the University of Virginia in Charlottesville, Virginia.

LCDR GROUPE: Hello. I am Cathy Groupe. I am the Designated Federal Official for the meeting and I would also like to note Dr. Kanwaljeet Anand, who I am sure will be joining us shortly, was omitted on the roster that you have with your handouts, but he is attending as a voting

member or a voting consultant, thank you.

DR. SHAFER: Steve Shafer, Professor of Anesthesia at Stanford University, Editor-in-Chief of Anesthesia and Analgesia.

DR. DESHPANDE: Jayant Deshpande. I am Chief of Pediatric Anesthesiology at Vanderbilt University.

DR. WLODY: David Wlody. I am an anesthesiologist at the State University of New York, Downstate Medical Center, and President of the Society for Obstetric Anesthesia and Perinatology.

DR. SORIANO: Good morning. I am Sul Soriano, a pediatric anesthesiologist at Children's Hospital, Boston, and Harvard Medical School. I am the President-Elect for the Society for Neurosurgical Anesthesia and Critical Care.

DR. ZUPPA: Good morning. I am Athena Zuppa. I am a pediatric intensivist and a clinical pharmacologist from the Children's Hospital at Philadelphia.

DR. ZELTERMAN: I am Dan Zeltermann,

Professor of Biostatistics at Yale.

DR. POLLOCK: I am Julie Pollock. I am a clinical anesthesiologist at Virginia Mason in Seattle, Washington.

DR. ARMSTRONG: I am Danny Armstrong. I am a pediatric psychologist, Senior Associate Chair of Pediatrics and Associate Chief of Staff at the University of Miami, Holtz Children's Hospital of Miami.

DR. SNODGRASS: I am Wayne Snodgrass. I am a pediatrician and clinical pharmacologist at the University of Texas Medical Branch in Galveston.

DR. MATTISON: Don Mattison from NICHD.

DR. KIRSCH: Good morning. I am Jeff Kirsch. I am the Chair and Professor of Anesthesiology at Oregon Health Sciences. I am the Chair of the Quality Executive Committee at Oregon Health Sciences, as well.

DR. McLESKEY: I am Charley McLeskey, previously an anesthesiologist, now a ZARS pharma industry representative on the committee.

Our next item here is to have the Conflict of Interest Statement.

**Conflict of Interest Statement**

LCDR GROUPE: The following announcement addresses the issue of conflict of interest and is made a part of the record to preclude even the appearance of such at this meeting.

The matter coming before the Anesthetic and Life Support Drugs Advisory Committee is a particular matter involving specific parties.

Based on the submitted agenda and all financial interests reported by the committee participants, it has been determined that all interests in firms regulated by the Center for Drug Evaluation and Research present no conflict for an appearance of a conflict of interest.

We would like to note that Dr. Charles McLeskey has been invited to participate as a non-voting industry representative acting on behalf of regulated industry.

Dr. McLeskey's role on this committee is to represent industry interests in general, and not

any one particular company.

Dr. McLeskey is employed by ZARS  
Pharmaceutical.

In the event that the discussion involves any other products or firms not already on the agenda for which an FDA participant has a financial interest, the participants are aware of the need to exclude themselves from such involvement and their exclusion will be noted for the record.

With respect to all participants, we ask in the interest of fairness that they address any current or previous financial involvement with any firm whose products they may wish to comment upon.

Thank you.

Our first presentation will be from Dr.  
Rappaport.

I will ask all of the presenters to please be careful to follow the timeline, however, Dr. Rappaport, we will be giving you an extra five minutes because we are running early.

#### **PRESENTATIONS**

**Introductory Remarks. Background.**

DR. RAPPAPORT: Good morning. Welcome, Dr. Shafer, members of the Committee, invited guests. Thank you for coming today.

In 1999, Dr. Chrysanthy Ikonomidou and her colleagues, including Dr. John Olney, published a landmark article in Science describing apoptotic neurodegeneration in juvenile rats exposed to NMDA-receptor blocking agents.

Additional reports by Drs. Todorovic, Olney and others added to the concerns over the potential clinical relevance of these findings by providing further evidence of neurotoxicity in the developing rodent brain associated with not only ketamine and other NMDA-receptor blocking agents, but the majority of general anesthetic and sedative drug products currently employed in pediatric anesthesia.

Additional studies have since documented that, in addition to histopathological changes, subtle and prolonged behavioral changes can be seen in rodents exposed to these agents as juveniles.

In response to these evolving and

concerning data, the FDA's Center for Drug Evaluation and Research and National Center for Toxicological Research developed a collaborative effort that has provided further confirmation in rodent models.

CDER and NCTR, with the assistance of other government agencies, then initiated studies in juvenile monkeys to determine the susceptibility of ketamine-induced neurotoxicity in a primate model during the period of synaptogenesis.

Recently, the preliminary results of FDA's non-human primate studies were accepted for publication in Toxicological Sciences. These early studies have demonstrated the presence of apoptotic neurodegeneration in juvenile monkeys which appears to be similar to that seen in the rodent studies.

These findings raise many concerning questions regarding the effects of exposure to general anesthetic and sedative drugs on the developing human brain. While we have no evidence to date that supports detrimental CNS effects in pediatric patients who have been exposed to these

agents, there have been no clearly well-designed studies to look at the possibility of neurological toxicity after exposure to anesthesia in this patient population.

Today's meeting will focus on describing the data from the animal studies, and we are fortunate to have the senior investigators for those studies here today to present their data.

Drs. Olney, Todorovic, Slikker and Soriano have each contributed important new facets to this evolving knowledge base. In addition, Dr. Soriano has also contributed to our current understanding with his clinical perspective on the available preclinical data.

This morning's presentations will begin with a brief review of the regulatory history of pediatric drug approval and the approval of anesthetic drug products for pediatric indications in particular.

Dr. Arthur Simone, an anesthesiologist in our Division, will present this information that will be important for you to understand as you

consider the potential impact of the relatively recent findings of anesthetic induced neurotoxicity in juvenile animals.

Dr. Daniel Mellon, one of our supervisory pharmacologists, will then present an overview of the preclinical studies performed to date, setting the stage for the presentations by the individual investigators.

Following presentations by Drs. Olney, Todorovic, Slikker and Soriano, and after our lunch break, we will continue with the Open Public Hearing portion of this meeting.

For the remainder of the afternoon you will be asked to address the discussion points that have been provided to you by the Division. We look forward to hearing your assessment of the available preclinical data, your thoughts on what further animal studies might be useful as we proceed with attempting to understand these findings, and your concerns regarding the applicability of the preclinical findings to the clinical setting.

We will also ask you to discuss what types

of clinical studies might provide useful data and to provide your recommendations regarding how the current knowledge base might impact the clinical practice of pediatric anesthesia today.

Although a large body of preclinical data does exist, we really don't know to what degree this data can be extrapolated to the clinical setting. While we don't expect you to provide us with a definitive answer to that question today, we do believe that it is important to bring these findings and the questions that they engender to you as thought leaders in the anesthesia and pediatrics communities.

It is essential that we at the FDA, as well as the pediatric anesthesia community and the parents of children undergoing procedures that require anesthesia, understand not only the existing information but the limitations of our knowledge and the risks of the alternative treatment options.

We are grateful for your willingness to assist us in this process and thank you for taking

time from your busy schedules to do so.

DR. SHAFER: Thank you, Dr. Rappaport.

Our next presentation will be an Overview and Regulatory Issues Regarding Anesthetic Agents for Pediatric Patients.

Dr. Simone.

**Overview and Regulatory Issues Regarding  
Anesthetic Agents for Pediatric Patients**

DR. SIMONE: Good morning. I, too, would like to extend my welcome to the members of the committee and the researchers that have joined us today. With my presentation, I would like to set the stage for today's topic, neurological implications associated with the use of anesthetic agents in pediatric patients.

I will also focus on FDA-related aspects of the issues.

[Slide.]

The concern for the neurodegenerative effects of anesthetic agents are related to the period of brain development marked by intensive synaptogenesis, often referred to as the

brain-growth spurt.

This time period seems to be relatively conserved among species. In rodents, the growth spurt occurs between the last 2 days of gestation and the second week of life after birth. In humans, the corresponding time frame spans from the third trimester of pregnancy through 3 years of age.

During this time period, neurons appear to be particularly vulnerable to environmental conditions that may adversely affect synaptogenesis and may thereby have an impact on cognitive function and behavior.

[Slide.]

There is a growing body of evidence which is the subject of presentations to follow that suggests certain substances which influence the release of glutamate and gamma-aminobutyric acid, or GABA, may affect the non-synaptic trophic actions of these compounds leading to altered differentiation of neurons and defective brain circuitry.

Exogenous stimulation, GABA-ergic or blockade of glutaminergic signaling pathways via N-methyl D-aspartate or NMDA receptors have been observed in animals to trigger a unique type of cell death during the synaptogenic period of development.

The type of cell death noted under these conditions, termed apoptosis or programmed cell death, differs from necrosis, and that is characterized by death of individual cells which retain or have blebbing of their plasma membranes.

The cells tend to shrink and fragment as they die and, unlike necrotic cell death, inflammation is not associated with apoptosis.

Although apoptosis is known to occur normally and thought to be a mechanism for eliminating defective cells, the extent to which it is observed with excessive GABA-ergic stimulation or NMDA receptor blockade is markedly increased over normal.

[Slide.]

The table in this slide is taken from the

white paper recently published by Mellon, et al., from the FDA and contains information derived from published in vitro studies. It indicates the relative NMDA antagonistic and GABA-mimetic and mu-receptor agonistic effects of many of the commonly used anesthetic agents and opioids.

The data to date demonstrating neuronal apoptosis in juvenile animals have been collected in studies conducted using some of these agents, in particular ketamine, isoflurane, midazolam, and nitrous oxide.

By their effects at the NMDA and GABA receptors, virtually all of the sedative hypnotic agents and inhaled anesthetic agents would appear to be capable of inducing neuronal apoptosis during synaptogenesis.

It is interesting to note that alcohol, listed on the bottom line, strongly affects both receptors and is associated, unlike all of the other agents listed, with a range of fetal abnormalities including cognitive and behavioral impairments in children born to mothers who

consumed substantial amounts during pregnancy.

It is worth noting that the effects observed in the consumption of alcohol during pregnancy have not been observed even to mild degrees in children exposed to anesthetic agents in utero.

[Slide.]

With even a theoretical concern for the safety of a vulnerable patient population, it is reasonable to ask how the risk may be minimized. With animal data suggesting a possible human risk for apoptosis with anesthesia exposure during synaptogenesis, it is appropriate to assess or reassess the necessity for exposure, the possible alternative therapies available, and what safety information is available for anesthesia drug products.

There are, generally speaking, four categories of exposure. Maternal surgery generally is limited to procedures for life-threatening conditions that cannot wait for the delivery of the baby. In some instances, the baby may be delivered

early in order to treat the mother and spare the infant from the untoward effects of the surgical procedure and the medications used perioperatively.

There is evidence to suggest that the benefit of surgery on the fetus versus the neonate for treating certain conditions, such as vessicoamniotic shunting as a treatment of urinary tract obstruction or resection of malformed pulmonary tissue or placement of a thoraco-amniotic shunt as a treatment for congenital or cystic adenomatoid malformation may also provide some benefit.

There is evidence that providing pain relief during labor and delivery has benefits for the mother, as well as the fetus. This is generally accomplished with opioids and local anesthetic agents. The same is true for cesarean section although use of general anesthesia agents is more likely for this procedure.

For pediatric patients less than 3 years of age, many of the surgical interventions that are performed are necessary to preserve the life or

health of the individual. Although some procedures, such as circumcision, are performed for reasons other than medical indication, that is associated with increasing risk if the procedure is further delayed.

In general, there are four alternatives to the use of hypnotic sedatives or inhaled anesthetic agents. These include the use of local anesthetic drugs or narcotics, the use of no anesthetic agents, and the delay of surgery until the vulnerable period has ended.

These will be discussed a bit later both in this presentation and in that of Dr. Sul Soriano.

[Slide.]

Let's first consider the anesthetic drugs and how they have been approved. This table in this slide contains many of the anesthetic and analgesic agents used perioperatively and in the ICU setting. It is not meant to be inclusive and may represent drugs that have been used over the last 20 years.

It is worth noting that two of these agents are readily available to clinicians although they have never been approved by the FDA. Specifically, thiopental has not been approved for parenteral use and chloral hydrate has not been approved for use by any route of administration.

While it is not important to appreciate all of the drugs that are on this slide, the same table is repeated in the following slides.

[Slide.]

In this slide, those drugs whose FDA-approved labels contain information specific to pediatric indications in dosing remain showing in white. Just notice they start to fade and the numbers dwindle.

[Slide.]

In this slide, those products whose FDA approved labels contain information specific to pediatric patients under the age of 3 years remain showing in white. For most of these products, studies assessing safety and efficacy have been conducted in patients within this age group,

thereby, informing the label on how best to use them in this population.

In some cases, such as curare primarily, the labeling address is used in pediatric populations in a generic fashion with guidelines that are not age specific but can be interpreted to be applicable down to the neonatal age.

It should also be noted that there is a paucity of drugs for pediatric patients that contain the level of information regarding safe and efficacious use that is available to adult patients.

[Slide.]

Let's consider for a moment drug use during pregnancy. The Code of Federal Regulations requires classifying products into one of five categories. These are generally based on teratogenic findings.

For Category A, adequate and well-controlled studies in pregnant women must have been conducted and have failed to demonstrate a risk for the fetus in the first trimester of

pregnancy, and there must be no evidence of risk in later trimesters.

In Category B, reproduction studies in animals have shown positive findings at doses greater than the human dose. Studies in pregnant women, however, have not shown increases in the risk of abnormalities when administered during the first, second, third, or all trimesters of pregnancy.

Despite the animal findings, it would appear that the possibility of fetal harm is remote if the drug is used during pregnancy. Nevertheless, because the studies in humans cannot rule out the possibility of harm, it is recommended that the drug should be used during pregnancy only if clearly needed.

Assignment to Category C is based on one of two conditions. First, if an animal-reproduction study has shown an adverse effect on the fetus, if there are no adequate and well-controlled studies in humans and if the benefits of the use of the drug in pregnant women

may be acceptable despite its potential risk, the category can be granted.

The alternative is if there are no animal reproduction studies and no adequate and well-controlled studies in humans, the category is also granted.

Pregnancy Category D is used if there is a positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans but the potential benefits from the use of the drug in pregnant women may be acceptable despite its potential risks--for example, if the risk is needed in a life-threatening situation or serious disease for which safer drugs cannot be used or are ineffective.

Lastly, Pregnancy Category X is assigned if studies in animals or humans have demonstrated fetal abnormalities or if there is positive evidence of fetal risk based on adverse-reaction reports from investigational or marketing experience or both, and the risk of the use of the

drug in a pregnant woman clearly outweighs any possible benefit--for example, safer drugs or other forms of therapy are available.

It should be noted that most drugs for all populations fall into Category C.

[Slide.]

This slide shows the anesthetic agents from the previous tables in the previous slides that are Pregnancy Category A or B. All of those drugs are Category B.

So, why is it that information available on the label regarding pediatric use and use during pregnancy is relatively limited especially for the older drugs?

In part, it reflects the growing role of the FDA in regulation of drug products and the evolution of what the agency could require of sponsors for approval of a product to be marketed in the United States.

[Slide.]

This chart shows the changes in the FDA's regulatory authority and requirements for marketing

approval over the past 70 years. It also includes the years marketing began for some key anesthesia drug products.

We start off with pentothal being marketed in 1934. In 1937, elixir of sulfonamide containing the poisonous solvent diethylene glycol killed 107 persons, many of whom were children. This dramatized the need to establish drug safety before marketing and it helped enact the pending Food and Drug law. In 1938, Congress passed the Federal Food, Drug, and Cosmetic Act and, most notably, it required that new drugs be shown to be safe before marketing and provided that safe tolerances be set for unavoidable poisonous substances.

During the next 22 years, halothane, meperidine, and methohexital were introduced to the market. In 1962, thalidomide, marketed as a new sleeping pill in Europe, was found to have caused birth defects in thousands of babies born in western Europe.

The role of Dr. Frances Kelsey, an FDA medical officer, in keeping the drug off the U.S.

market, gave rise to public support for stronger drug regulation; to wit, the Kefauver-Harris Drug Amendments were passed in the same year to ensure drug efficacy and greater drug safety.

For the first time, drug manufacturers were required to prove to FDA the effectiveness of their products before marketing them. That evidence had to consist of adequate and well-controlled studies, a revolutionary requirement.

The 1962 Amendments also required that FDA specifically approve the marketing application before the drug could be marketed and asked the Secretary to establish rules of investigational new drugs including a requirement for informed consent of study subjects.

In 1966, FDA contracted with the National Academy of Sciences and National Research Council to evaluate the effectiveness of 4,000 drugs approved on the basis of safety alone between 1938 and 1962.

In 1968, FDA initiated the Drug Efficacy

Study Implementation to follow through with the recommendations of the National Academy of Sciences. That same year, fentanyl was approved and two years later so was ketamine.

In 1971, the National Center for Toxicological Research was established to examine biological effects of chemicals in the environment and extrapolate data from experimental animals to human health.

In 1973, the U.S. Supreme Court upheld the 1962 drug effectiveness laws and endorsed FDA action to control entire classes of products by regulation rather than to rely solely on time-consuming litigation.

Between '79 and '92, several important anesthetic agents were approved. Most notable are Propofol, Midazolam, Isoflurane, and later Desflurane.

In 1989, the FDA issued guidelines asking manufacturers to determine whether a drug is likely to have significant use in older people and to include older patients in clinical trials.

In '93, the FDA issued the gender guideline which called for assessment of medication responses in both genders and revoked a previous guideline that excluded women of child-bearing potential from clinical trials.

Regulations were promulgated stating that there must be a pediatric use section in the label--this was in 1994--even if it stated only that safety and efficacy of the drug product had not been evaluated in pediatric patients.

In 1995, Sevoflurane was approved.

In 1997, the Food and Drug Modernization Act, or FDAMA, supported accelerated approval and gave an extra period, a six-month period, of marketing exclusivity to manufacturers that carried out studies in children.

In 1998, the FDA promulgated the Pediatric Rule, a regulation that required manufacturers of selected new and extant drug and biological products to conduct studies to assess their safety and efficacy in children. A federal district court later overturned the Rule.

In 1998, the FDA also promulgated the demographic rule which required that the marketing application analyze data on safety and effectiveness by age, gender, and race.

In 2002, the Best Pharmaceuticals for Children Act improved safety and efficacy of patented and off-patent medications for children. It continues the exclusivity provisions for pediatric drugs as mandated under FDAMA in which market exclusivity of a drug is extended by six months in exchange for the studies conducted in children.

In 2003, the FDA was given clear authority under the Pediatric Research Equity Act to require that sponsors conduct clinical research into pediatric applications for new drugs and biological products.

It is interesting to note that most of the regulatory efforts related to pediatric patients have occurred over the past 10 years. Let's look at how pediatric indications for a new drug product are generally secured.

[Slide.]

Typically, studies are conducted first in adult populations and the drug is approved for marketing with that limited population.

Often pediatric studies are performed as a postmarketing or Phase IV commitment by the sponsor. Preclinical trials in juvenile animals are not always required prior to study in pediatric patients.

In February of last year, a Guidance for Industry titled "Nonclinical Safety Evaluation of Pediatric Drug Products" was issued to provide input as to when such studies are appropriate.

Pediatric clinical trials are generally designed to provide proper dosing guidelines for the different segments of the pediatric population.

An indication is granted if efficacious dosing regimens are identified and safety concerns that would outweigh the benefit of the drug are not identified.

When safety data are analyzed, efforts are made to identify both the adverse events noted in

the studies of adult patients and those which are unique to pediatric patients.

The bottom line is that the requirements for approval with the pediatric indication have grown more rigorous in recent years with the ability to detect rare or very subtle adverse events is limited at the time of approval, as is the ability to identify such events if they will not express themselves for up to several years following exposure especially to an acute administered drug.

Again, this is all related specifically to the time of approval.

[Slide.]

So, let's go back and look again at the alternatives to using the sedative hypnotics or inhaled anesthetic agents.

[Slide.]

Local anesthetics which can be administered in topical, regional, or neuraxial fashion may be suitable for some procedures. However, the toxicities in pediatric patients have

not been fully elucidated for all of these drugs, and sedation is generally utilized for anxiolysis, amnesia and the ability to apply the local anesthetic with patient comfort.

[Slide.]

Similarly for opioids, they may be suitable for some procedures, as well. However, here, too, toxicities for pediatric patients have not been fully elucidated, and sedation is also generally utilized for anxiolysis and amnesia.

[Slide.]

No anesthesia, although it may work for some procedures, has become less of a viable alternative especially with research that has demonstrated sensation by the fetus and young children of pain and morphological changes that have been found in rodent brains for animals that have not been treated with analgesia during stressful procedures.

There is other data to suggest that premature infants showed metabolic stress responses postoperatively that can be blocked with

intravenous opioids, and further information that shows that stress response can be reduced both with analgesia and anesthesia.

There is further study to show additional evidence of bad outcomes for children who are not properly anesthetized, and I believe that will be a topic Dr. Soriano will cover in more detail later on.

The option of delaying surgery is pretty much a no-go as most of the surgical procedures are performed either for life-threatening or urgent type of procedures that are needed to be done, and cannot be delayed.

[Slide.]

We know that we do not have sufficient data to detect a problem that has been seen in the animal studies at the time of approval. What happens post approval? The agency does have a means of finding adverse events and collecting them post approval and this is referred to as the "Adverse Event Reporting System Database" or AERS.

There are limitations to this type of

database. Reporting to it is strictly voluntary. People that submit their data do so from all walks of life. There are clinicians who submit data, patients, friends and family members of patients, lawyers. So, those who submit the data are not necessarily those who have close contact with the patient or fully appreciate the clinical status of the situation.

The data captured is also a hit-and-miss type of an ordeal. Timing of the adverse event tends to be critical. If it is temporally related to the administration of drug, it is more likely to be reported than if it happens distant in time to the administration.

The nature of the events, those that are more dramatic tend to be reported, those that are subtle tend to be looked over.

There is also a significant amount of data in the AERS database submissions that is missing, everything ranging from patient age, gender, comorbidities, comedications and these are significant in terms of the ability of the agency

to comment on the type of data that is received.

Lastly, because this is a voluntary system, having a true numerator for the incidence of these adverse events is difficult to assess. It is likely that the data from the AERS database underestimates the numerator, but in addition to that, there is no way to make an accurate determination or assessment of what the denominator would be in terms of exposure of these populations, so it is quite limited, yet it is all we have.

[Slide.]

Nonetheless, we asked the Office of Surveillance and Epidemiology to examine the AERS database for events related to ketamine between the time of approval and the end of January this year.

In all they were able to identify 153 pediatric reports, those in patients less than or equal to 16 years of age.

They specifically looked at certain organ classifications for the adverse events. Those were musculoskeletal, nervous, and psychiatric.

From that over 150 reports, they were able

to narrow that down to 58 that applied to those organ systems. They were surprisingly a little bit more heavily weighted towards later years than the earlier. Typically, adverse events are more frequently reported following initial approval of a drug rather than later on.

[Slide.]

Among these 58 reports, there were 4 fatalities, and 25 reports involved at least one other drug that acted at the NMDA or GABA receptors.

[Slide.]

These are the adverse events that were noted. Many of these are labeled adverse events by the product, all of them are rather dramatic in nature, and all of them occurred at the time of administration.

So, from at least the AERS database, we don't have a whole lot of information that would be helpful with regard to the issue at hand, this neuroapoptosis in this vulnerable population.

[Slide.]

So, where do we stand? A safety signal has been identified in animals for many drugs used to provide sedation and anesthesia. This database is growing.

The relevance of these animal findings to the pediatric patient population is unknown, yet we still have a need to provide anesthesia to these patients and, for the most part, the situation cannot be avoided.

There is also no available alternative therapy that has been shown to be safer.

Where do we go from here? We will have further discussion, a more detailed discussion, on both the preclinical data and the clinical relevance this morning and then where we go from here will be the topic of this afternoon's discussion.

Thank you.

DR. SHAFER: Thank you, Dr. Simone.

We have time for some questions if people would like to direct questions to Dr. Simone about his presentation.

[No response.]

DR. SHAFER: Dr. Simone, thank you very much.

The next presenter is Dr. Dan Mellon, who will talk about the history of the preclinical data and anesthetic-induced neuroapoptosis.

**History of Preclinical Data:**

**Anesthetic-induced Neuroapoptosis**

DR. MELLON: Good morning and welcome.

[Slide.]

What I would like to do today over the next 20 minutes or so is to summarize some of the studies that have been conducted predominantly and published in the literature, focusing on the in vivo studies that have been presented so far to characterize some of the effects of anesthetic drugs on the developing brain.

I would also like to outline from a historical perspective some of the steps taken by the agency to further characterize the potential clinical significance of these findings.

[Slide.]

The first paper that I think you will be seeing several times today was a paper published in Science in 1999 by Drs. Ikonomidou et al. and forgive me if I am mispronouncing that, in collaboration with Dr. John Olney, who we are fortunate to have with us today.

This particular paper was interesting because they utilized a 7-day-old rat model, and although the primary data that was presented was based upon a drug called MK-801, which was not a clinically approved drug but was a very potent NMDA receptor antagonist.

This particular report described some pretty significant changes in the developing brain in this particular model, and also of importance is that they noted that several studies were conducted utilizing ketamine, in this case a regimen that consisted of 20 mg/kg subcutaneously, 7 different injections administered 90 minutes apart over a 9-hour period.

[Slide.]

I borrowed a figure from that particular

paper, and this is a figure that you will be seeing actually something very similar to over and over again today.

What this particular diagram illustrates is a brain slice from these 7-day-old rat pups. In panel A, this particular pup was treated with a saline administration and, in panel B, the animal was treated with MK-801, an NMDA receptor antagonist.

The staining here is utilizing a method to try to detect apoptosis. The dark spots that you see and the dark areas that you see in panel B represent neurons that are undergoing an apoptotic phenomena leading to the removal of those neurons from the brain.

You can see from this particular slide that the apoptotic neurons are actually occurring in a widespread number of tissues within the brain, and in marked contrast to the observations in panel A where, although there is some staining which is indicative of the natural process that takes place during brain development, the instance of this

particular phenomena is pragmatic.

As noted in this particular paper, ketamine administration at 20 mg/kg sub-Q over every 90 minutes for a total of 7 injections was reported to produce similar results.

This is one of the first papers that I actually was handed when I arrived at the FDA, trying to understand what this particular phenomenon was and how it may impact our understanding of the safety of drugs.

[Slide.]

Based a large part upon these particular observations, in 2000 the Agency raised some concerns regarding a proposed NIH clinical trial to study ketamine in children.

This particular paper, as is clear from this, was not unnoticed by the Agency at this time, and ultimately has led to the establishment of an FDA-wide Expert Working Group that consisted of FDA neurotoxicologists representing both the Center for Drug Evaluation and Research, and the National Center for Toxicological Research, as well as

CDER's Office of Pharmaceutical Sciences, which is a branch that is involved in laboratory research, and they established a Rapid Response Team to try to further characterize and understand this particular phenomena.

In addition, lengthy discussions have taken place since this particular paper publication, as well as subsequent papers, including discussions with the Research Subcommittee of the Pharmacology Toxicology Coordinating Committee.

The PTCC is composed of supervisory pharm-tox individuals who are involved in all of the new drug-review divisions and this subcommittee also contributed their thoughts and input on how best to try to approach this particular problem and understand it.

[Slide.]

The FDA investigations have actually been, in many regards, published and one of the primary efforts that has taken place was actually a very distinct and clear review of the literature at the

time on NMDA receptor systems and the potential for these systems to have a role in neurotoxicity of the developing brain. This review was published by Dr. Haberny et al. in 2002.

In addition, the Office of Pharmaceutical Sciences noted that they were to duplicate and extend the findings that were reported by Dr. Olney's group in the 7-day-old rat and confirmed that these findings indeed were reproducible. These findings were reported by Dr. Scallet et al. in 2004.

Based upon considerable discussions within the Agency, it was determined that, since duplication of Dr. Olney's findings was able to be obtained, this would support the need for studies in a nonhuman primate model.

It was recommended that the rat model could certainly be used to help further pursue our understanding of this particular phenomena, particularly with a focus on some of the mechanistic perspectives of how this is occurring and how it could be interpreted.

Ultimately, CDER and NCTR decided that it was best to nominate ketamine to the National Toxicology Program to obtain funds to support the nonhuman primate studies.

The National Toxicology Program is an interagency program that is designed to try to study and further evaluate our understanding of how various environmental compounds can affect human health.

[Slide.]

Ultimately, in 2002, the nomination was reviewed by the National Toxicology Program with the approach to try to characterize the potential effects of ketamine on neurodegeneration in the developing nonhuman primate, as well as include some behavioral assessments to try to understand what the long-term implications are of some of these findings in primate infants that were exposed to ketamine during development.

These studies were approved for conduction by the NTP but ultimately were unable to be funded.

However, the Agency has pursued these studies

nonetheless and they are currently being completed by the National Center for Toxicological Research.

Dr. William Slikker, who will be joining us a little bit later, and will be presenting later this morning, will describe to you the progress of these studies in the nonhuman primate.

[Slide.]

I borrowed this slide from Dr. Slikker, so I must thank him. What I would like to try to describe here is the time window for vulnerability to the neurotoxic effects of an NMDA receptor antagonist specifically focusing on these red sections here, which were the apoptotic neurodegeneration as described by Dr. Olney.

In fact, based on upon work conducted by Dr. Olney, it is clear that the window of vulnerability in the rat model appears to be within the first weeks after birth, and I note that the time scale here between the rat, the Rhesus monkey, and the human is obviously very different reflecting the expected life expectancy of these particular species. But what is important to note

is that this period of synaptogenesis, as identified in the vulnerability as identified by Dr. Olney's group, appears to correlate as an approximate time frame that is longer in the human that actually starts in the third trimester and goes out to what we believe to be approximately three years.

In the Rhesus monkey, this time period also occurs later in gestation and extends out to about two months. So, what we can see is that although this is a very critical time period, it does occur at slightly different durations of time frame that depends upon the development of the species.

Also, in this slide is a section of a time period where the vulnerability to excitotoxic neurodegeneration is noted. I point this out specifically because today's focus is primarily on the apoptotic neurodegeneration and a lot of information has been obtained and reviewed over the years regarding this potential for these compounds to produce excitotoxicity.

[Slide.]

Just to try to contrast that, the apoptotic neurodegeneration, which is today's focus, has primarily been focusing on the effects on the developing brain, whereas excitotoxicity, in large part, although it can occur in multiple areas, has been shown with ketamine and other compounds to be focusing on the adult brain.

The response to apoptosis is really a cell death that does not involve necrosis, as Dr. Simone indicated, and you will be hearing more about as well.

The excitotoxic neurodegeneration is really manifesting itself histopathologically as vacuolization of the neurons and eventual necrosis if the dose and the duration and depending upon the drug are increased.

The apoptotic neurodegeneration phenomena that we are focusing on today has been shown to be occurring in widespread areas of the brain. In terms of MK-801's effects and some of the other NMDA receptor antagonists, the excitotoxicity

degeneration occurring in the adult is really focusing in some distinct brain regions.

Apoptosis, as will be described to you further, can actually be a very physiological phenomenon and is part of the normal brain development. It is possible, however, as Dr. Olney will describe to you, that environmental insults may perhaps accelerate this process and it is unclear at this point in time whether or not some of these neurons that are being removed are neurons that would have been removed normally and simply this process may accelerate that or if these neurons would not have normally been removed.

In terms of excitotoxic degeneration, it has primarily been focused as more of a pathological response--for example, ischemia--and what is actually very interesting and challenging about this particular question is that an NMDA receptor antagonist, and indeed a lot of anesthetics, have actually been looked at as potential means of blocking excitotoxicity produced by ischemia.

So, it is a very distinct contrast to the potential toxicity of these compounds at different time periods of development.

[Slide.]

What I would like to do next is focus on some of the primary studies that have been published in the literature that have looked at in vivo models to determine whether or not anesthetic agents have produced this phenomenon. I am going to be focusing on some of these papers just simply by pushing some highlights as to how this information has been accumulating over the years.

We are fortunate today to have some of the primary investigators with us who will be describing their findings in much more detail as time proceeds. So, I am only going to be focusing on some highlights and filling in some of the studies that have actually been published that are not necessarily available--we don't have the primary investigators available to us today to contribute.

But what I would like to illustrate is how

this information is slowly accumulating and leading to a greater understanding of the phenomenon.

The first paper that I would focus on after Dr. Ikonomidou's paper is by Hayashi et al. in 2002, co-authored by Dr. Soriano, who is with us today. Their model was a neonatal rat post-natal day 7 exactly as was used in the Science paper of 1999. They utilized intraperitoneal injections, histopathology at 24 hours after the last injection.

Here, they compared saline injection with a single dose of ketamine as well as the repeated dose phenomenon that was utilized in the procedures reported in '99.

[Slide.]

The important aspect of this particular paper is that they noted that single doses of ketamine did not appear to produce evidence of neurodegeneration. They confirmed that the repeated doses of ketamine can produce evidence of neurodegeneration in this particular rat model. But what is important here is that it suggests that

there are exposure conditions that do not produce neurodegeneration.

[Slide.]

In 2003, a very important paper was published by Dr. Todorovic. This is a neonatal rat model. Of course, Dr. Olney is very much involved in these, and you will see a lot more about this, so I am only going to give you a highlight. But the important part about this particular publication is that they utilized 6-hours worth of anesthesia and, rather than simply looking at ketamine or some of the other compounds, such as MK-801, they utilized an anesthetic regimen that consisted of nitrous oxide, isoflurane, and midazolam. The endpoints included histopathology as well as behavioral testing up to 160 days post treatment, an electrophysiological evaluation of hippocampal slices.

[Slide.]

This is one of the first published papers that actually suggested nitrous oxide, isoflurane and midazolam can also produce neuroapoptosis in

the rat model extending the number of compounds that perhaps may be implicated in this particular phenomenon.

This was one of the first studies to really try to mimic the clinical anesthetic setting which, as we all know, in anesthesia, in multiple times, multiple compounds are used, not just simply one.

The findings report that for the neonatal rats exposed to 6 hours of this mock anesthetic cocktail produced widespread apoptotic neurodegeneration in the developing brain, deficits in hippocampal synaptic function, and persistent memory and learning impairments.

[Slide.]

This paper sparked a lot of discussion and it illustrates one of the challenges that we face when we look at animal models and try to understand how we can extrapolate risk to humans.

In many circumstances, what I am describing in here is true with all drugs and during development is true every time we deal with

these from a preclinical perspective and that, in many regards, there are various species differences. We understand that.

We know that in many circumstances we can identify most sensitive species and in toxicology, where we look at the development of a new drug, that is one of the reasons why we require two species, at least one non-rodent, to try to evaluate toxicological phenomena.

In some circumstances, the most sensitive species may not be the most appropriate species--for example, if there are metabolic differences and one species may produce a different metabolite than is produced in humans and, if that metabolite is causing some of the toxicities, that toxicity may not be relevant.

These are things that have to be taken into consideration when we evaluate toxicological findings. There are certainly developmental differences between species that may impact the outcome and the interpretation of the study and there are always technical study design challenges.

One of the things that we recommend to sponsors all the time is that we encourage them to try to develop their toxicological studies to mimic the clinical setting as closely as possible.

In the anesthetic world, that is even more challenging than other areas and that is because there are a lot of concurrent medications that are utilized in a clinical setting. In anesthesia, there is obviously tight control over blood gases and nutritional support, hemodynamic stability.

These are not simply mimicked in the animal model. However, efforts have been underway to do so and it is very difficult to try to extrapolate the doses administered through a clinical setting.

From a nonclinical perspective, initially, when we don't have any data in humans whatsoever, we base that on body surface area. But once we obtain some pharmacokinetic comparisons, we can allow a much more comfortable establishment of the potential implications of these findings.

In the initial studies that were reported

in the literature, it was not clear exactly what blood levels were obtained in those animals to try to interpret it and that is where the Office of Pharmaceutical Sciences has helped us contribute some of the understanding of the effects of ketamine, which we will discuss in a moment.

Ultimately, one of the other challenges in this particular arena is trying to understand what the pharmacodynamic effects are and how they are different potentially between species.

When you look at some anesthetics, the anesthetic effect may require different physiological concentrations than are occurring in humans, and it is not entirely clear exactly what levels are necessary in order to produce an anesthetic state and how that may ultimately be interpreted in terms of trying to understand the toxicological phenomena observed.

These are challenges that we face and are particularly difficult with this particular question.

[Slide.]

As I mentioned, the Agency itself was conducting studies on their own to try to further understand and better interpret some of the findings that were reported by Dr. Olney's group, and this particular paper, first offered by Dr. Scallet et al. and published in 2004, was designed to confirm and extend the original findings of Dr. Ikonomidou, et al.

Again, they utilized the neonatal rat model, post-natal day 7. They compared subcutaneous injections, histopathology at 24 hours. They compared saline with repeated doses of ketamine, 7 total, once every 90 minutes of 10 mg/kg and 20 mg/kg with that of a single dose of ketamine at 20 mg/kg.

The important part about this particular study is that they looked at plasma levels of ketamine shortly after administration to try to understand what types of exposures were occurring.

[Slide.]

This table summarizes the findings. Ketamine administered at 10 mg/kg, 7 times, did not

produce evidence of neuroapoptosis. When you looked at the plasma levels that were obtained in these particular animals, and in this case I am comparing it to what has been reported for ketamine levels necessary clinically for major surgery, which we view as a worst case scenario, of about 2 mcg/ml, the exposures were approximately equivalent.

Ketamine, a single dose at 20 mg/kg, did not produce evidence of neuroapoptosis. Comparing plasma levels that were obtained in this particular study provided an exposure margin of approximately 2.7.

The regimen that was reported by Drs. Ikonomidou, as well as Dr. Hayashi et al., of 20 mg/kg, 7 times, did produce evidence of neuroapoptosis, confirming these findings a third time.

The levels that were obtained with that regimen were approximately 7-fold higher than those that were required for major surgery. So, it suggests that there is perhaps a threshold level

where exposure to ketamine may not produce this response and exposure to ketamine at higher levels may produce these responses both from a dose and perhaps a duration perspective.

[Slide.]

Drs. Fredriksson et al. conducted some studies in Sweden, and I mention these because they unfortunately will not be able to be here today. But what is interesting about this particular study was that it added a second species.

It was conducted using mouse pups neonatal day 10. Here, they combined ketamine, 50 mg/kg, diazepam plus the combination of ketamine and diazepam to vehicle.

[Slide.]

The important findings that were noted in this particular report was that this is adding a second rodent species where neuroapoptosis was noted with these compounds.

They noted that ketamine and diazepam alone produced neurodegeneration in the mouse. Ketamine and diazepam interestingly enough produced

different neuroanatomical patterns of neurodegeneration suggesting that perhaps compounds that act at different targets, although they may produce similar responses, may have subtle differences.

They also reported that the combination of both ketamine and diazepam produced a greater degree of neurodegeneration than either drug alone.

They reported functional deficits at 2 months of age in motor activity and learning performance in the animals that were treated with ketamine as well as the combination of ketamine and diazepam.

[Slide.]

Mickley et al. 2004. The model embryonic rat fetuses treated through the maternal circulation. They examined not neuroapoptosis but conditioned taste aversion for learning and memory.

[Slide.]

The results of the findings are interesting. Rat fetuses treated on embryonic day 18 with ketamine through the maternal circulation

taught and learned conditioned taste aversion, remembered them appropriately, whereas, animals that were treated on embryonic day 19 did not. Exposure of the rat fetus to ketamine in utero resulted in long-term behavioral deficits in the adult animals.

The data suggest that there are critical periods of gestational development in which the rat is susceptible to long-term behavioral neurotoxicity, similar to the findings that Dr. Olney's group also reported.

[Slide.]

Again, some studies that you will hear more about in a little while from Drs. Todorovic and Olney, the potential of ketamine and midazolam, individually or in combination, to produce apoptotic neurodegeneration in the mouse. Ketamine was administered at 10, 20, 30, 40 mg/kg, midazolam at 9, and ketamine combination with midazolam at 40 and 9.

[Slide.]

The findings report that ketamine at 10

mg/kg produced a slight, but nonsignificant, increase in neuroapoptosis. 20 mg/kg and higher reproduced the findings.

Interestingly, they noted that doses between 30 and 40 actually showed a sharp increase in the incidence of neuroapoptosis, again supporting the concept that there is certainly perhaps a threshold and a level of these particular compounds that are more problematic than others. Midazolam produced a dose-dependent increase in neuroapoptosis. Ketamine plus midazolam produced a greater degree of an increase in neuroapoptosis than either drug alone.

[Slide.]

Rudin et al. 2005, studies conducted in Israel. They report findings in the mouse model with ketamine administered at various doses subcutaneously.

[Slide.]

In this particular study, ketamine produced neuroapoptotic response at 5 mg/kg and above. Neuroapoptotic neurons in this particular

model actually peaked at 72 hours. In the rat they appeared to peak between 24 and 48 hours. They were able to still detect evidence of apoptosis 7 days after treatment.

This paper published and reported that there were no gross neurobehavioral effects noted at day 7 although I have to note that the neurobehavioral phenomena that were evaluated were relatively limited. Nonetheless, additional findings.

[Slide.]

Now, I mentioned at the very beginning I was going to report information that has been in the published literature about in vivo models so I am going to hint at this particular publication by Dr. Slikker et al. coming from the National Center for Toxicological Research.

This has recently been accepted for publication; the title of the paper, "Ketamine-induced Neurodegeneration in the Perinatal Rhesus Monkey." The model that was evaluated was the Rhesus monkey models that have

been established by the Agency looking at gestational day 122, post-natal day 5 and 35. Ketamine administration was conducted for a 24-hour period with a 6-hour withdrawal period, and comparing that also to 3 hours in post-natal day 5 animals.

Now, because I mentioned I am going to report published information and this is not technically published, I am going to allow Dr. Slikker to tell you more about his findings.

[Slide.]

However, what I do what to leave you with at this point in time is that, based upon the growing amount of information both within the public domain and in the published literature, from studies coming from academia as well as studies that have been conducted by the Agency since the beginning of this particular question, it is becoming clear that multiple anesthetic drugs have been implicated in this phenomenon, NMDA receptor antagonists have been implicated, GABA-ergic drugs have been implicated.

Other compounds have yet to be tested. Opioids have not been evaluated. Local anesthetics have not been evaluated, but at this point in time, we have more compounds that have been implicated.

We now have evidence in the published literature, and you will see today, that multiple species have shown this phenomenon--rats, mice. You will see information today in a guinea pig model, you will see information today in the monkey.

Long-term behavioral changes have been reported with several of these particular studies, and the data that is accumulating so far suggest that perhaps the combination of drugs that are commonly also utilized in an anesthetic regimen may actually be more problematic than the individual drug alone.

You will also see some information--not that I have presented here--but you will see some information and there are some growing studies in this regard to looking at ways of trying to potentially block these responses. The challenge

that we face is ultimately how to interpret this information based on preclinical studies when an endpoint in a clinical setting is not clear.

Ultimately, from a regulatory perspective, these studies are fascinating and very important in terms of trying to understand the phenomena. Exactly how we are going to utilize that information is not clear.

At this point in time, I would like to allow the primary investigators who have been conducting these particular studies over the years to give you their interpretations from their perspectives and present that information.

Thank you very much.

DR. SHAFER: We have about two to three minutes for questions.

DR. ANAND: I have two questions. One is all of the preclinical studies have been done in the absence of any painful stimulation or surgical injury, that is my understanding. So, this is anesthesia in the absence of any type of other surgical intervention. Is that correct?

DR. MELLON: That is correct.

DR. ANAND: The other question; you had mentioned that the neuroapoptosis in humans extends from the third trimester of pregnancy to three years of age. What data is that based on?

DR. MELLON: That is an extrapolation from what we understand of the development of the brain itself, and it is an estimate. It is purely an estimate. We do not completely understand that phenomenon but, based upon the existing information that we have--and I am sure Dr. Slikker will provide some additional insights into that--that is what we are predicting, as well as Dr. Soriano.

DR. ANAND: I would submit most humbly that it is pure speculation at this point in time.

DR. MELLON: Fair enough.

DR. WLODY: I have a question about the Fredriksson study, which was on Slide 18. I think it is interesting. It is comparing a dose of ketamine 50 mg/kg, diazepam 5 mg/kg, with a combination of both drugs at the same dose.

I wonder, it seems you would assume that,

when you are combining two drugs at the same dosage level, two drugs that have different methods of action, that you would see--and the toxicity--and I wonder if it wouldn't make more sense to use a smaller dose of each drug to obtain the same total anesthetic action. I wonder if that might not decrease the toxicity by using subtoxic doses of each of those drugs that have toxicity at two different mechanisms.

DR. MELLON: Since I have not conducted that study, I certainly acknowledge your point. I think it is a very good point, it is certainly something that I think needs to be taken into consideration as we pursue further evaluations of combinations of these particular products.

In this particular publication, that was what they had examined. If I recall correctly, I believe that those were additive effects. I don't have the paper in front of me, but your point is well taken.

DR. SHAFER: Dr. Henthorn.

DR. HENTHORN: Thanks for a nice

presentation. I was struck by your question. Is there any relation to this--to fetal alcohol syndrome?

DR. MELLON: That is something that you will see more about, that Dr. Olney will be presenting as well, and Dr. Simone did mention that effect as well. I think from the pharmacological perspective, it is clear that ethanol does block an NMDA receptor antagonist, and it also potentiates GABA. Exactly how that compares to the anesthetic regimen is not exactly clear.

As Dr. Simone noted, there is a phenotype with fetal alcohol syndrome that has become obvious. We have not noted that to date with anesthetics.

DR. SHAFER: Dr. Armstrong.

DR. ARMSTRONG: It wasn't clear to me, I don't see it in the presentation, but maybe you can clarify it.

One of the concerns that we would have in looking at the pediatric population certainly would be the child who has the single exposure, but there

is a large population of children where the risk would be cumulative exposure because of repeated procedures, the child with leukemia, the child with a GI or bronchoscopy issue.

Do we have any data in the preclinical models that has looked at cumulative repeated exposures rather than the single exposure that seems to be presented in the data?

DR. MELLON: At this point in time, we do not have that data. I think certainly these are things that would have to be taken into consideration and it would be nice to be able to obtain that data. Possibly understanding some of the mechanisms mediating this response can help us interpret and predict what those challenges are and how we can try to interpret that. That is information that would be very beneficial.

DR. SHAFER: Two last questions. First, Dr. Raja.

DR. RAJA: Dan, you presented the data on the Agency studies by Scallet et al. reported in 2004, and you presented the exposure margins for

those, and then you gave a worst case scenario of 2 mcg/ml from human studies. Were those data obtained from adults or pediatric population?

DR. MELLON: They were obtained predominantly from adults, as well as pediatrics. We were looking at what was available and reported, plasma levels for major abdominal surgery if you were going to use ketamine alone. And the value that we based that exposure margin on was approximately 2 mcg/ml.

DR. SHAFER: Last question. Dr. Kirsch.

DR. KIRSCH: I am struck by the lack of definition of gender in any of the studies. It is well known that, even in cell culture, neurons from female pups react differently than from male pups.

Is there a gender effect of this finding?

DR. MELLON: I would defer that to some of the primary investigators at this point in time, but I think that you also raise a very interesting point. I think these are questions that still need to be addressed fully as time goes on.

DR. SHAFER: Thank you very much, Dr.

Mellon.

Our next presentation will be from Dr. John Olney, who will be talking about the Preclinical Developmental Neurotoxicity.

Dr. Olney, thanks for coming to the meeting today.

**Preclinical Developmental Neurotoxicity**

DR. OLNEY: Thank you for inviting me to this important meeting.

[Slide.]

This is the topic I will be presenting on and I have my coworkers listed here, not in order of importance, but in order required to make a nice futuristic pyramid. You will notice that Vesna Jevtovik-Todorovic is at the bottom holding the entire structure up.

[Slide.]

Well, I wanted to give some conceptual orientation to begin with. Developing neurons are programmed to commit suicide if they are unsuccessful in meeting important developmental milestones. This has been known for a number of

years. Normally, most differentiated neurons successfully meet these milestones, so only a small percentage are obliged to commit suicide.

But there are aberrant circumstances, at least we have postulated that in the past, that can interfere and cause failure of many neurons to meet important milestones, death of many neurons that would have normally survived, and neurodevelopmental disturbances that normally do not occur.

So, that is the conceptual orientation here, and we want to know, then, are there aberrant circumstances that can indeed trigger neuroapoptosis.

[Slide.]

In 1999, we observed that drug-induced developmental neuroapoptosis can be produced using drugs that block NMDA glutamate receptors.

[Slide.]

We studied that phenomenon for its window of vulnerability and has been already described, we found that it coincides with the period that has

been described as the brain growth spurt period, also pretty synonymous with the period of rapid synaptogenesis, and that period occurs at different times in different species relative to birth--in the rodent, approximately the first three weeks after birth and, in the human, from somewhere around mid-gestation to several years after birth.

[Slide.]

The first thing we studied was whether other disturbances in other transmitter systems could trigger this phenomenon. And we studied several systems and drew a blank until we came to the GABA system. We studied several GABA-A agonists, agents that activate GABA-A receptors, and found that they did trigger this phenomenon.

[Slide.]

So, we had two classes of drugs, an NMDA antagonist and GABA-A agonist, and that caused us to turn our attention to ethanol which has both NMDA antagonist and GABA-mimetic properties and, over the millennia, has damaged more fetal brains than any agent in the human environment.

[Slide.]

So, we studied ethanol and found that it quite robustly triggered neuroapoptosis in the developing rat brain. We postulated that provides a potential explanation, at least partially, for the reduced brain mass and neurobehavioral disturbances associated with the fetal alcohol syndrome.

[Slide.]

This is what it looks like in the infant mouse brain following administration of ethanol. In the saline-treated animal, at this magnification you can't see an effect. However, this a sprinkling here and there of individual neurons undergoing physiological cell death, the natural phenomenon that does occur during development.

When that process is accelerated and increased in magnitude by ethanol, you have regions of the brain that are having such dense degeneration that it stands out using any of a number of stains. This is a silver stain. We also used TUNEL staining in that study and showed

exactly the same pattern of degeneration. I will show you some other types of stains later.

You can see that when the brain is sectioned at three different levels, at each level there is a prominent pattern of degeneration involving a number of different neuronal groups.

[Slide.]

We studied this phenomenon by electron microscopy, which is the gold standard for identifying apoptotic neurodegeneration. Early in the process, the cytoplasm doesn't show many changes while the nucleus is just beginning to show changes. However, in the cytoplasm, we can find subtle effects when we look at the mitochondria.

Here is a mitochondrion, for example, magnified here to show that there is a stripping of its external membrane and a dissolution of the internal matrix. Those are the first signs that we see of this neurotoxic process.

Later, about an hour or so later, the cell starts showing these spherical balls of clumped chromatin and the nucleolus disaggregates into

wormlike structures that disperse throughout the cell.

Notably, the nuclear membrane, which should look like this, has become fragmented and discontinuous, allowing intermixing of the nucleoplasm and cytoplasm and the entire cell becomes condensed. These are the classical features of neuroapoptosis.

[Slide.]

We studied the mechanism using mutant mice and western blotting and other methods. The pathway is what has been identified previously in many different studies--not pertaining to this phenomenon, but pertaining to apoptosis--has been identified as the intrinsic bax-dependent mitochondrial pathway whereby bax is caused to translocate to the mitochondrial membrane where it causes increased permeability allowing cytochrome c to leak out, which causes activation of Apaf-1 and caspase-9, and that, in turn, activates caspase-3, the executioner of the cell.

We have determined that commitment to cell

death occurs back here, and this is important because it indicates that, by the time the process has arrived at caspase-3 activation, the cell is already committed to cell death. That means that you can use immunohistochemical marking using antibodies against activated caspase-3 to identify the cell that is already committed to cell death.

[Slide.]

So, we have used that staining method. This is the infant mouse brain 8 hours following ethanol treatment and you can see the many neurons that are labeled using caspase-3 activation immunohistochemistry.

It is a remarkably bilaterally symmetrical reaction in which, on each side of the brain, the same neuronal groups are involved, and that is the kind of pattern we see with this type of pathology.

[Slide.]

This is the same activated caspase-3 immunocytochemistry of the mouse brain following ethanol, and here I am showing what the saline control brain looks like. There are cells that are

staining by this method because there are cells that are degenerating naturally during development.

But you can see that they are sparse and scattered in contrast to the pattern following ethanol which is concentrated, highly concentrated, in specific neuronal groups and layers and is much more dense in much higher numbers.

[Slide.]

We have studied the process, the time course of degeneration over time, by a combined light- and electron-microscopic method. These are plastic sections that would be used to view the brain prior to cutting electron microscopic sections.

We are looking here at the anterodorsal thalamic nucleus, which is outlined like this in the developing mouse brain. The normal picture is here. Twelve hours after ethanol, you can see these many dark structures which, under magnification, are apoptotic profiles and, by three days following ethanol treatment, the region is pretty well cleared out of the degenerative

debris--a few stragglng phagocytic cells--and there aren't any traces left, such as fibrosis or glial hypertrophy.

The only traces are an absence or a lack of many of the neurons that previously populated that region. The decrease, we cut these sections across the plane of maximal diameter or dimension of that nucleus, and when you compare this picture with that, there is approximately a 62 percent decrease in neuronal mass.

This is permanent deletion of neurons from the brain that ethanol is causing.

[Slide.]

Well, we have studied a number of different classes of agents, and this is the list currently that we have found will reproduce this phenomenon; NMDA glutamate antagonists, GABAA agonists, and ethanol.

We studied anticonvulsant drugs, many of which are GABA-mimetic agents, but some of which are sodium channel blockers, and we found that the sodium channel blockers also produced the

phenomenon.

We also found that commercial solvents do and we are not sure exactly how they relate to the other classes of drugs that produce this phenomenon. The first commercial solvent that we studied was DMSO. It is a quite robust producer of this phenomenon. I will mention another one, propylene glycol, in a moment

[Slide.]

The practical implications are that many agents in the human environment produce or possess apoptogenic properties. Drugs of abuse would include; ketamine, known as "Special K" on the street; PCP, known as "angel dust" on the street; nitrous oxide, laughing gas, has been around for 150 years and has been abused periodically over those years; benzodiazepines; barbiturates; and finally ethanol. So, there are many drugs of abuse that have these properties.

Anticonvulsants, as I said, both GABA-mimetics, and sodium channel blockers produce the phenomenon. General anesthetics, although they

have many different properties, all of them basically do have either NMDA antagonist or GABA-mimetic properties.

Commercial solvents were early in the period of studying these phenomena, these agents, but the one I will mention that is of particular interest is propylene glycol because it is used to dissolve these agents that are sometimes not soluble in water. For example, benzodiazepines aren't very soluble in water and they are often solubilized in 70 percent propylene glycol and 10 percent ethanol or alcohol.

So, you have a primary drug that has apoptogenic properties being dissolved in a solvent that also independently has apoptogenic properties.

[Slide.]

I am not going to say anything about the anesthetic cocktails. I am just flashing this slide to introduce the anesthesia issue that I am going to discuss, I will let Vesna discuss this anesthetic cocktail study that she performed when she was working with me in St. Louis.

[Slide.]

I want to discuss threshold conditions. Initially, when we studied these phenomena, we were using relatively high doses or long exposure periods in order to produce the phenomenon in a dramatic way and be able to study it. But we now, for the last several years, have been focusing on threshold conditions--that is, the minimal effective condition that will produce the phenomenon.

We know that a single one-time exposure to an apoptogenic drug during synaptogenesis is sufficient to trigger widespread neuroapoptosis. So we want to know for each apoptogenic drug or drug combination how much is required which, if exceeded only one time, will result in neuroapoptosis.

[Slide.]

We started out studying ethanol and the question we asked was how high and for how long must blood alcohol levels be elevated to cause a significant increase in neuroapoptosis.

The answer for the C57BL/6 mouse, how high, 50 mg/dl, and how long it has to be elevated at that level for 30 minutes.

For a perspective, the legal intoxication level is defined as 80 mg/dl.

[Slide.]

We then studied ketamine and found its sub-anesthetic doses cause neuroapoptosis. Ketamine around the world is used as a single dose to produce sedation or induce anesthesia. So, the question we are asking; can one-time exposure to ketamine trigger a significant increase in neuroapoptosis? The answer for the infant mouse was, yes, a sub-anesthetic dose of ketamine triggers a significant increase in neuroapoptosis.

The fully anesthetizing dose for ketamine in the mouse is 80 mg/kg. I believe that has been established for the adult mouse. I don't know that there is published evidence for the infant mouse, but 80 mg/kg is what has been published for the adult mouse.

We are using doses in the range of 10 to

40 mg/kg and any dose above 20 produced a significant increase in neuroapoptosis and, as was mentioned earlier, there was a jump between 30 and 40, appearing to be an accelerated rate when the dose gets in this range.

[Slide.]

This is what that looks like. In the neocortex, its superficial layer, layer 2 in the saline-treated animal does have some occasional neuronal profiles that are undergoing physiological cell death, but that is stepped up by ketamine, so that a number of additional neurons are staining. In the caudate putamen, there is a sparse distribution of physiological cell death but, following ketamine, that is stepped up to a higher rate.

[Slide.]

We did study ketamine and midazolam individually and in combination and found that the combination did cause an approximate additive effect.

[Slide.]

We have also studied propofol and we find that a fully anesthetizing dose of propofol--this is given intraperitoneally to the infant mouse--is approximately 200 mg/kg. We are studying doses in the range of 25 to 100 and find that there is a dose-response relationship in which any dose above 25 mg/kg causes significant neuroapoptosis in the caudate and cortex.

[Slide.]

We have also studied isoflurane as an individual agent. In Vesna's study, using a triple cocktail that included isoflurane as one ingredient, she used a dose or a concentration of 0.75 percent. Loepke et al. recently reported that, in the infant mouse, the MAC for isoflurane, the minimal anesthetic concentration for isoflurane, is 2.3 percent, or 2.26 percent is what they reported.

So, 0.75 percent is about one-third of that, and that is the concentration that Vesna used in her study. She exposed her animals to the triple cocktail for 6 hours. So we decided we

would test a reduced duration, bringing it down to 4 hours, and would study the individual agent isoflurane at this 0.75 percent concentration.

It caused a significant increase in neuroapoptosis. We then decided to reduce the duration further, but increase the concentration to 1.5 percent, the duration at 2 hours. Still, this concentration is sub-MAC and we found that that produced a significant increase in neuroapoptosis.

So, then, we reduced the time factor further to 1 hour and increased the concentration to 2 percent, still within a sub-MAC range, and found that that produced a significant increase in neuroapoptosis.

[Slide.]

Now, I am going to address some arguments that you will be hearing in a moment from Dr. Soriano and I am going to give my perspective on those arguments.

The argument has been raised that hypoxia/ischemia is the culprit and not the anesthetic drug. We believe that that is not a

valid argument because we have shown that ketamine induces neuroapoptosis in the absence of hypoxia/ischemia in this paper here, and Vesna Todorovic was the one who did the blood gas measurements in this study, showing that the arterial oxygen saturation following ketamine across a 4-hour period while the neuroapoptosis was occurring remained in the range of 97 to 99 percent.

Recently, Loepke et al. have reported that isoflurane under conditions similar to the conditions that we show produce neuroapoptosis, that these conditions do not cause hypoxia/ischemia.

Finally, the third reason why I think this is not a valid argument is that hypoxia/ischemia, when you intentionally induce it in the infant rodent brain--and we have done this a number of times and published studies on it--it kills neurons rapidly by a process that does not entail caspase-3 activation and by electron microscopy is not apoptosis. It is excitotoxic cell death, which we

can distinguish from apoptosis.

[Slide.]

The second argument, one which was just raised very recently, is that hypoglycemia is the culprit. Loepke et al. reported in 2006 that, in infant mice, isoflurane does not cause hypoxia/ischemia but does cause hypoglycemia.

Their hypoglycemic animals--they had an n of 4--were subjected to 3 percent isoflurane for 30 minutes for cannulation of the carotid artery, followed by 1.8 percent isoflurane for 1 hour.

Using a larger number of animals, we have determined that isoflurane triggers neuroapoptosis in the absence of hypoglycemia, so we don't think that hypoglycemia is a valid explanation for the neuroapoptotic response.

Here are our data. We studied the infant mice immediately after being removed from the maternal cage--that would be at zero hours--and studied the blood glucose levels, and then at 1 hour--this is the control at 1 hour--at 2 hours, and 4 hours. There are no significant differences

among these control levels.

Then, the isoflurane conditions were 2 percent isoflurane for 1 hour, 1.5 percent isoflurane for 2 hours, or 0.75 percent isoflurane for 4 hours. There are no significant differences in the blood glucose levels compared to the control values except for the 4-hour, 0.75 percent, isoflurane condition in which there was a significant increase in blood glucose.

So, we do not believe that there is hypoglycemia accompanying the neuroapoptosis response in this infant mouse model.

[Slide.]

The third major argument that has been raised is that the mouse time scale is the culprit.

The hypothesis is that mouse neurons succumb quickly, within hours, to an apoptogenic stimulus because mouse synaptogenesis happens quickly in a compressed time frame or an accelerated time frame whereas human neurons succumb slowly. It has been postulated that it would require 2 weeks for a human neuron to succumb to apoptosis because human

synaptogenesis happens slowly over a protracted time frame.

Can this be tested? It is being tested currently in non-human primates whose synaptogenesis period is longer than in the mouse.

I am going to show you some data in primates pertaining to both ethanol and ketamine. These data are highly preliminary and we don't have a large enough number of animals to draw scientific conclusions, but I will show you the preliminary data.

Primarily, I want to emphasize that the preliminary data I believe indicate that most likely the primate neuron does respond in the same time frame that the rodent neuron does.

[Slide.]

This is the fetal monkey experiment that we have performed pertaining to ethanol. The protocol is to expose a pregnant monkey during a particular period of gestation. We have studied several periods of gestation, in this case, gestation day 122. The total term period for the

fascicularis monkey is about 160 days, so this is roughly between the second and third trimester.

After an infusion, a steady-state infusion, of ethanol maintaining blood levels at a certain level for approximately 6 hours. Then, in 2 hours, we do a cesarean section and remove the fetus and under anesthesia euthanize the fetus and look at its brain.

The caudate putamen is being shown here and every little black speck you see is a caspase-3-positive neuron. By higher magnification, here is what the field looks like and, in the saline control extracted from the same putamen region, this is what the saline control looks like.

All of the saline controls we have studied so far have only a sparse showing for neuroapoptosis in any brain region that we have studied, and we study these brains by serial section throughout the brain.

[Slide.]

Now, ketamine, the protocol we have used for ketamine--and we are just beginning these

experiments, because we just obtained the grant to study this--is fascicularis monkeys. We have infused ketamine intravenously for 5.5 hours at a dose required to produce a light plane of surgical anesthesia and we maintain it at that for 5 1/2 hours. Then, in 2 to 2 1/2 hours, we do the cesarean section, remove the fetus, and study its brain.

I am showing you here the frontal cortex and the saline control. There is no appreciable caspase activation pattern at all. In the ketamine-treated animal, there are deep layer pyramidal neurons showing a degenerative effect.

[Slide.]

In the cerebellum, there are a few--this is the same ketamine protocol--there are a few profiles that are positive, but in the ketamine-treated animal, there are many, many more.

[Slide.]

In the hippocampus, there is a row of neurons that are caspase-positive in the ketamine animal, but we cannot find comparable staining in

the control animal.

[Slide.]

In the temporal cortex, I am showing you three different regions that have different staining patterns. Here are neurons in the very deep layers showing caspase positivity and, in a different region, some slightly different shaped neurons that are also deep, but not quite as deep, showing caspase positivity.

In this region, it is a superficial layer of neurons that are showing caspase positivity. In the saline control, there are just a couple of faint profiles showing caspase positivity.

[Slide.]

Finally, in the caudate nucleus, in the saline control, I can see two profiles that are definitely caspase-positive, but in the ketamine animal, many more.

[Slide.]

I am going to present some tentative conclusions, which of course are simply my opinion.

These findings have potentially important

public health implications and pose a therapeutic dilemma for physicians. How real the problem may be depends on how sensitive the immature human brain is to anesthesia-induced neuroapoptosis. Unfortunately, in my opinion, it is not possible, by either human or animal research, to resolve this question in a definitive and timely manner.

[Slide.]

The only solutions that come readily to my mind are a constructive partial solution which would be to evaluate, in animal studies, the neuroapoptogenic potential of all anesthetic protocols and, for pediatric anesthesia, employ only those that have the widest margin of safety.

A more satisfactory long-term solution would be to develop adjunctive agents that do not interfere with the beneficial properties of anesthetic drugs, but can intercept and inactivate the intracellular signals that trigger neuroapoptosis.

I will just one last slide to try to illustrate what I have in mind.

[Slide.]

I showed you this cartoon before in which after the surface receptors are interacted with and the neuron is in an abnormally inhibited state, there are a series of intracellular signals that occur between this point and Bax translocation.

I am illustrating them as dominoes, which, once the first one is toppled, then there is a chain reaction and the rest of them are toppled until finally, Bax is translocated to the mitochondrial membrane. And, after that, it is just plain an apoptosis cascade that we don't want to have happen.

I think that it is going to be possible to identify these dominoes and stabilize one or more so that they cannot be toppled and I think that that is a way of having our cake and eating it, too, because I think that you can produce anesthesia of the subject and of the neurons and still interfere with the intercellular signals that trigger neuroapoptosis.

I think we can have our cake and eat it,

too, and I think that is the challenge to both academia and the pharmaceutical industry to develop these adjunctive drugs that can allow us to have our cake and eat it, too.

Thank you.

DR. SHAFER: Thank you, Dr. Olney.

We have a few minutes for questions. I am actually going to start with the first one. In the fetal monkey study of the ketamine that you showed us, notably absent was both the infusion rate of the ketamine and the concentration.

Do you have those data?

DR. OLNEY: We have blood levels on ketamine and the infusion rate. They are roughly comparable to the data that Bill Slikker will be showing you. He has studied a larger number of ketamine-treated monkeys, and I think he will probably will be giving you full data on that, but we have followed his protocol as far as the dose and we measured the blood ketamine levels and they are in the same range as he has produced.

DR. SHAFER: Dr. Zuppa.

DR. ZUPPA: I have a comment first and then a question. It is concerning to me that the metric that we are using to define exposure is a single time point, a single plasma concentration, and I would just like to point out that maybe, moving forward, we should define that exposure better. It may be an area under the time-concentration curve or something like that, just a more uniform definition of exposure.

My second question is when you were looking at, when you reported the hemoglobin concentrations, the oxygen saturations, where was that measured? Was it arterial? Was that tissue concentrations or tissue saturations?

DR. OLNEY: Arterial.

DR. ZUPPA: Okay.

DR. SHAFER: Dr. Anand.

DR. ANAND: Dr. Olney, thank you for that beautiful review. Going back to this slide, you say that there is an abnormally inhibited neuron. Surgical operations produce an abnormally excited neuron. Do you think the two would cancel each

other, so that the protection is achieved during surgery?

DR. OLNEY: I don't know the answer to that, but I think it should be studyable in mice and rats. They don't cost very much and there are lots of them around, so I think that is a wonderful study for anyone who would like to pursue it.

DR. ANAND: Could I also just caution about the comparison between species, because, you know, rats and mice are altricial species. Humans and guinea pigs are precocial species so the rates of brain development are very different in those species.

The other is that when you look at human brain development, cortical events occur later in developmental time whereas limbic events occur earlier in developmental time, and other parts of the brain develop at other rates.

So, to take a broad representation of this species as comparable to a certain age in humans is very difficult. The old studies by John Dobbing and colleagues were just based on brain weight and

water content. There was no real science related to the development of the brain in that.

So, I would just like to caution that, although those studies are important, even the primate studies are not very relevant to the human.

The subplate zone in the human is 4 times that of the Rhesus monkey. So, how do you jump from all these experimental species to the human condition?

I would like to caution that.

DR. SHAFER: Do you care to respond or not?

DR. OLNEY: I would just say that there is no perfect way to make that extrapolation. However, what we are trying to do in our monthly studies is we study the monkey brain at different developmental stages. And we are very interested in determining what is the pattern of degeneration at each stage because we believe that it is different at each stage because the neurons are in a different developmental stage of development at each age.

So, they are in a different period of

their sensitivity. So you will have one combination of neuronal groups that will be sensitive early in synaptogenesis, another combination of neuron groups that will be sensitive in the mid-gestation, in the mid-synaptogenesis period, and still other combinations of neurons that will be sensitive in the late period. And we want to understand what combinations of neurons is going to be sensitive at each stage so that we can have some correlation to neurobehavioral disturbances that might be produced by those different combinations of neuron dropouts.

DR. SHAFER: Dr. Eisenach.

DR. EISENACH: Thank you, Dr. Olney. I think we are struggling here with the absence of a phenotype for the anesthetic exposure.

That isn't to say that it doesn't exist. We just haven't recognized it if it does. But we do know about fetal alcohol syndrome and I wondered if you could expand on the study that you presented where an equivalent to a single glass of wine would increase the alcohol concentration in the blood for

a sufficient period of time to induce apoptosis.

What do we know about single large or small exposures of alcohol in the human and risk of a phenotype such as fetal alcohol syndrome?

DR. OLNEY: I think the biggest problem here is we don't really have good measurements in the human for precisely and accurately determining neurocognitive deficits that could be produced by a small dropout of neurons during development.

Our tools for measuring the neurocognitive deficits are really crude. Even though we have been working at it for a long time trying to tune them, they are really crude when it comes to detecting small amounts of deficits, for example, 2 or 3 IQ points decreased.

Well, you cannot make such measurements accurately and then correlate them to small amounts of neuronal dropout. Nevertheless, you can show in neurohistological studies, on developing monkey brains at least, that the neuronal dropout is happening. It's a dilemma, how do you make that extrapolation.

For the fetal alcohol syndrome, it was occurring for at least 1,000 years before we were able to detect the effect and relate it to exposure of the fetus to alcohol. When the effect was detected, it wasn't detected because of the neurocognitive deficits. It was detected because there were craniofacial malformations that were much more obvious and we were able to make that correlation.

It was subsequent that they determined, oh, there are also neurocognitive deficits, and they are sometimes so severe as to be frank mental retardation.

Now, in the case where there was frank mental retardation, that was because it was a severely alcoholic mother who exposed her fetus to repeated binges with markedly elevated blood alcohol levels going on for hours, if not days so, obviously, you are seeing a very dramatic, severe syndrome here. But in the fetal alcohol syndrome, there are all degrees of variations to mild, learning deficits, hyperactivity, attention deficit

disorder, mild to severe, all gradients.

DR. SHAFER: Last question. Dr. Snodgrass.

DR. SNODGRASS: A couple of things come to mind here for comparisons. There is a fair bit of data about lead and childhood development, and that appears I think to be a different mechanism than GABA-ergic or the NMDA receptor. And so I think that raises the issue of are those the only two kind of biomarkers that should be used in further studies in this area, or do you think that there are other biomarkers that could be correlated with possible behavioral outcomes.

DR. OLNEY: Actually, there is some evidence that lead might interact with an NMDA receptor. We have lead on our list for evaluating whether it can induce neuroapoptosis. I do think that neuroapoptosis may be a kind of final common pathway for neurodevelopmental disturbances produced by a number of different environmental agents.

It might be that each agent has a somewhat

different mechanism for triggering apoptosis but I do think that, as a cell death mechanism, neuroapoptosis is a very likely mechanism.

DR. SNODGRASS: Also, I want to just add to the "cake and eat it, too" kind of issue you raised here. The idea of altering self-signaling pathways to block expression perhaps of genes that are activated that result in further damage, there is data. I am aware of data in the military actually looking at organophosphate-induced brain injury in an animal model where they looked at the gene activation and gene array studies and they have identified a pathway--that they have now found a small molecule inhibitor that gives them about a 20 percent improvement in the rodent studies outcome on a behavioral test. The molecule turns out to be a cannabinoid type substance.

DR. SHAFER: Dr. Olney, thank you very much.

Our next speaker is Dr. Todorovic, who will be talking about Preclinical Model of Anesthetic-Induced Neurotoxicity.

**Preclinical Model  
of Anesthetic-Induced Neurotoxicity**

[Slide.]

DR. JEVTOVIC-TODOROVIC: Over the last 50 years or so, we have seen significant advances in pediatric anesthesia, in anesthesiology in general, but in pediatric anesthesia in particular.

We know now surgeries that were inconceivable only 20 years ago are happening routinely in pediatric OR suites, and we also know that this is making our surgeons and obstetricians quite cocky. So there is more to be seen and more to be done.

We also know that, as of the last 20 years, what is considered viable pregnancy is actually changing dramatically. When I was a resident, and that wasn't too long ago, we did not monitor fetuses that were less than 25 weeks of postconceptual age. Now, I am being told that 20 weeks is viable as well, so we are routinely monitoring them as well.

What that basically means is that with the

increased rate of premature births, and increased survival of premature babies, we are going to see more and more surgeries done in very, very young children.

Of course, all of you agree with me whether you know much about anesthesia or not, that it will be certainly overly simplistic to consider a child to be a small adult.

[Slide.]

Now, I have listed here a few complications that are fairly common, and certainly Dr. Soriano, being the expert, can tell you more about it later. And it is not meant to be the most exhaustive list of complications that you see in children and not as commonly in adults, but it does give you some idea how they differ from adults when it comes to anesthesia or procedures.

You can see here that laryngospasm and bronchospasm, oversedation, and problems due to unrecognized heart murmurs is actually quite high on our list.

Another one that they are very cognizant

of in anesthesiology and in medicine in general, in pediatrics in particular, is that small kids or very young children do not do very well clearing different anesthetic drugs and we believe that this is largely due to the immaturity of their hepatic and renal functions.

[Slide.]

Now, the organ that I would like to focus on today, and we all have been focusing on for the whole day, is basically the central nervous system.

We know now that central nervous system in humans is not fully developed at birth, and you have heard about it today.

We know the development, the synaptogenesis or brain growth spurts starts in the last trimester of pregnancy and. at the birth, the brain weight is about 300 grams. Brain enjoys enormous growth early on and during the first year of human life, it triples in size, which is basically the majority of growth that really happens.

[Slide.]

What is brain growth? People refer to that as synaptogenesis brain growth spurt. And two hallmarks of brain development that we are all focusing on today are neuronal synapses formation and glial cell proliferation because we all quite a bit believe that neurons do not proliferate or divide once the human is born.

What adds to this enormous growth that you saw, which is basically tripling in size of brain, is mainly massive synapses formation and glial proliferation to provide a nutritional milieu for the neurons to do what they need to do, and what they need to do is quite a lot.

A lot of them have to migrate to their final destination so that they can mature, differentiate and form meaningful connections with neurons so the meaningful circuitries could be eventually formed.

We have heard before that all of that has to happen or is happening during the last trimester of pregnancy and mainly during the first two years of post-natal life.

[Slide.]

Now, we have seen some of the timelines and comparisons today, and this is a very simplistic way of just trying to give you some idea how different species differ as far as synaptogenesis and. because I will be talking about rats and guinea pigs today vis-a-vis humans, I am offering this timeline. However, it is certainly debatable.

We believe that the peak of synaptogenesis in humans happens during the very last couple of weeks of in-utero life and first month of post-natal life. Neonates are particularly sensitive because massive synapses formation is believed to happen at this time.

We believe that, in rats, that is the beginning second week of post-natal life, about 7 to 10 days. In guinea pigs, brain development is a completely in-utero phenomenon. Their pregnancy is 68 to 70 days long and, by the time they are born, the brain is fully developed.

We also believe that peak of

synaptogenesis in guinea pigs and their fetuses occurs midway in pregnancy and that is about 40 days of in-utero life.

So, if you really want to look at the peak of synaptogenesis in mammalian species that I will be talking about today in rats in particular, you are probably looking at 7 to 10 days and, in guinea pigs, you are looking at about 35 to 40 days of in-utero life.

[Slide.]

Now, what happens to neurons that did not make it in the process of synaptogenesis? They do not migrate to their final destination. They do not form meaningful synapses. They are basically considered redundant. Nature, as you have heard today, created a way to get rid of them and it is called "suicide." People alluded to that earlier.

And I will show you why actually pathologists consider that calling it suicide seems a little dramatic but it actually makes the point appropriately.

They actually die by the process of

apoptosis or neuronal suicide or programmed cell death or physiological cell death. You have heard all of these terms.

Although it is a physiological phenomenon and is expected to occur during normal synaptogenesis, we really cannot afford to lose a lot of neurons in that process. I think I was generous when I put that up to 1 percent of neuronal population although there is some regional variation can actually be sacrificed that way. So, it is a very tightly--although physiological, very tightly controlled phenomenon.

[Slide.]

Now, some of the elements of this story you have heard this morning; what are the actual histomorphological hallmarks of apoptosis? These happen to be actually our slides, slides that we have done when I was in Dr. Olney's lab. These are the effects of anesthetics.

But if you were to pull any paper in pathology or any textbook, this is actually a typical example of physiological cell death when

you study neurons.

We know now that changes happen mainly in the nucleus and it is actually shown as a clumping of nuclear chromatin here and the formation of chromatin balls. We know at this point, which is the early stage, the nuclear membrane is intact. However, in the later stage--that is, again, anesthesia-induced apoptosis--you can see that there is no nuclear membrane. There is intermixing between cytoplasm and nucleoplasm and formation of apoptotic bodies. It is actually a very obvious phenomenon, kind of difficult to miss.

But the most important element, and the reason why this phenomenon is referred to as "suicide," is the fact that there is no massive inflammatory or phagocytosis reaction. These neurons die alone without dragging others around them with them.

[Slide.]

Now, we also know based on the work that has been done by Dr. Rockich and many others that two main neurotransmitters in the brain, glutamate

and GABA, are very important in promoting all key elements of neuronal development.

Now, what happens when you start tampering with these two very important neurotransmitters? Obviously, the balancing act between GABA and glutamate is crucial for these neurons to be able to signal to each other to move, to connect, to form synapses, what happens when you start meddling with that?

Well, that is exactly what we do when we expose developing neurons to anesthesia. Although the field of mechanism is still developing, it has come a long way and now we do know that the majority of general anesthetics--I should say all of the general anesthetics--that we are currently using in anesthesia medicine and dentistry actually have been shown to either manipulate GABA system or to manipulate an NMDA system, which is actually a subtype of glutamate receptor.

We know that they can either potentiate GABA inhibition and/or inhibit glutamate excitation.

[Slide.]

I have listed some of them for you here that both intravenous anesthetics and inhalational anesthetics have been shown to potentiate GABA. And we also know that ketamine, we have heard a lot about today, is a well-known NMDA antagonist.

Studies that we have done in Dr. Olney's lab when I was a fellow showed that nitrous oxide or laughing gas is an NMDA antagonist, very similar to ketamine and a study done by Dr. Franks about 6 months later showed that xenon, an inhalational anesthetic more commonly used in Japan and Europe, is also an NMDA antagonist, very similar to nitrous oxide.

So, if I tell you that a fine balance between GABA and glutamate is crucially important for the developing neuron to know what they need to know, and to do it properly, and then if I tell you that all the anesthetics that we commonly use in daily practice actually manipulate these two neurotransmitters to a certain endpoint that is desired in clinical setting, the natural question

is: What does that really mean for the developing neuron?

[Slide.]

So, the study we have done in Dr. Olney's lab was actually a fairly simple study because all we wanted to know is how everything that has been known up to that point applies to clinically relevant anesthesia and, more importantly, how does it apply to clinically relevant anesthesia protocols.

So, we have designed a very simple experiment with either isoflurane alone or we did a dose-response experiment with equipotent concentrations and then we combined it with either nitrous oxide or midazolam, or nitrous oxide and midazolam, creating what we used to call affectionately a triple cocktail.

What we found when we exposed 7-day-old rats that are the peak of their brain development to this anesthetic combination, we found that there was a widespread neuroapoptosis that happens at the peak, as I said, at 7 days of age.

We found changes in long-term potentiation, which is nothing more than studying memory in a dish, and then we followed these animals for a long time, up to 6 months of age, and monitored their cognitive development. We found that that was affected as well, and I will tell you more about it later.

[Slide.]

Now, I said we studied 7-day-old animals because they were at the peak of brain development.

But then we also wanted to know how that particular combination would actually affect animals that are of different age and we decided to work with pretty much the whole span of brain development for rats.

We included 1-day-old rats, 3-day-old rats, 7-, and you will see a lot about that, 10- and 14-, which is pretty much at the end of brain development for rats.

In order to confirm that our animals were not severely hypoxic or hypocarbic, severely hypoventilated, we performed arterial blood-gas

analysis to kind of set the stage for actually looking at the anesthetic type effect, and not hypoxia and hypocarbia.

What we did here, we collected arterial blood gas at zero time, 2 hours into the anesthesia, 4 and 6 hours. If you look at these numbers, and they are kind of hard to follow, all you need to see is that, basically, none of the animals was hypoxic, none of the animals was hypocarbic; if anything, they were hypercarbic, because they had a little bit of respiratory alkylosis.

The way this was performed, and the only way you could do it was obtaining arterial blood from the left ventricle during transcardiac puncture, which is certainly far from how we obtained it in a clinical setting, and I will show you some studies that address that later on.

[Slide.]

Now, these animals are very small. When you work with a 1-day-old rat, it is about 2 to 3 grams so there is really very little you can do

and you have a lot of technical limitations. Certainly, observing vital signs and blood pressure, blood pressure being one of them, becomes a big issue.

The best we could come up at the time, having animals this small, was to purchase a laser probe that would allow us to measure cortical cerebral blood flow. It was technically, relatively easy.

Animals needed to be anesthetized for that which was perfect for our experimental paradigm, and we could put that probe anywhere on the top of the cortex we desired to measure the flow, thinking that if there is severe hyperperfusion and hypervolemia, that we would notice changes in cortical cerebral flow during our anesthesia.

We performed anesthesia using the triple combination that I told you before for up to 6 hours and we carefully recorded cerebral blood flows over the course of time.

As you can see, when we looked at animals ages from 1 to 14 days, we found that compared to

the baseline recording of cerebral blood flow, there were absolutely no changes except for slight variations that occurred during our anesthesia protocol.

[Slide.]

However, when we studied the degree of neurodegeneration or apoptosis that occurs after 6 hours of this triple anesthesia protocol, we found that the degree or the vulnerability or sensitivity to the very same anesthesia protocol differed dramatically across the age.

What we did here is actually we compared the severity of damage as a fold increase to age-matched controls, because synaptogenesis is a physiologically occurring phenomenon. You really cannot lump them all together. You cannot compare 7-day-old experimental animal with a 1-day-old control, and vice versa.

So, all of these experiments were done with a 7-day-old experimental compared with a 7-day control and, thus, we came up with a fold increase.

If you look at this, this is cortex and

this is anterior thalamus. These are cumulative scores because we looked at 4 cortices and 6 thalamic nuclei.

You can see that the peak of vulnerability is at 7 days of age, which coincides with the peak of synaptogenesis in rats. The very same, the very same triple anesthesia cocktail, did not cause much damage in p.14, neither that it did in p.1. So, the conclusion for us at the time was that what you give to these rats matter less than when you actually do it.

[Slide.]

This is the picture actually of the typical scene that you can have after exposing, in this case, a 7-day-old rat to anesthesia, triple anesthesia combination. You really do not have to be a pathologist to see the difference.

If you look at the left panel, there are thalamic nuclei here. You do occasional staining--this happens to be silver staining--that stains dead or dying neurons and it gives you a cumulative picture of cell death.

When you look at the right panel, Panel B--this is triple anesthesia combination--it is simply devastated with number of cells that are either dying or dead.

[Slide.]

We have a cingulate cortex, as well. Again, on the left side is the saline control. Actually, I say "saline control." This is actually saline and DMSO control because we use DMSO to dissolve midazolam.

On the right side, it's a cingulate cortex. You see a pretty typical bilateral pattern, second and fourth cortical layer very much affected, and actually, this picture reminds me of when Dr. Olney was showing with a TUNEL when I was in his lab.

[Slide.]

Now, the question remains, though, even though we see a lot of histological damage, there is no doubt that morphology changes. The question is so what. You know, this is a very compliant system. This is a very young animal. We know that

young individuals can recover incredibly well with extensive occupational and physical therapy. So who cares? You know, yes, there is a damage, but if there is no functional significance, we should probably move on.

Well, this is why we designed a study, because the damage was so profound that I just could not move on without figuring out whether that matters at all considering the fact that these are very much commonly used anesthetics, and the protocol that we used was very much clinically relevant as far as at the potency of the doses and concentrations.

So, with the help of Dr. Dave Wozniak and Rich Hartman, we designed a whole panel of behavioral studies where we actually followed these animals for up to 6 months after exposing them for only 6 hours to anesthesia at age of 7 days.

We couldn't find that these animals were different from controls in any other experimental paradigm behaviorally that we did except with cognitive paradigms. I can certainly spend a whole

hour talking about this slide, and I don't have that luxury today, but just to tell you briefly that we use two main tasks, which are Morris Water Maze and Radial Arm Maze, and they are very good tasks for spatial learning in rats.

The Morris Maze is actually based on the basic principle that rats are excellent swimmers but they hate to be in the water. So, for them it is a great motivator to get out of the water and get on the platform.

So, what you do is you put a platform inside the water tank and you allow these animals to swim around and find the platform. The training actually starts with the platform being visible and then the platform is submerged, so they have to remember where the platform had been, so that they can go and find the rescue or refuge on the platform.

It is a very complex learning actually task that has to be done by people that know what they are doing but basically what we were able to show when we used the little more complex design,

which is platform was submerged and animal could not see it which is called "place trials," we found actually that control animals take very little time after initial pretraining to actually zero in on where they should be to get out of the water.

It takes them about 2 days to reach a very quick path to the platform, whereas, our experimental animals at two days were no better than a day 1, and were still kind of struggling to figure out what to do. It took them twice as long to comprehend this same task compared to controls.

When we retested, this is when they were about 30 days of age, when we retested them at 130 days of age, again, with the submerged platform, we were not quite happy how--although experimental animals performed worse than controls, we were not quite happy with the control animal performance after 5 days of testing, so the testing was extended to 10 days.

As you can see, control animals managed to master the task, however, experimental animals were having a lot of problems, and never actually

learned it.

This is a little more complex called the "probes trial," where you actually remove the platform completely. What you are looking for is actually how much time animals spent in the quadrant where a platform had been and how many times an animal crosses the very same spot.

We found again that our anesthetic animals were actually more lost than control animals and were actually pretty much all over the place and not really knowing where they should go.

Radial Arm Maze, which is another way to test learning and cognition in rats, showed pretty much the same results, that these animals were slow learners.

[Slide.]

Dr. Olney alluded to some of the aspects of apoptotic pathways and I was asked by the panel to just mention a few more things about that. I am going to go briefly through that.

We know right now that apoptosis could be activated at least by two pathways, intrinsic and

extrinsic, and intrinsic is mitochondrial dependent as you have seen previously.

[Slide.]

What we have done actually, when you look at the pathway for the mitochondrial activation, as Dr. Olney said, it is actually based on changes in Bcl-XL that is in the membrane of mitochondria. This kind of protects mitochondria from being leaky. If level of Bcl goes down, cytochrome leaks out of the mitochondria, activates caspase-9 and caspase-3 leading to DNA fragmentation.

So, if the anesthetics can activate this particular pathway, what would you expect? Well, the expectation was that the anesthetic cocktail would decrease the level of Bcl-X, would increase the level of cytochrome c, would activate caspase-9 and ultimately activate caspase-3. This is where we started.

[Slide.]

So, as you can see, anesthesia cocktail indeed did that. It took about 2 hours in thalamus, about 4 hours before Bcl-XL levels were

50 percent of what was in a control state, which is zero time with a slow recovery.

When we looked at 14-day-old animals, if you can recall, these animals were not as susceptible to the very same anesthesia protocol. We see absolutely no decrease in Bcl-X. If anything, although not significant, they are showing compensatory increase, possibly to protect the cell from cytochrome c leakage. If you look at cortex, pretty much the same phenomenon was observed except that the time point for Bcl-X to decrease was 4 hours, and not 2.

[Slide.]

What happened to cytochrome c? When we looked at cytochrome c, and in this case again, two most vulnerable areas, thalamus and cortex, we found that cytochrome c leak was maximal at 4 hours. It was 4-fold higher than what you observe in zero point or control point. When you look at 14-day-old animals, they had absolutely no response regarding cytochrome c release.

In cortex, we found a similar finding

except that it took 6 hours for cytochrome c leakage to be significant in this many fold increase.

In 14-day-old animals, again no response.

[Slide.]

This is caspase-9 activation in 7-day-old animals. As you can see, some signs of caspase-9 activation at 2 hours, quite a bit at 4 hours, and just as much, quite less at 6 hours. And this is anterior thalamus.

[Slide.]

Now, as far as the extrinsic pathway, again, we know that this is important, which is association of proteins that I have here for you for the activation of caspase-8 and, ultimately, activation of caspase-3.

So, again, what would be the expectations is that somehow anesthetics would manipulate Fas, would activate caspase-8 and activate caspase-3.

[Slide.]

Again, when we looked at 7-day-old animals at the peak of synaptogenesis that are most

vulnerable and, compared to 14-day-old animals, you can see the activation of Fas at 6 hours in anterior thalamus, 6 hours in cortex, in 7-day-old animals, where there is no such thing occurring in 14-day-old animals, caspase-8 activation in parietal cortex.

[Slide.]

This is caspase-3 activation followed over the course of 6 hours. As you can see when you compare it to controls, you see quite a bit at 4 hours and quite a bit at 6 hours, again, on thalamic nuclei.

[Slide.]

Now, when the study came out, needless to say that we were absolutely crucified, and when you add the time--that we did the best we could, but it was far from being sufficient. There were a lot of technical limitations and, for me, being an anesthesiologist, they were actually very significant.

For example, we dealt with the very small-size animals. Even 7-day-old animals are no

more than 18 grams. There is not much you can do with an 18-gram animal. When we dealt with smaller, such as p-1 or p-3, they are barely 3 to 5 grams.

Well, this is certainly not how we do anesthesia. We don't throw kids in a jar and walk away and come back 6 hours later to see what happened. So something has to be done about that.

If we were even close to mimicking what we normally do, we had to have a better way of staying on top of all the hemostasis, just the way we do it in the operating room, or at least to give it the best shot.

Secondly, as Dr. Olney alluded this morning, it is a short synaptogenesis. It takes only 2 to 3 weeks in comparison to humans. How does that relate? Do we have a direct relation, time versus duration of anesthesia? We couldn't really answer that question using only mice or rats.

Finally, is it only rats?

Now, this was in 2003. A lot has been

done since then, and certainly primate work is coming more and more but, at the time, the best I could do with the resources that I had was to actually use guinea pigs.

[Slide.]

Guinea pigs are very interesting in a sense that, as I told you, their brain development is an in-utero phenomenon. So what you have actually is you have a pregnant mother to work with. They are fairly large. They could be as much as kilo and a half.

Well, that is really like a candy store for an anesthesiologist because you can do whatever your heart desires. You can intubate them, you can mechanically ventilate them. You can put in an arterial line. You can put a continuous intravenous line. You can paralyze them. You can stay on top of everything and never let go of a single vital sign. That was just perfect for us, so that is what we did.

We took pregnant guinea pigs that were about 35 to 40 days of pregnancy. We studied

earlier pregnancy and we studied later pregnancy. I don't have time to talk about it today, but if you have any questions, I will be more than happy to go over that. This hasn't been published as of yet. It is currently being reviewed.

We had real controls. They got absolutely nothing, and the only thing we did is we did not feed the mother. We did sham controls, because a lot has to happen to that mother.

We had to come up with sham controls--and I am calling them sham controls; they are really not even controls, because you have to have something. We decided to use fentanyl infusion. Fentanyl is a very commonly used neuro-opioid-agonist narcotic that is incredibly often used in pediatric anesthesia and is part of the sedation protocols in many intensive-care units throughout the nation.

So, we used fentanyl infusion starting with the bolus over a course of 4 hours and then compared that with experimental. In this case, we used midazolam at 1 mg/kg IM, which is the sedative

dose for a guinea pig, moved on to nitrous oxide 75 percent and isoflurane 0.55 percent, which is about half a mg for guinea pigs.

We have done, as I said, everything we could to mimic the operating room setting and this time, actually, what we wanted to do is to look at shorter anesthesia exposure.

Guinea pigs have brain development--it takes almost 5 times longer than rats, so if we have a direct time correlation, you would expect that in the species that has 5 times longer time it takes to develop brain that, if you do 4 hours of anesthesia, you are not going to get anything. So, that is what we did.

[Slide.]

Now, if you look at this, what we have here--these dotted lines are actually physiological range for a pregnant--for guinea pigs, not necessarily for a pregnancy guinea pig. But we kind of made an assumption that these values are relatively within the range of what you would see with pregnant animals.

What we did here, we went through great pain of not allowing any of these parameters to wander too much outside of this physiological range and, as you can see, we were pretty successful at that.

We measured pH and PCO<sub>2</sub> and pressure of oxygen, bicarbonate, saturation oxygen, saturation using arterial blood gas. We measured glucose very tightly. We looked at all the vital signs end-tidal oxygen, end-tidal CO<sub>2</sub>. We looked at heart rate and blood pressure and we tightly controlled the temperature, exactly what we would do in the operating room setting.

[Slide.]

This is what we got. Now, this was a very extensive experimental protocol that did not only include the combination isoflurane and nitrous and midazolam, but it is listed here for you just to get an idea how isoflurane alone, for example--and as soon as you start adding more to it, how actually the picture tends to change.

When we did caspase-3 staining and used an

optical dissector to quantify the number of neurons that were actually labeled with activated caspase-3, you can see that in all cortical structures that we studied, when you compare controls to a combination of anesthetics, we find a many, many fold increase in caspase-3 labeling.

We do see some increase in isoflurane alone. But, interestingly enough, fentanyl did give us, although not statistically significant, which is something I don't know what it means in biological systems, but mathematically, it wasn't.

But, as you can see, there were sometimes even twice the increase of caspase-3 staining with a fairly low dose of fentanyl infusion if used over the course of 4 hours.

[Slide.]

When we looked at thalamus, we found a similar finding actually that when you compare, this time actually, isoflurane and midazolam were pretty much as bad, if not worse, than the whole triple combination. And other structures, as well were quite affected.

[Slide.]

So this is the animal with synaptogenesis that is quite a bit longer than the rat, exposed to anesthesia for 4 hours, everything very tightly controlled, and yet we have seen caspase-3 activation everywhere.

Now, this happens to be a parietal cortex.

I don't know how well you can see, but these are actually caspase-stained cells compared to sham controls and here we don't see as many.

[Slide.]

This is the larger magnification.

[Slide.]

Now, the question is what is the fate of these neurons? We say that when caspase-3 is activated, the cell is pretty well committed to die and goes on to really die. Well, how do we know that?

Well, we wanted to confirm that that is indeed the case after exposure to anesthesia. So this experimental paradigm included exposing controls, shams, and experimentals exactly the way

I showed you before using triple anesthesia combination, letting these animals stay pregnant, letting these animals deliver, and then take their babies when they are 5 days of age. At that point, guinea pig brain is fully developed. What happens? What goes on after that?

Well, this is what we found, that in the vulnerable regions that we zeroed in on again--because they were most impressively damaged midway in pregnancy, we actually found that the number of neurons, when you study the neuronal density, is significantly decreased in the neurons and, in some cases, as much as 50 percent compared to control.

So, these neurons do go on to die and actually cannot be accounted for when brain is fully developed.

[Slide.]

Now, what does this all mean? Dr. Soriano will tell you much more today about clinical implications, and certainly there is not much known about unwanted effects in cognitive respect. I

just want to impress upon you how little is actually known about cognitive effects of anesthetics per se.

I had to search long and hard to come up with three references--three--and this is going back to 1945. In 1945, Dr. Levy published a study where he looked at 124 children mainly having tonsillectomy and appendectomy. There are some other smaller cases, but the majority, the bulk, of the cases were T and A's and appendectomies.

At the time, what they used was ether anesthesia. So the endpoint was the same, knocking the child down so the procedure could be performed.

But how that was done was quite different because it was always exclusively mask induction so you have to keep that in mind. it was quite dramatic just the way it was done by itself.

What he found, and I thought that was interesting--and that was an observation by somebody who was not an anesthesiologist or anesthetist--he found that actually the most susceptible kids were kids that were younger than 2

years of age, and up to 58 percent of them developed behavioral sequelae.

Now, these sequelae were so severe that actually these parents sought professional help. All of these kids were seen repeatedly in psychiatric clinic, so it was beyond the point when parents could control what was going on.

[Slide.]

The second study was almost 10 years later, and it is done by Dr. Eckenhoff. Actually, this is the first study where he alluded that there is a possibility that some of the personality changes could be connected to anesthesia. That was a relatively novel--actually, totally novel--concept at the time.

He did a retrospective study looking at 612 patients under the age of 12 and he studied them 2 months after the operation, mainly tonsillectomies and ears, relatively short procedures.

At this time, this is the cocktail that was used. This is closer to home, something we can

actually recognize. They used pentobarbital, scopolamine, and morphine for sedation because they realized it is not a good idea to plop a kid on a table and slap a mask on a face and let them cry themselves to sleep.

So, they started sedating them actually, but they used cyclopropane and nitrous, and ether and nitrous for induction of maintenance, and the only one we really use nowadays is nitrous.

But they also noticed the personality changes were actually, in some kids, very dramatic, and again, his totally independent observation was that the most sensitive were kids younger than 3 years of age, that up to 57 percent of them had personality changes having done the same procedure as 3 to 8 or older than 8-year-old kids and a very similar anesthetic protocol.

[Slide.]

Now, we are traveling to 1986, still quite a bit long ago, and this is a study again not done by an anesthesiologist. It was done actually by a group of dermatologists who studied actually the

effect--they actually had to deal with kids that had these nevi that needed a lot of surgical, a lot of actually interventions under anesthesia.

It was a relatively short procedure, but required repetitive, many, many, many times to bring these kids back for the intervention to be done. So, they actually, for the first time, suggested that maybe there is a relationship between anesthesia in long-term impairment of cognitive development.

These kids underwent anesthesia for 15, 20 minutes, no longer than an hour at the time, but they were frequently exposed. They looked at 107 kids and actually, the main anesthetics used--this is very familiar--ketamine and halothane.

So, what they found actually is that cognitive impairments were linked to regressive behavioral changes that were present up to 18 months post-anesthesia. Again, children younger than 3 years of age were most sensitive.

[Slide.]

What they say in their paper, and I

thought that was very interesting and I took the whole report out for you to see, is they say that, "Although the clinical impression of many anesthesiologists is that there are probably no long-term effects, the authors of this paper were unable to locate any published data concerning the possible long-term effects on memory, problem solving, conceptualization, or learning disabilities in children who have undergone general anesthesia." They were completely healthy kids otherwise.

[Slide.]

What are the conclusions?

We don't really have conclusions. We know it is a work in progress.

We know that animal studies confirm morphological and long-term behavioral impairments caused by general anesthesia especially if anesthesia--if they were exposed to anesthesia at the peak of their brain development when they are most vulnerable.

We also know that the duration of

synaptogenesis, the biggest criticism initially, it is very unlikely to have much to do with anesthesia-induced neuronal damage.

We also know that even if you closely maintain homeostasis, which is what we strive for, and are very proud of in anesthesia field, it doesn't seem to prevent anesthesia-induced neuronal damage.

We don't know, based on limited studies that are available right now in anesthesia literature or otherwise, what are the long-term cognitive effects of anesthetics. So, Dr. Soriano will shed some light on that today, and I hope we can do a little more to determine one way or another, or at least attempt to, whether there is anything of concern for humans.

Of course, all of these studies need to be done in a well-designed and organized fashion, preferably focusing on anesthesia exposure in very, very young children.

Thank you.

DR. SHAFER: Thank you, Dr. Todorovic.

In the interest of time, I will ask that we withhold questions and we can discuss the paper during the discussion this afternoon. Thank you very much.

It is now time on our schedule for a break. Actually, it is time for the next talk, but I thought that was cruel and unusual for the group.

We will take a 10-minute break. If I could ask people to be back here at 10:55, we will begin promptly.

[Break.]

DR. SHAFER: Our next speaker will be Dr. William Slikker who will speak on Ketamine Effects on the Developing Nervous System. Dr. Slikker is Director of the Division of Neurotoxicology at FDA National Center for Toxicological Research, NCTR.

**Overview of FDA (CDER/NCTR)**

**Studies to Evaluate the Potential for  
Anesthesia-Induced Neurotoxicity**

DR. SLIKKER: Well, this is really a privilege to be here and get a chance to present some of our work that we have been doing in this

area over the last several years, and it has really been a pleasure to listen to the presentations. Since this set the ground so nicely, the stage so nicely, for me, I can go much more quickly through some of these initial slides.

[Slide.]

Certainly, I don't think there is any doubt in this room that the NMDA receptor system plays a major role in many of the developmental aspects of the nervous system. It is important to realize that this involves both what we think as functional as well as structural kinds of development.

[Slide.]

One point I wanted to make about the NMDA receptor itself is that it does have usually four components to it, as seen here and, in each one of those functional NMDA receptors, you do have to have the NR1 subunit. But you could have it matched with other subunit types, such as 2A and 2B, for example.

So, this is what really is going on with

this receptor and that it does control this course of especially calcium into the cell. That is a very critical feature of its activity and it functions normally very well for us in the developing nervous system.

[Slide.]

Now, of course, you are very familiar with this work, very groundbreaking work, by John Olney and his coworkers and it certainly did raise a lot of issues with this area.

What we began at that point in time is to try to understand how we might be able to replicate that in our own laboratory setting in conjunction with our colleagues at CDER within FDA and to see something about the dose-response phenomena involved here as well the blood levels involved with this kind of effect.

So, this work published in Toxicological Sciences by Andy Scallet and others working together between NCTR and CDER demonstrated that, indeed, if you had a dose of 20 mg/kg of ketamine, given on 7 occasions over about a 9 to 12-hour

period, that you could produce anesthesia in that rodent model at post-natal day 7. And, as Vesna pointed out so clearly, it is a very sharp peak, that 7 seems to be the most sensitive age postnatally.

Here, just look looking at the thalamus, although this happened in several different brain areas, you can see that you can get this tremendous increase in cells that are dying through apoptotic mechanisms.

But I think what was interesting about this work is that it did point out that, compared to controls here, that if you gave multiple doses of 10 mg/kg, which is half this dose, but you gave it over the same period of time, you got only a very mild, if any, increase at all in apoptosis or, if you gave a single dose of 20, we did not see any massive increase.

So, really, it was this multiple dose over this period of time that resulted in these kinds of effects. So, this gave us some idea that perhaps there was some dose-relatedness here and that this

deserved further study.

We also were able to show that the plasma levels of ketamine were in the range of about 10 to 15 mcg/ml to reach this particular effect as seen here.

[Slide.]

So, one of the things that we wanted to do was sort of understand this time course phenomena more carefully and start to get a handle on really what the levels were doing in the plasma of these animals.

So, this is data that we are seeing here from rodents. We are looking at animals that were dosed with 20 mg/kg, 6 times, at 2-hour intervals, so it is giving us the effects that we have seen before. But what is interesting about it is that, after the last dose here, 5 minutes after the last dose, you can see that you do start to drop off the levels in the plasma.

So, this is after the sixth dose, you can see that the level is maintained fairly well out to 1 hour, but drops off very quickly. So, by 4

hours, you are pretty much down to zero levels in the plasma.

What we also were able to do then was to look at the animals at these various times and realize that control in 2 hours and 4 hours after withdrawal showed virtually no effect by using this particular cell-death type assay that we are looking at here. But, when we got out to 6 hours of withdrawal, then we saw the jump up of effect that we thought we would experience, and also it was there at 18 hours.

So, there is a time delay that has to be taken into account before you can see these effects. And what was very interesting about it in our eyes is that you do not start seeing the apoptosis until the actual plasma levels of ketamine were pretty close to zero. So, this is something to keep in mind in doing these studies.

[Slide.]

Now, just to get a little bit closer to the target. Here are brain-tissue concentrations, and they are relatively close as far as their

levels in brain and plasma but, again, the idea being that after the last dose, after all 6 doses were given, you have these brain levels there for an hour, out to 2 hours. By 4 hours, they are pretty much down to zero, essentially by 6 hours. Again it takes this delay before you get the effect, but you do see it out here after 6 hours of withdrawal, again with the actual target tissue levels of ketamine being very, very low.

So this I think is important information to keep in mind as you are designing and interpreting studies.

[Slide.]

One of the ways to sort of get further information about how things are occurring within this system is to do some in vitro studies. So we did some studies with primary cell culture using, in this case, frontal cortex.

What we are able to do here is actually achieve the cells from very young infant mice and then grow them for several days before we expose them to various levels of ketamine and other agents

and then look at their response.

[Slide.]

Using this rat-forebrain culture approach and using a cell-death ELISA assay, you can see that these cells in culture are very sensitive to ketamine. As you move from control to 0.1, up to 1.0, it is only just a very subtle increase, if any. But, when you go to 10 micromolar, then you jump up and see this increase in cell death. And it is there with 20, as well.

Now, a very important feature of this particular slide is showing the importance of the NMDA receptor and its responsiveness to this treatment. What you see here is that if you add in antisense to the NR1 subunit, that subunit of an NMDA receptor that is essential for its function, and block its upregulation, you can also protect the cells for this toxicity.

So, these are cells that were exposed to ketamine for a number of hours, anywhere from 6 up to 24 hours, and then examined somewhere between 6 and 24 hours later, and you get this effect. But

you could block it by blocking the upregulation of the NMDA receptor subunit NR1.

[Slide.]

Just to go along with this, and sort of fitting in with some of the work that Vesna showed, it is important to think about Bcl-2 and Bax which, as you know, are two of the important proteins when it comes to apoptosis.

What we were able to show here is that, as you increase the dose of ketamine in these cell cultures, again, you have got an increase here of the NR1 protein showing up at the 10 and 20 levels.

Those are the areas where we know we are getting cell death, and also the Bax protein comes up markedly at that point in time, as well. All of these can be blocked by using the NR1 antisense.

So, again, if you can block the upregulation of that NMDA receptor component, then, you also can block the toxicity.

[Slide.]

So, of course, one of those ways in which you can help demonstrate that cells are dying, and

dying due to apoptosis, is use the TUNEL approach and what we are seeing here is either a control or low doses of ketamine with these cells in culture.

That is quite different than at 10 micromolar where you start to see the cell death. Again, if you add the antisense, you can block that effect.

We feel like there are several different ways you can evaluate these cells and they will all tell you the same thing, that you can certainly produce cell death with the ketamine and you can block it by using the antisense.

[Slide.]

Now, another thing that we wanted to do was to evaluate this in the frontal cortex of Rhesus monkeys. The reason for this is that we were interested in this question that had to do with is there a different time required to affect primate cells versus rodent cells.

So, we thought, by using these in vitro approaches, we could get to some of those issues. Here, we are using a primary cell-culture system in monkey frontal cortex cells, and these are

collected on post-natal day 3. This work has been published by our colleague Cheng Wang there at NCTR. This came out in Toxicological Sciences.

What we are looking at here--first, let's take a look at the cell death, cell-death assay, up here that we have been looking at in the rat cultures. You can see that we have an increase in cell death as you move to the 10 and 20 micromolar concentrations of ketamine. So this is consistent with the rodent data.

Also, if you use the antisense with that, you can block the effect. If you use sense, which, of course, is just sort of a kind of control, it is no effect, which would be correct in assuming that you have to have the correct antisense to make this function work. Of course, just sense alone has no effect.

So, these results are very consistent with the rodent data using the same kinds of time-course exposures and the same kinds of washout periods, you get these same effects as if you were using rodent cells. It made us believe that these two

different cell types were going to respond in very similar fashion.

One thing that was different between the rodent data and the primate data is that we did get a more dramatic LDH response, which is indicative of perhaps the cells going through not only apoptosis, but perhaps also necrosis. So, this is something that we wanted to look for in the in vivo studies that we were planning.

Then, this is just another assay down here looking at mitochondrial function, which we know is involved in this process and is showing the same kind of dose dependency and the same kind of blockade with the antisense.

So, we felt like the cells in culture between the rodent and the primate were giving us the same kind of information.

[Slide.]

Again, you can look at this time course phenomena and get a good idea about this--that is, compared to control cells in culture, looking at the MTT assay, that mitochondrial function test,

you can see that if you just simply look in a time-course way and look for exposures that are in the 2-hour range, you don't get anything that is different from control but the mitochondrial function is dramatically affected if you expose the cells to ketamine for 6 hours.

This is using that standard 10 micromolar concentration of ketamine, which gives you values that are in the same range as 10 to 15 micromoles per milliliter if you did it in an in vivo study.

So, what we are seeing here is that you have to expose at least 6 hours, and you don't get a much different effect if you go out to 24. But you certainly need 6 hours exposure to get an effect.

The withdrawal time was also critical in that if you looked immediately after you withdrew, this is in the control situation, or if you looked 2 hours later, you didn't get much difference, But if you have the washout that at least lasts for 6 hours, then, you start to see the effect, and it is there also at 12 and 24.

So, this gives you some idea about when you should be looking for effects. You can't look immediately after you expose the animals, you have to give some time for washout.

[Slide.]

Just going through comparing between the primate and the rodent, just to make sure things were consistent, you can see here controls with TUNEL exposure, TUNEL assessment, the 10 micromolar of ketamine showing dramatic effect, and again the NR1 antisense blockading effect.

[Slide.]

Sort of where we are right now with these studies is these in-vitro studies, they give us certain parameters to be used in the development of the primate study. Ketamine does induce cell death after 6 hours of exposure. That is what we have been seeing with these cells of the culture.

This takes time to manifest itself. You have to wait at least 6 hours or so afterwards to see these effects. It appears that certainly apoptosis is involved based on the TUNEL and the

cell-death ELISA, but also the LDH release sort of suggests there could be necrosis going on, as well.

Then, of course, we figured out that NMDA NR1 subunit, its synthesis and upregulation is very critical. Without that upregulation, you are not going to get this effect, you can block it.

[Slide.]

This is the model that we put together as our working model. This was published in Reproductive Tox a couple of years ago.

What we think is going on here, based on the data that I have been showing you, is that you have a normally functioning system where you have an NMDA receptor on the outside and, helping to guide calcium into the cell, you can then treat those cells with ketamine. Then, in doing so, of course, you blockade that ability to bring influx of calcium into the cell.

Now, after you remove ketamine and get rid of it, what we think is going on is that you have then an upregulation of an NMDA receptor on the outside of the cell and, now the ketamine is gone,

you no longer have the blockade of that receptor that can allow even additional amounts of calcium, more than in a normal situation.

So, this compensatory upregulation of an NMDA receptor would allow for a greater accumulation of calcium within the cell. This is our working hypothesis and one that we have been trying to generate data to see if we can either support it or refute it.

[Slide.]

Now, getting to the part that Vesna spoke so nicely about, and that is the idea of trying to work between species, it is not an easy task.

We developed four or five years ago this idea that there would be some comparability perhaps between the rats, and as Vesna pointed out. They are sensitive in this period between birth and about 14 days of age, but certainly a dramatic peak at day 7.

We postulated what may be going on in the monkey and the human. This is simply based on the anatomical development of the nervous system and

not based on any data at this point in time, so this is postulated.

We thought that the monkey probably would be sensitive somewhere around mid-gestation up to about 2 months of age and, for the human, this could range anywhere from, you know, mid-gestation up to 2 or 3 years of age. But that is just based on general brain development and time for eye-opening and a number of different endpoints of that nature.

So, we thought that if we are going to do studies in monkeys, we needed to get some idea about whether or not the time window of sensitivity was something that we could relate back to this diagram, and that may help us then do further interpretation between the rat and the monkey and then eventually to the human situation.

[Slide.]

The experimental design that we came up with was one in which we needed to do some initial preliminary experiments in the non-human primate to make sure we could anesthetize the animals for a

length of time and maintain them in a physiologically stable state. We found out after several preliminary experiments that that would be possible.

We also wanted then to look at this sensitive stage; that is, at what time points during development would you have your greatest sensitivity or what time points may not be that sensitive.

So, we began this search, and it is just a start in this direction, by looking at three different time points. We looked at about 75 percent of term, which is right around 122, 123, days of gestation in the Rhesus monkey. We looked at 5 days after birth, a relatively young animal, and then we looked at 35 days after birth. So, those are our three stages that we began with.

We also wanted to look at dose-response, not sort of dose-response in the classic sense, on a mg/kg basis, but using dose more on the idea of duration of anesthesia.

We wanted to look at whether we are going

to have 24 hours of anesthesia, 3 hours of anesthesia, 9 hours of anesthesia, look at dose in that regard, and always trying to hit the same light surgical plane of anesthesia as John described, try to hit that particular level throughout, maintain that in a physiological way but use the duration of anesthesia as the dose proponent.

Then, we also wanted to look at reversibility--we are just loading animals into the reversibility set at this point in time--and that is, look at cognitive function, other kinds of behavioral endpoints in these animals after they are exposed to ketamine, follow them for 1 or 2 years to see how it may be affecting these animals' ability to learn very complex behavioral tasks. So, that study is ongoing now.

We also were interested in developing some imaging approaches that would provide non-invasive biomarkers and that is also going on in this Phase III. Then, eventually, we will get to Phase IV, trying to understand more about how we can compare

across different agent categories by using both genomic and proteomic effects.

So, that is the study design right now, and I will just show you the Phase I and Phase II data at this point.

[Slide.]

So, what we did is to--in these in vivo monkey studies, is to use the Rhesus monkey, as I mentioned, gestational day 122, which is about 75 percent term in this species, day 5, and post-natal day 35.

They were infused intravenously with ketamine to maintain a light surgical plane and this was either done for 3 hours or for 24 hours. And, in each case, it was followed by a 6-hour withdrawal.

The animals were initially given a dose of 20 mg/kg IM ketamine, and then the infusion was begun at that point in time. Usually, the rate varied between 20 and 50 mg/kg per hour but it was actually done to effect, not to a particular flow rate.

Then, we examined the brains of these animals in a histological way using silver stain, which is your classic indicator for cell death of the nervous system, caspase-3, which you have heard a lot about already, and we also used a technique called Fluoro-Jade that was developed at the NCTR some years ago.

Now, the physiological monitoring is very critical. I think Vesna did a really great job introducing this, so I can go over this relatively quickly, but the idea is that because you have an animal that weighs half a kilogram, you can certainly do a lot of monitoring. And our OR, within our primate facility, it looks a lot like an OR within many clinical facilities, because we have equipment that we can do all these measurements with and do it on a continuous basis.

I will show you some data from the oxygen saturation curve, and I will also show you some plasma ketamine levels. I won't go through all the rest of these because we just don't really have time, but they are now accepted by Toxicological

Sciences, and that article will be coming out soon, but that data has been reviewed and accepted.

One thing I will point out, blood glucose was brought up, and we did not see any significant changes in blood glucose in any of these animals that we looked at.

[Slide.]

Now, here is just the data looking at hemoglobin saturation with oxygen. And this was done with a CO-oximeter placed on the tail of the animal, and you can, of course, monitor this very easily.

What we are looking at here is effects in the pregnant female. The ketamine values are these open triangles, and the red ones are the control animals that were done in a parallel fashion but the animals, of course, in control were not anesthetized.

These samples were taken with animals that were chair conditioned, and in chairs at the time in which these samples were taken from the control animals.

So, what you can see here is that they range right around 94 percent and there is no difference between your ketamine-treated and your control animals. And these are pregnant females who were anesthetized for 24 hours or were control state for 24 hours. And the same for the post-natal- day 5 animals, again right around 94, 95 percent across the way for both control and treated, and the same way for the post-natal day-35 animals.

So, certainly, these kinds of data indicate that you can maintain these animals for long periods of time in an anesthetized state without having any of these alterations in the physiological parameters. These data now, in a table form, are available on line or will be shortly in that manuscript.

[Slide.]

So, then another area to look at, of course, is plasma concentrations. Of course, this is a critical feature because you want to sort of know how to relate this back to the human situation

and also to the rodent work that has been done.

Here, we are looking at ketamine values in mg/ml, and you can see that here, with the dark blue line, is the 122-day gestational animal. And you can see the ketamine values. They come up relatively quickly. There is a slow rise here. This is a n of 3 animals, and these are standard error bars. You can see it fairly tightly comes up to right around 10 to 12 mcg/ml.

You can also see in the post-natal day-5 animals, it is right about the same range coming up here to about 10 or 12 mcg/ml. What is interesting is that the post-natal day-35 animals require a bit more ketamine to maintain the same sort of plane of anesthesia. You can see that it jumps up to around 20 or 22 or so mcg/ml to obtain the same stage of anesthesia.

So, these are the values, and also for norketamine you can see the same sort of thing occurring here, which is one of the major metabolites of ketamine.

Now, I wish the lights could be dampened a

bit--I don't know if that is possible--but what I want to do is show the histological data from these studies and to point out that we use three different kinds of assessment probes.

This is the caspase-3 work, and you can see that compared to control, where you have occasional cells--and here is higher amplification as shown inside this box--you do get some cell death in these control animals. And these are fetuses, of course, that were killed 6 hours after their dose was discontinued, so we gave them a washout period. We gave them 24 hours exposure and then 6 hours washout, and then evaluated these brains.

You can see--with ketamine given for 24 hours, you can see an increased number of these cells, here a little bit higher amplification. With the post-natal day-5 animals, control here versus treated, you can see the increase, as well, in these animals that were anesthetized with ketamine.

So, this is in the frontal cortex area,

about in the same area in the cortex that both Vesna and John were talking about. So, it looked like with the caspase-3, we were getting responsiveness in animals that had been anesthetized for 24 hours compared to control.

[Slide.]

This is post-natal day 35, which is kind of interesting. You can see occasional cell-- here in the control post-natal day-35 animals, but what was kind of interesting to me is that we did not see any real increase in cell death based on this caspase-3 indicator.

[Slide.]

We also looked at silver stain. As you know, this is a classical kind of indicator for cell death in the nervous system, and you can see that compared to control here, 24 hours exposure to ketamine, here, the ketamine-exposed animals, there is a nice band of cells there that light up. Those dark cells are the ones that are dying.

Another thing that was kind of interesting is that, when we went to a shorter period of

exposure, and here we are using the 3-hour exposure, we did not see this increase as we did in the 24-hour exposure. So, we think there is certainly some time-course effects here, as well.

[Slide.]

Here is looking at the Fluoro-Jade indicator, which is another indication of cell death in the nervous system. Control levels, occasional cells, which you would expect, the brain is going through apoptosis as far as normal developmental sequence, so you can have a few of those.

With ketamine, however, it is dramatically increased and this was 24 hours of ketamine with 3 hours, not a dramatic increase. If anything, it looks a lot more like control than it does the ketamine-treated animals for 24 hours.

[Slide.]

So, these are the data that we have, and sort of putting them together with the EM work here. I think Vesna was the one that talked about the EM effects. We do have the EM in all these

animals, as well, and what is interesting is that indeed the EM did support the concept that, in the monkey, you may also be having some necrosis along with apoptosis.

You can see here the control monkey. Here, in B, it shows some condensation and fragmented nuclei. This has to do very much with the apoptotic type of cell death.

Here, you do get some of the swelling that would be consistent with a level of necrosis. So, we think that, in the monkey, you are probably getting a lot of apoptosis and some necrosis along with that.

[Slide.]

Just to finish this up, then, in terms of looking at the importance of this 3-hour versus 24-hour, you can also look at this in-situ hybridization and get a feel for the effect on the induction of this NR1 subunit of the NMDA receptor and you can see that it has increased with ketamine at 24 hours. But what is very interesting, if you just do control or 3-hour ketamine treatment, you

do not see this increase in the induction of this upregulation of the NMDA receptors.

We think that does tie the in vivo to the in vitro work that I showed you earlier. It is necessary to have the upregulation of that receptor in order to have effects.

[Slide.]

Here is a nice summary slide. This was, like I said, just accepted by Toxicological Sciences. It gives you sort of an overview of the studies I have just been speaking of.

Here is your caspase-3 data, your silver stain data, and your Fluoro-Jade data. So, we have three different kinds of approaches to detect cell death, and we can just sort of summarize it here.

With the silver stain, you certainly have a dramatic increase on gestational day 122, which is about 75 percent term, with a 24-hour exposure to ketamine. It is also there on day 5, and you can see it there with caspase-3, as well as the Fluoro-Jade.

However, when you go to post-natal day 35,

a little bit older animal, we do not see an increase. And, if you go to post-natal day 5, which is sensitive to the 24 hours exposure, but only give it 3 hours of exposure to ketamine, we do not see an increase. In fact, I mean, here, you can see that the exposed animals are not any greater than the control animals.

So, what we are looking at here is a nice effect that is a first attempt to describe something about a sensitive window, and one of the first attempts to try to look at duration of exposure in a way in which we can start to understand the effects in the in vivo non-human primate.

[Slide.]

So, just to summarize this, then, we feel like these data are still consistent with the idea that you have to have the upregulation of the NMDA receptor, and that you have to allow amount of time for that to occur and for the influx of calcium to occur, so you can get this cell death.

[Slide.]

The time-course seems to be consistent with this model. It also is consistent with the idea that the upregulator receptor allows in calcium, which then can have effects, of course, on mitochondrial function.

I won't go into the rest of this at this point in time. We do have data that supports each one of these steps. But I think the main thing is to think about this, not only producing apoptosis, but in the primate we also think there is some necrosis occurring, as well.

[Slide.]

Just to summarize then, we feel these results with ketamine administration can result in a dose-related increase in neurotoxicity both from the in vitro data and from the in vivo data.

Ketamine-induced neuronal cell death in the monkey is probably both apoptotic and necrotic in nature. This seems to be a little bit different than the rodent that seems to be primarily apoptotic in nature.

Ketamine-induced cell death may involve

upregulation in NMDA receptor especially in NR1 subunit, and we have recognized this in several different levels. But certainly if you use antisense to that NR1 subunit and block its upregulation, you can block the sensitivity or toxicity of ketamine.

[Slide.]

Also, we realized from these data that the earlier stages of development in the monkey--that is, either gestational day 122 or post-natal day 5--are more sensitive to these anesthetic effects than the later stages, say, post-natal day 35. Now, this is the first attempt at defining the sensitive window, but it does give us some initial steps to think about.

Also, that shorter duration of ketamine exposure, say, around 3 hours, does not produce the kind of effects that you see with 24 hours of exposure.

[Slide.]

But there is a lot to be done and many of you, of course, realize that in this room. What

about the threshold phenomena, is there a threshold of duration before you get an effect?

We are also now looking at 9 hours of anesthesia, trying to understand where the break may occur, because we don't think there is much going on at 3, certainly at 24--let's try to fill it in, in between to get a better handle on that.

What about the real periods of development that are sensitive? We don't know really what the front end sensitivity is, we are only at 75 percent term that seems to be sensitive. Can you go to mid-term or even earlier than that and see sensitivity? Well, we are not sure yet.

We have a little idea about what has happened on the older aged animals, and certainly the monkey at post-natal day 35, the animals don't seem nearly as sensitive.

Is there neurohistological evidence for long-lasting effects? Vesna spoke to this--I think has some of the only data suggesting that there are those long-term effects. I think it is very interesting. We want to be able to look at those,

as well, in the primate situation.

We want to do that with a number of different modes. One of those approaches is, of course, using the functional assessment as Vesna also talked about. We are developing and have developed a really complex cognitive function assessment tool for the primate and we will be applying that to these animals.

Those studies are already loaded and we will just be waiting to assess those as we move along. Also, we think there are ways to develop non-invasive markers that can help us with not only this issue but perhaps, sometime in the future, could even be available perhaps for clinical studies.

We are using microPET approaches and attempting to develop a non-invasive biomarker of the cell death that we could use not only in our animal studies but perhaps could be translated to the clinic.

Then, of course, you know, what sort of strategies can we develop for decreasing or

blocking these effects, and certainly, Vesna spoke to some of those kinds of issues and so did John.

I think that there are many different ways as we understand more about the mechanism, I think we can find ways that will allow us to block this effect. Certainly, those kinds of things that have to do with oxidative stress seem to have some initial effects and it could well be that that is one approach that could be used. But other approaches could be used, as well.

[Slide.]

Just to finish up, we have just sort of begun what is really a long road here. You have seen work in all these areas this morning, but I think that what we would like to do is think about the possibility of defining this as we are starting to do with ketamine in the non-human primate, but then think about other possibilities and those could be some of the mixtures that we heard about from Vesna's work and also could be some of the interactions between the GABA agonists and the NMDA antagonists. And I think that Vesna's idea about a

fentanyl control is a very good one and we would like to consider that one, as well, at our studies.

[Slide.]

With that, I want to thank some of the people who were responsible for this work; Cheng Wang and colleagues from NCTR--all have been working very hard on this over the last several years; our good friends at CDER and FDA and also CBER; and then support from a variety of sources for the rodent studies, NTP, and for the primate studies, NICHD, CDER, and NCTR all working together to get these done.

I thank all of them and I thank all of you for your attention.

DR. SHAFER: Dr. Slikker, thank you very much.

In the interest of staying on schedule, I will ask the committee members to hold their questions. When we begin our discussion at 2 o'clock, we will devote questions to the last three speakers here.

Our next speaker is Dr. Sul Soriano, who

will be talking about Clinical Perspectives:  
Implications of Non-Clinical Findings.

**Clinical Perspectives: Implications of  
Non-Clinical Findings**

DR. SORIANO: Thank you, Steve.

I would like to thank Dr. Rappaport and the FDA group for inviting us to this panel to re-discuss Initiative 3 has a wide implication in the practice of anesthesia.

They asked me to talk about the clinical perspective or pediatric anesthesia and this whole issue about anesthetic-induced neurotoxicity in the developing brain. I would like to give you my perspective as a pediatric anesthesiologist.

[Slide.]

Basically, you can summarize our concerns by this quote from Hamlet. "...for there is nothing either good or bad, but thinking makes it so."

[Slide.]

Why do I say that? Certainly, when you look at the management of a neonate, it has changed

quite a bit over the years. Back in the early fifties, sixties, seventies, it was thought that the neonates had no ability to perceive pain and therefore didn't require much analgesia or anesthesia.

Furthermore, these neonates, when they presented for surgery, were in extremis and were frequently in septic shock and required profound human dynamic support.

[Slide.]

It has been a practice during those days to use the Liverpool technique when you anesthetize these patients during surgery. It is composed of nitrous oxide, a muscle relaxant, then curare, and perhaps hyperventilation, but basically, this was chemical paralysis.

[Slide.]

It was the landmark studies by my colleague Dr. Sunny Anand, when he was a Fellow at Oxford, who looked at the effect of analgesia/anesthesia in this group of patients. He basically randomized premature neonates undergoing

surgery for ligation of patent ductus arteriosus and randomized them to either receive the Liverpool technique or fentanyl at a fairly high dose.

He found that the non-anesthetized group developed metabolic acidosis, hyperglycemia and several postoperative morbidities including long ventilatory requirements, bradycardia, intraventricular hemorrhages and hypotension.

[Slide.]

We were fortunate to have Dr. Anand join our group in Boston Children's Hospital, and he, along with Dr. Hickey, looked at the effect of pain and its effects on the neonate and fetus, and found that indeed these neonates and fetuses had a neuronal circuitry to perceive pain.

In this nice review article that they put together for the New England Journal of Medicine. You can see that, in fact, even before birth, you had all the neuronal circuitry to both perceive and feel pain.

[Slide.]

Further work from the Cardiac Group in our

institution showed that these infants who were subjected to a light anesthetic versus a deep anesthetic, which was a narcotic anesthetic, those who were anesthetized slightly mounted a stress response as indicated by these rises in stress hormones during cardiac surgery.

So, the thinking now switched over to the fact that it is indeed safe using a more sophisticated human dynamic monitoring and available drugs to anesthetize these fetuses during surgery.

[Slide.]

Then, came the sentinel article from Dr. Olney's group, and certainly this has made us think about this practice because, in their abstract, they said these findings may have relevance to human neurodevelopmental disorders including postnatal anesthesia exposure to drugs that block NMDA receptor.

It was thought perhaps maybe this process just induced or accelerated apoptosis which would not have any functional consequences.

[Slide.]

However, Dr. Todorovic's group, along with Dr. Olney, showed that indeed not only ketamine but some commonly used anesthetic drugs induced this process. But, more importantly, it showed that this had functional consequences, as shown by Dr. Todorovic.

[Slide.]

But I submit that perhaps maybe this artificial model that we use to examine this may be not completely reflect what we see in the operating room when we manage these patients. There are certainly differences in the way the disease process, as well as the conditions that we face when we are anesthetizing patients.

[Slide.]

As Dr. Todorovic said, infants aren't small adults. I submit to the neuroscientists in the group that human babies are not just large rat pups, and there are some problems with this experimental paradigm or model. First of all, as indicated by some of the other panelists, the

duration exposure to the drugs may not be clinically relevant in this case.

[Slide.]

We thought, when we designed the study by Dr. Hayashi and my group, when we designed the study, we wanted to duplicate what we actually do in clinical practice.

Ketamine is typically given as a single dose, an induction dose to either sedate or anesthetize a patient to allow that patient to enter the operating room, and also being frequently used now by emergency-room physicians to reduce long-bone fractures as part of the routine analgesia they provide, but ketamine is typically given in single dose.

When we did that, we found that there was no increase in neurodegeneration in all areas of the brain. When we increased the duration to 4 doses, which was approximately about 5 hours, we still did not see any changes. However, when we gave the dose for over 9 hours, duplicating a continuous infusion of this drug to these rat pups,

we found the neurodegenerative changes that had been previously reported.

[Slide.]

Furthermore, there is the whole issue about duration in rat days and human days. Certainly, 7 rat days probably extrapolates to about 27 to 30 human months. So, therefore the experimental paradigm was giving ketamine for 6 hours, may equate to an exposure for about a month and certainly we do not do this in operating room.

But perhaps, in the setting of the ICU where you have a chronically ventilated neonate, that may be occurring, but not in typical pediatric anesthesia practices.

[Slide.]

The lack of precise physiological monitoring is important. And Dr. Todorovic showed you that indeed in these models, they have precise physiological monitoring.

[Slide.]

You can see here one of my patients I took care of a couple of weeks ago. You can see that we

had specialized ventilators measuring every single breath that this patient took and gave out. We monitored arterial blood pressure saturation throughout the surgery.

More importantly, we need to do that to assure end-organ perfusion, because the requirements of the surgery itself varies from different points in the surgery. Certainly, you need a deep level of anesthesia when you are intubating the patient's trachea. You may need a deep level of anesthesia when they are making the surgical incision and even manipulation of internal organs.

However, during most of the surgery, we use subanesthetic doses or subMAC doses during the course of that. Certainly, we also adjust our intravenous drugs accordingly, giving high doses or anesthetic doses when that stimulus is highest. But most of these cases are done in a subanesthetic dose fashion.

[Slide.]

Again, there is species variation,

something that has been pointed out by my predecessors in this panel, and there are differences in dose-response as you can show. We use 1 mg/kg of ketamine for humans, whereas, these rat pups require up to 20 mg/kg to get the same effect, so there are differences.

There are also differences in drug metabolism of this drug, perhaps maybe metabolized fraction of the primary drug may be different, and also you have to understand that the area under the curve, the dose and duration of the drug has a huge role to play in this.

I would like to finally address the issue about peak susceptibility.

[Slide.]

Now, you have seen Dobbing and Sands, but you haven't seen the actual chart that they put on the paper. This is the conglomeration of about 7 studies looking at different peak points of peak synaptogenesis in various species. You can divide it pretty much in 3 different groups: the prenatal group, the perinatal group where peak

synaptogenesis occurs, and that is where man sits, and the postnatal group where you have rats, rabbits.

You have to look at, as Dr. Todorovic said, the peak period is when you would see these neurodevelopmental changes. Certainly, when you look at the timeline, the peak period of synaptogenesis occurs around 7 days in the postnatal rat, however, in human, it straddles the point of birth.

So, what do we do about these periods afterwards? Dr. Todorovic has shown that about at 14 days, you see less of an effect of these drug exposures. So, certainly, when you look at the fetus that is undergoing fetal surgery, or the neonate that is undergoing emergency surgery at birth, they may be at the peak period of susceptibility or vulnerability to this problem.

What do we do about this 2 1/2-year-old child who is getting anesthetized by her mother? Is her mother poisoning her child's brain? We don't know and certainly a lot of anesthetics are

done in this age group.

So, therefore, you should consider and think about the implications of this broad range of susceptibility. It peaks at 7 days, perhaps it peaks in the rats, and it peaks at birth in humans.

[Slide.]

Again, Dr. Anand pointed out the whole issue about anesthesia with and without painful stimulation. All the models we spoke about so far is an artificial model of anesthesia and surgery, because there is no surgical stimulus. Certainly, there is a lot of data including work from Dr. Anand's group that showed that uncontrolled pain during the neonatal period leads to neurodevelopmental problems in the later age.

Furthermore, transmission of these impulses to the cortex, the immature cortex, has caused some toxic damage as shown by previous investigators. Certainly, during surgery, the whole issue about injury, overstimulation, excitatory toxicities involved with the stimulation may be a problem.

Another point is the whole issue about neuroprotective effects of anesthetics. Many of these drugs that we use are neuroprotectant anesthetics. There are inhaled agents, as well as the barbiturates, and the benzodiazepines all have some neuroprotectant abilities. Perhaps they may be causing some neurotoxicity in an unstimulated brain. But, in the case of an infant or a neonate undergoing surgery where there is massive stimulation, massive changes in the stress response, these anesthetics may perhaps be neuroprotective. That has to be looked at.

[Slide.]

So, we come back to the question is anesthesia harmful to the neonate's brain.

[Slide.]

Certainly, we looked at ethanol in some of the organ studies by Dr. Olney's group that show that it does produce fetal alcohol syndrome. Furthermore, Dr. Olney's group has shown that anti-epileptic drugs, such as phenytoin, phenobarbital, benzodiazepines, and valproic acid

has the phenotype that produces fetal malformation, developmental delays, and microcephaly.

But this far, as Dr. Eisenach has pointed out, there is no phenotype yet. Perhaps we are not looking closely enough. We may not have had the right measures of it, but there is no phenotype that we know of of toxicity involved with exposure to anesthetic drugs.

[Slide.]

Now, just to point out that these neonates need surgery. You can see how small and how sick these neonates are, that indeed they had the highest morbidity and mortality rates in any subgroup of patients that we anesthetized.

These surgeries are done on an emergent basis. They are lifesaving surgeries if the neonates can survive. They frequently will present with numerous congenital anomalies which can also be parts of genetic syndromes we may not have uncovered at the point of surgery.

Many of these neonates also have pre-existing brain injury. Some of the studies

done in our group looking at fetal MRI show that they may have brain lesions pre-birth, so that is something to consider, and certainly during the course and the conduct of anesthesia, we are faced with the transitional circulation, the change from fetal to neonatal circulation, as well as respiratory instability due to the patient's condition, as well as surgical manipulations are things they have to deal with.

[Slide.]

When you look at the outcome of these neonates undergoing surgery, many of them will show that they aren't quite right when they are looked at later in life. Neonates who have undergone laparotomy, esophageal atresia repairs, patent ductus arteriosus, in all these core studies, they have been able to show some neurodevelopmental outcome of deficits.

You have to consider that when you conduct these types of studies, there are confounding variables such as prematurity which, indeed, has its own set of debilitating problems. Coexisting

malformations are noted, genetic syndromes, the state of the patient in extremis frequently when he presents for surgery, the surgery itself, and management of them in the intensive care unit, and perhaps the anesthetic drugs, maybe the depressant quality of the drugs may contribute to the injury.

[Slide.]

Looking at some indirect measures of neurodevelopmental outcome after surgery, I will point to some of the studies that we have done again in our institution, Boston Children's Hospital, looking at infants who have undergone transposition of the great arteries surgeries. These patients were all anesthetized in the standard fashion with a fentanyl-based anesthetic, fentanyl infusions for sedation.

In this extensive study, they looked at postoperative seizures and neurodevelopmental assessments.

[Slide.]

You can see when they looked at the neurocognitive score both 4 years and 8 years out,

they weren't very different. They pretty much straddled what the normal control is here, which is 100 percent. You can see they don't stray too far although I might point out that there may be some infants who had problems down here.

If you compare this to some of the functional data that Dr. Todorovic showed in her Water Maze studies, you would expect some of their deficits to be way down here, 50 percent depressed rather than closer to the normal mean.

[Slide.]

Certainly, there are initiatives by the pediatric anaesthesia community, and many of them are present here both as observers, as well as members of the panel.

I would like to point out that CDER, as well as NICDH, have sponsored neonatal drug development initiatives and included us in that group. The Society for Pediatric Anesthesia has awareness and educational programs, as well as a journal that Dr. Shafer is the editor of, where they actually published papers on this, and they

are putting together a registry and complications database.

The American Society of Anesthesiology again have increased awareness through various panels in their annual meetings and also Dr. Eisenach's journal, which he edits, Anesthesiology.

Also, there are some groups that are funding different studies within our community.

Certainly, the thing to do, though, is to perform a randomized controlled trial to remove all the confounding variables with a cohort study may be good.

[Slide.]

Just to summarize the proceedings from the Neonatal Pain Control Group, Dr. Maxwell, Dr. Anand, and I, and Dr. Mattison, who was involved in helping plan this, determined that we should look at this.

This is an important issue and when these long-term developmental outcomes are performed in humans, we should various endpoints and outcome skills to show whether or not indeed this occurs.

Now, the issue is whether or not if we do that at 2 years of age, 4 years of age, 8 years of age, or at 32 years of age when all synaptogenesis and plasticity is completed. We don't know what the answer is, and hopefully, this panel and members of the audience can help out and determine that measuring point.

[Slide.]

I would like to let you know that also the international pediatric community has starred this as an important problem, and they formed a multi-national, multi-center collaborative group looking at the effect of anesthesia on the developing neonate. This is headed by Dr. Andrew Davidson in Melbourne, Australia, Mary Ellen McCann in my institution in Boston, and Neil Morton in Glasgow, and each center has several collaborations with other pediatric hospitals in their region.

What they plan on doing is they want to look at inguinal hernias performed in neonates and infants, and they plan on randomizing them to receive either spinal anesthetic alone versus a

general sevoflurane anesthesia, and they plan on doing neurodevelopmental assessments at 2 and 5 years.

We started recruiting patients for this and we expect the comment period to last until 2009, the analysis and data collection will occur then, so we probably won't have any data available until 2015.

Again, this study is being funded by private grants and private seed money from various institutions.

[Slide.]

So, what do you think?

"...for there is nothing either good or bad, but thinking makes it so."

Certainly, as humane practitioners, we all should consider the alleviation of pain and suffering is important in all aspects of our care of patients, and blunting the stress response during surgery is certainly important particularly in minimizing morbidities after surgery.

The laboratory data are irrefutable and we

should recognize this and believe it because it is true and, even in my own hands, I see it occurring.

However, the long-term effects in humans are still unknown and need to be investigated further.

Basically, we have no other alternative to use to anesthetize these patients during the perioperative period.

[Slide.]

As a member of the pediatric community, I would like to give my gratitude to the work by Drs. Olney, Todorovic, and also the FDA group for relooking at this issue in an isolated model to show us that perhaps this may be correct, that there is the potential for this to occur in the laboratory animal. It is up to us as practitioners, as clinicians, to see if indeed it occurs in our patients, and to look for funding, design the appropriate studies and conduct them fully to see if indeed we are harming these patients.

In the meantime, before we get that data done, we should continue to anesthetize, sedate,

provide analgesia for these patients, and not throw the baby out with the bath water.

Thank you.

DR. SHAFER: Dr. Soriano, thank you very much.

We have 10 minutes before the lunch break.

What I would like to do to open the presentations by Dr. Todorovic, Dr. Slikker, and Dr. Soriano, open it to questions from the members of the panel.

Dr. Mattison.

DR. MATTISON: The question that I have is for Bill, but it is for Bill and John Olney together.

Bill, you suggest in your non-human primate data that exposures up to 3 hours have no effect. Dr. Olney, in your non-human primate data, you are seeing effects at doses which are 5.5 hour duration. I don't remember that I can judge what the dose unit, area under the curve, or peak concentrations would be.

So, Bill, it seems like what you are observing is sort of the maximally tolerated dose

at the 3-hour interval if we are getting adverse neurodevelopmental consequence at 5.5 hours. I mean, sure, it is about double the time, but a 3- to a 5-hour period isn't, in my mind, a substantial difference.

Perhaps the two of you can help me understand how you view those two differences in exposure durations.

DR. SLIKKER: I think this is an important issue. I think that if you remember the time course data that I showed you with cells in culture, and also the study that we did in the rodent in vivo as well, that time does make a difference, and actually, it is a relatively steep change when you move from 2 to 4 hours up to 6 hours.

So, actually, it could well be that these data reflect a different sensitivity based on duration of exposure and that perhaps we sort of have to begin to bracket that series of exposures necessary to produce an effect.

Now, that has to be cautioned in the sense that you have actual level of exposure also be

considered. You have the conditions for that exposure, the animal model, et cetera, et cetera, but I think that there is evidence from the in vitro data that you have a relative important window of time, and it has been described in vitro.

It seems to hold up in vivo and that is critical in determining whether or not there is going to be this outcome that we see with the cell death. I think that that has to be considered in light of the various kinds of assessment tools that are being used to look for cell death. Obviously, we have used a variety and John has used a variety, and some of them overlap, some of them don't, but I think that that is also a consideration, the kind of assessment tool that you are using.

But I don't think our data are incompatible based on what I understand what John presented and what I presented.

DR. SHAFER: I would like to actually follow up with a question myself, if I may.

Just to calibrate the concentrations, Dr. Mellon presented that 2 mcg/ml was considered to be

a clinical dose, and if I am stating it correctly, you said that is kind of a high clinical dose.

My own looking into the question in the last few days has suggested that clinical dosing that we are likely to see in the doses we typically use are more in the area of around 0.5, maybe 1 mcg/ml.

The concentrations that I saw in your study were about at least 5-fold higher than that, sort of in the 5 to 10X range, so I actually got a sense of safety by saying that you can give a 5- to 10-fold overdose for 3 hours, and not have effect.

Did I properly interpret your data?

DR. SLIKKER: Well, I think this is an interesting question and one that you sort of have to go back to the basic physiology to maybe understand more fully.

The thing is that you are absolutely right, the levels in the plasma to reach a stable surgical plan of anesthesia are higher for the monkey than they are for the human.

We will have to go further than probably

even this group to understand why that is the case.

But that is the way it is and, if you look at the doses that are recommended for anesthesia in the various species, you can see that it increases as you move from the human to the monkey to the rodent. So, it has been known for a long time.

So, I think the difficult thing that this panel has to grapple with is do you look at a comparison between species based on the concentration of ketamine to the plasma or do you look at it as the basis for that comparison, the plane of anesthesia that is achieved.

Our monkeys were not any different than the kind of plane of anesthesia you would prefer to use if you were going to use this agent as one to induce anesthesia for a surgery.

So, we are using the same physiological endpoints and getting that plane of anesthesia. It happens to result that those plasma levels are different, and they are higher in the monkey than in the human.

DR. DESHPANDE: I have a comment and a

question for Dr. Todorovic. First, a comment for all of the investigators. Thank you for bringing up a very important and crucial point that we need to clarify for our patients. Dr. Soriano, thank you for a very comprehensive assessment of the challenge we face.

The comment for Dr. Todorovic is that she pointed out the behavioral problems that children face after undergoing anesthesia. Recent studies have shown that pretreatment with midazolam, a benzodiazepine, actually can improve the behavioral problems that patients afterward, so that much of this is appropriate pre-op preparation and appropriate pre-op medications. Some of the studies that were pointed to earlier really have been at least clarified.

The question that I have is that a lot of premise, and actually an alarm that was brought up in me when I was re-reading much of this material, was the analogy between fetal alcohol syndrome and what is going on here.

Is the alcohol effect solely through the

NMDA mechanism as I would assume from what things had been presented today?

DR. JEVTOVIC-TODOROVIC: Well, I haven't done work with alcohol. Dr. Olney did with Dr. Ikonomidou and Dr. Dave Wozniak, and I am sure he could talk more about the effects of alcohol on behavior.

Histologically, actually, when you look at the effects of alcohol, I find it morphologically very similar to what we see when we start combining anesthetics.

Now, our behavioral study was done with triple cocktail because at the time we wanted to establish whether there is a phenomenon to study or not, so we went for what was most toxic morphologically. So, unfortunately, at the time we couldn't address each anesthesia separately, so I couldn't tell you whether NMDA antagonists as opposed to GABA-ergic is important.

However, when you look at each and every anesthetic that we use, we try to say that they have one mechanism of action sometimes, but it is

again overly simplistic. As you know, they are dirty drugs and they do a lot of things to a lot of receptors.

For example, we were very proud of our nitrous oxide finding to say it's an NMDA antagonist. But then you have studies that it is very effective in blocking nicotinic receptors, maybe has an effect on GABA. We couldn't see that in our hands, but it is popping.

So, there is simply no anesthetic that we use nowadays that is a clean drug. So, to separate NMDA antagonism from GABA-ergic effects from nicotinic effects, from sodium channel effects would be very difficult.

So, I really cannot say how that would affect the behavior if you were to do that. I know Dr. Wozniak has done a lot of work with alcohol and I think you have done some work with MK-801, as well, and MK-801 is as close to being a pure NMDA antagonist as you get.

I think that there were indications of behavioral effects. Another thing we have to

understand, the studies with rats really showed that these animals were incredibly similar to controls. It was only after we studied specifically cognitive deficit and we really zeroed in on that, that we could see the difference.

So, when you do any cognitive studies in humans, it is incredibly important to be very, very precise about doing these fine cognitive tests.

You know, the element of motivation is very important in humans. If you have seen the study we have shown with juvenile rats, they were 30 days of age, they did eventually learn the task, but it took them twice as long as it did for controls.

So, if you are looking at a child in school environment that is taking twice as long for any cognitive task to grasp, well, what is going to be his or her level of motivation? And we cannot ignore that.

Eventually, that child may just give up, because it takes so--for rats, they just went on happily about their business. What got me even

more concerned, that although they eventually learned at the age of 30, when there was some compensatory mechanism in place, when they were only 6 months of age, which is adulthood for rats, they struggled with complex tasks.

So, what does that mean, are we going to follow people until they are 40 years of age? Are we going to be concerned that it takes them 3 readings to get something? I don't know.

It is a very difficult phenomenon to study in humans, but to ignore the fact that the task was accomplished in twice as long of a time, we cannot do that because the motivation is very important especially early on for cognitive development.

That is the only thing I could say. But we did use cocktails, and the reason we did that, I thought that it perhaps matters clinically, because I have never had a patient who got a single anesthetic.

I know that even in the intensive-care units, we do combine them on a regular basis, midazolam/fentanyl infusion, a little bit or

morphine here and there, maybe ketamine is needed, and I have heard talk about putting vaporizers in intensive care units where we can actually keep babies anesthetized with isoflurane and sevoflurane.

I just want to say regarding these anesthetics, it is a good point. You know, I am a little concerned about the clinical study that is being designed in Australia. I am aware of that study. We are again clumping all inhalational anesthetics together. The studies using sevoflurane, it is commonly now pretty much almost the only inhaled anesthetic used in clinical practice in pediatrics, but really, some of the studies done in Dr. Eckenhoff's lab showed that sevoflurane is not as toxic as isoflurane.

So, yes, we are going to get a very important clinical answer if, indeed, sevoflurane doesn't cause any cognitive deficits later on. But, no, it is not going to point at anything that we have presented today because it is becoming irrelevant.

If you are going to compare the cognitive effects of isoflurane in humans to rats or guinea pigs or monkeys, you really have to use isoflurane in humans as well. To assume that they are the same is a wrong assumption.

DR. SHAFER: Or sevoflurane in rats.

DR. JEVTOVIC-TODOROVIC: Right.

DR. SHAFER: One last question. This will be the last question and we will have lunch.

DR. ZUPPA: I thought it was very interesting that the monkeys that were 35 days post-natal had the highest plasma concentrations but had the least amount of neurotoxicity. I was just wondering if the higher plasma concentrations were associated with a higher dose that was delivered or an altered clearance mechanism.

DR. SLIKKER: I don't believe with the data that we have at hand that we can fully answer that question. But you are absolutely right, and it was a nice observation that indeed the 35-day animals required more, higher plasma levels of ketamine to maintain the same stage of anesthesia.

But I really couldn't tell you right now what the mechanism is behind that, and I can't tell you about the clearance of the compound and whether or not it is altered because of disposition or because of metabolism, et cetera.

These are plasma level studies to compare against levels in other species including human, but they weren't designed to be pharmacokinetic studies per se.

DR. ZUPPA: Just the other interesting point is the gestation day 122, it looks as if they never really reached steady state, so I don't know if the dose is being titrated up and titrated up, and that is why, or whether or not you saw that clinically, and they were just harder to anesthetize.

DR. SLIKKER: That is another really good observation and what we are looking at there, of course, is a mean of 3 animals with a standard error around them, so it would be better probably to look at the individual animals to try to get a feel for that.

But, indeed, what we were trying to do, as I mentioned, was to maintain the same level of anesthesia, and it seem as though there was more anesthetic necessary as the procedure progressed through the 24 hours.

However, there could be some time of day phenomenon here, because obviously, these animals and all the people involved are there for 24 hours-plus, and it could well be that it seemed like the amount that was necessary to be infused did require to be incremented to maintain the same level, and then perhaps, near the end of the study, things sort of plateaued off as far as that is concerned, or even dropped down a bit.

But the goal was to maintain the level of anesthesia plane consistently and we adjusted the flow accordingly and it is reflected to some extent in those values with ketamine that you see in the plasma.

DR. SHAFER: It is now time for our lunch break.

We will return and start promptly with the

open public session at 1 o'clock.

[Whereupon, at 12:00 Noon, the proceedings  
were recessed, to be resumed at 1:00 p.m.]

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A F T E R N O O N P R O C E E D I N G S**Open Public Hearing**

DR. SHAFER: This is the Open Public Hearing.

The following is to be read by the Chair for the Open Public Hearing.

Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision-making. To ensure such transparency at the Open Public Hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the Open Public Hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with the sponsor, its product, and, if known, its direct competitors.

For example, the financial information may include the sponsor's payment of your travel, lodging or other expenses in connection with your

attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have any such financial relationships.

If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

For the speakers at the open public forum, you will notice some lights at the podium. The green light means you are good to go. When you see the yellow light, it means you have 30 seconds, and when you see the red light, the power is cut to your microphone.

It's a fairly insistent system of keeping the program on schedule.

Each presenter will have six minutes.

The first presenter is Peter Jackson with an introduction by the Honorable Congressman Mark Kirk, is that correct? Okay. He will not attend so Peter Jackson will speak.

MR. JACKSON: Distinguished Committee

Members, thank you for the opportunity to speak to you today regarding the problem of prescription opioid drug abuse.

I do have no financial interests, by the way.

My name is Peter W. Jackson. I am a biologist with the U.S. Environmental Protection Agency in Chicago. My wife, who is with me today, and I reside in Arlington Heights, Illinois where we have raised two children, one boy and one girl.

During the week of August 13, 2006, our daughter Emily, whose picture you see here on the screen, only eighteen years of age and days from her first day in college, was staying with relatives to console the family as they had just lost their father and husband due to cancer.

On August 18th Emily was killed accidentally when she consumed a pain killer that had been prescribed for her late uncle. The coroner conducted an inquest and concluded that Emily died from a combination of this drug, OxyContin, and other things in her system which

included alcohol and her own prescribed medication.

While this combination presumably enhanced the toxic effect of the OxyContin, if you take away the OxyContin, Emily would likely not have died. Emily was not a drug addict. She clearly did not know what she was getting involved with, and she made a simple but tragic mistake.

The circumstances of Emily's tragedy, ready access to a dangerous prescription drug and a lack of knowledge about its true danger, are very common elements of a growing problem of prescription drug abuse in this county.

Without question, personal responsibility and drug-safety awareness play an important role for this problem, but if we do nothing more than preach the importance of personal responsibility, young people will continue to die in increasing numbers.

Each of you should have received a full statement containing details about our daughter's tragedy and why my wife and I feel that FDA needs to do something about the high level of abuse with

OxyContin and other prescription opioids. My comments today will be limited to a statement of recommended actions that the FDA should seriously consider.

We call on the FDA to schedule a special public meeting to explicitly address the problem of OxyContin abuse, along with other opioids which may have a high documented rate of abuse. The goal of this meeting should be to develop a comprehensive policy designed to substantially limit non-patient access to, and abuse potential of, OxyContin and other widely abused prescription opioids.

Following are several strongly recommended strategies for FDA's consideration insofar as OxyContin is concerned.

1. Per FDA's Citizen Petition 2005P-0076, temporarily recall OxyContin from the market until the drug can be reformulated to minimize abuse and overdose potential. Reformulation should address the realities that OxyContin is killing our children even when swallowed whole and is often fatal when consumed with alcohol, which was the

very reason that Palladone was removed from the market by FDA.

2. For the same petition, make a label change that would limit the use of OxyContin to severe chronic pain from documented peripheral tissue diseases such as cancer.

3. Place additional controls on OxyContin so that individual prescriptions are more carefully issued and monitored.

4. Require mandatory specialized pain addiction and abuse training of all doctors who are authorized to prescribe the drug.

5. Implement long-term studies of addiction risks and the relative effectiveness of different dosages versus other therapeutic options.

In short, we would like to see OxyContin administered to patients in need in a manner that reflects a documented threat to public safety posed by this drug. The most effective policy will require a number of these regulatory actions, if not some form of all of them.

In closing, Emily was a compassionate,

friendly, and loving person, who cared more about the people around her than herself. She was a vital person in the lives of many people, and she was very excited about the life ahead.

In my daughter's name I beg of you to please address this issue, so that other families may be spared this tragedy, and other people may live full and happy lives.

Finally, I would like to point out I am here to represent not only Emily, but a number of other kids who have been killed by OxyContin, and I would like to conclude by showing you a short set of pictures as a reminder that the statistics you have seen regarding OxyContin-related deaths represent real wonderful people.

Thank you.

DR. SHAFER: Thank you.

The next speaker is Art Van Zee.

DR. VAN ZEE: I am Dr. Art Van Zee. I have no financial disclosures. I appreciate the opportunity to speak with the committee today.

I want to talk just briefly about the

national growing and huge prescription opioid abuse problem. I want to render up some possible suggestions that the FDA can do to impact the problem and specifically, like Peter, I would ask this committee, whose responsibility and oversight is the opioids, to schedule a very dedicated specific meeting to deal with the prescription opioid abuse crisis in general and all the issues that are involved.

I am a general internist, practiced 30 years in a small coal mining town in southwest Virginia. I have been involved in pain and addiction issues over the last 8 years since OxyContin came into the coal fields. It is hard to overstate the pain, suffering, and tragedy that OxyContin brought our region, and as you well know, this has spread to become a national problem.

I am not anti-opioid. I prescribe opioids in whatever dose it takes to treat my cancer patients. I use opioids for a select subpopulation of chronic non-cancer pain patients and, for the last four years, have used bupinorphine in

treatment of opioid-addicted patients.

I think one of the resounding messages from the whole OxyContin problem has been that very high potency sustained release opioids, when abused, are much more addictive and highly addictive. This has been certainly my experience, our experience, in that region where I have seen many hundreds of young people who had recreational-use Percocet or Lortabs; that is, you snort Percocet or Lortabs at parties just as the previous generation would drink beer. They were able to do that and walk away from that but, once they were exposed to and abused a very highly potent sustained released opioid, they became rapidly addicted.

We had a virtual tsunami of opioid addiction which took recreational opioid users to become opioid addicted.

These drugs here, Palladone, Avinza, Opana have been ones that have come to the market through the FDA since the whole OxyContin problem has come.

Palladone, as you know, was taken off the market

in regards to the alcohol interaction problem, but I think, to me, it is just a total disconnect from the level of the FDA and bringing these drugs to the market with the background of the several years of experience of what we have had with OxyContin.

These are high potency, long-acting opioids that have no demonstrable improved therapeutic efficacy or safety as opposed to the immediate release preparations or to other already available sustained release preparations.

So, what are the efficacy safety risks of sustained release versus immediate release preparation with OxyCotin?

Immediate release and sustained release have been comparable efficacy and safety and back pain and cancer pain. Curtis Wright was the medical review officer at the FDA who reviewed the New Drug Application of OxyContin in 1995 and concluded that there was no superiority to OxyContin other than the BID dosing.

The same is true of morphine, immediate release versus controlled release, comparable

efficacy and safety in cancer pain patients, and then hydromorphone, as well, comparable efficacy and safety.

Then, Dr. Chou and his colleagues from the University of Oregon had reviewed this in 2003, reviewed all the literature at that time and again concluded that there was no superiority of either sustained release opioids over immediate release opioids when dosed appropriately or between sustained release opioids and immediate release opioids, no clear superiority in compound.

So, what are the risks of sustained-release opioids over immediate-release opioid? Clearly, as we refer to, there is a higher addiction risk when abused and then as you have heard this tragic story with Pete and Ellen and Emily, there is a higher risk of inadvertent overdose and death very clearly.

This is Patrick Steward, a young man in California, bright, college educated, promising future, not a drug user, actually the grandson of a physician, founder of U.C. Davis Medical School,

who took one offered OxyContin at a party, had one beer, and died from consequences of that.

So, I would offer some suggestions at the FDA level what can be done to significantly impact this problem.

DR. SHAFER: Thank you, Doctor.

The next speaker is Dr. Rod Eckenhoff.

DR. ECKENHOFF: First, a comment. The main focus of my laboratory has been on how the inhaled anesthetics actually work and I would say that calling these drugs glutamatergic, GABA-ergic, or any other "ergic" is a vast oversimplification of how they work - vast.

It is very probable that at least, in my opinion, it is probable at this point that the desirable effects of the drugs are not produced through any of those mechanisms. The undesirable effects, I don't know. I hesitate to say right now the undesirable effects are not, but I don't see any reason. We certainly shouldn't be considering GABA-ergic activity or glutamatergic activity as a biomarker for the fact that we are talking about

here today.

That is me. No conflicts.

I think the reason that this meeting is here is not so much--I know the focus here is the perinatal period necessarily, but durable effects on cognition. So, the focus here is not going to be on the population that is the focus today necessarily, but it is on another group of patients.

We are hypothesizing that inhaled anesthetics promote basically neurodegenerative disorders such as Alzheimer's disease, and I am going to go through some background and tell you a little bit about why we think that.

First, in isolated proteins, in our Protein Chemistry Laboratory in looking at these drugs, we have noticed that inhaled anesthetics make proteins in general stickier, and there are a whole series of neurodegenerative disorders that are based on protein stickiness, to make it simple, and so we asked the obvious question: does it make amyloid beta the harbinger of Alzheimer's disease

stickier as well, and the answer is on this slide; indeed it does, dramatically. It does it in a durable fashion, long outlasting the period of the drug exposure itself.

It does it with a rank-order potency, and this is essential for this group to consider. We should not be looking at studies that only look at a single drug anymore. We need to look at all of the drugs in our armamentarium because we have to come up with alternatives.

So, in this case, halothane was the most potent at doing this, isoflurane next, and ethanol and propofol were the least, or, in fact, a little protective. And this just shows that the oligomer being produced is indeed the toxic oligomer thought implicated in Alzheimer's disease.

Cell culture showed the same thing. I am not going to go through this in detail except to say--this is a published work--except to say that the combination of anesthetic plus amyloid beta, whether produced in the cell or whether exogenously, enhanced toxicity, enhanced

cytotoxicity.

We then moved to animals. This work was just published. This is a transgenic animal that overproduces amyloid beta responsible for Alzheimer's disease, and they develop a cognitive disorder much like Alzheimer's disease.

This is the Morris Water Maze data. Vesna has already talked a bit about this. I won't go into it in detail. There are about 100 different metrics you can derive from those data and, in this case, we looked at the percent of the animals reaching criterion at each platform position, which can be moved periodically .

This is the transgenic animal at 12 months. These are the control wild-type animals. These are both controls and neither one of these had received anesthetics, showing that the transgene causes a cognitive decline very clearly.

This way is bad. This way is good.

If we look at only the transgenics and expose them to anesthetics, the anesthetics do nothing further to this cognitive decline,

suggesting they either don't interact with this disease progression or that we have reached a floor of cognitive ability in this animal model.

In the wild type, however, this is isoflurane. Isoflurane produced a state much like the Alzheimer animal in the wild-type animal, a phenotype much like the transgenic animal.

Halothane did neither. Halothane was just like the control. So, this is a very important message for this group to consider especially what I said initially about GABA-ergic drugs. These are equally GABA-ergic, halothane and isoflurane, yet there is something distinct about isoflurane's ability to produce cognitive decline, at least again in this animal model, and everybody has been showing caspase, so I will show the caspase data here.

In the wild type, very modest levels of caspase generation enhanced in halothane. In the transgenic animal, overall it is enhanced compared to wild type. But again in halothane it's a little higher, probably being explained by enhanced plaque

formation in the halothane animal and that fits the hypothesis of enhanced aggregation of these peptides.

This is just to show some clinical data looking at this question specifically years ago. This data is not statistically significant at this point but it hardly gives one a sense of comfort, and that is that if you look at the development of--a group of patients that had documented Alzheimer's disease, and you look at their prior history and when they developed the disease and how often they had anesthesia and surgery before, 1 to 5 anesthetics, this is the odds ratio for developing Alzheimer's disease, greater than--

DR. SHAFER: Dr. Eckenhoff, thank you very much.

The next speaker will be Dr. Xie.

DR. XIE: Thank you for the opportunity. I don't have any financial relationship with any company.

So I am going to follow Dr. Eckenhoff's talk to talk about the common use of the anesthetic

isoflurane and Alzheimer's disease neuropathogenesis. We know that Alzheimer's disease is one of the greatest public-health problems in the United States and in the world and the impact will only increase with anticipated changes, anticipated in the coming decade.

Currently, A.D. affects about 4.5 million Americans. It is estimated about it will increase to 13.2 million by the year of 2050 if no treatment are found.

A beta, which is a special protein in the brain, A beta production and accumulation is an important part of Alzheimer's disease neuropathogenesis. Also, the evidence is increasing that the caspase activation and apoptosis are also important in the Alzheimer's disease neuropathogenesis.

About 100 million patients worldwide have surgery each year. There are several reports that Dr. Eckenhoff just mentioned that have suggested that anesthesia and surgery and other factors such as hypoxia and hypocapnia may facilitate the

development of Alzheimer's disease.

Therefore, we set up to study the commonly used inhalation anesthetic isoflurane on this apoptosis and also on this A-beta levels. What we found here is that the clinical relevant concentration of isoflurane, which is 2 percent for 6 hours in the test tube only in the cultured cells can induce caspase-3 activation, reduce the cell viability, affect APP processing, which is the protein to produce A-beta, and also dramatically increases the A-beta levels in the cell culture.

We further found that this increase of isoflurane-induced caspase activation in the cell is dose dependent, as you can see here, for 1 percent isoflurane does not induce apoptosis and 2 percent did increase the caspase-3 activation.

We further found that the inhalation of isoflurane can induce a vicious cycle of apoptosis and A-beta accumulation. As we know, and as many people have already showed, isoflurane can induce apoptosis but what happens next. You know, we know that apoptosis may not be closely associated with

cognition dysfunction. But A-beta does. A-beta has been shown by many studies either critical card for the dementia or any cognitive dysfunction. We, therefore, studied the relationship between this apoptosis and A-beta increase induced by isoflurane.

We first found that apoptosis comes first.

Why? Because we can see that isoflurane can induce caspase activation and apoptosis in this naive H4 cell without detectable changes in APP processing and A-beta generation indicating that the induced caspase activation by isoflurane is independent of APP processing and A-beta generation. So this must come first, will come A-beta--apoptosis first.

We also found that the E-VAD, which is the inhibitors for caspase activation and apoptosis, can also attenuate the isoflurane effect on caspase activation, APP processing and A-beta generation.

We also found that isoflurane can enhance two enzymes, one called BACE, one called gamma-secretase. These two enzymes are responsible

for A-beta generation.

Finally we found that the A-beta-5-CQ, which are two compounds, can attenuate the A-beta to stick together. If we can decrease A-beta stick-together, we can see that we can also attenuate or reduce the isoflurane-induced apoptosis.

We also found that A-beta cells can come back to potentiate this isoflurane-induced apoptosis to form a vicious cycle of apoptosis and A-beta generation.

So, for summary, we found that isoflurane, the commonly used anesthetic, can first induce caspase-3 activation and apoptosis and this increase will facilitate or increase enzymes or activity of both BACE and gamma secretase therefore to facilitate the APP processing in order to increase more A-beta generation, the more A-beta generation will come back to potentiate this caspase activation and apoptosis to form a vicious cycle of apoptosis and A-beta generation.

We also found that the isoflurane may also

cause apoptosis through A-beta aggregation, as Dr. Eckenhoff just mentioned, that isoflurane can make A-beta stick together, and, as they stick together, make them more toxic.

Now, the conclusion is that the acute insults like hypoxia, hypocapnia may affect the A-beta metabolism and apoptosis and therefore facilitate A.D. generation and also induce a vicious cycle of apoptosis and A-beta generation. We must emphasize that this is only an in vitro study, a test-tube study. We need to do more studies to determine the in vivo relevance before we can make any conclusion.

You know, these studies are alarming and we take it seriously. However, it is too premature to call to stop using isoflurane in the O.R.

Thank you.

DR. SHAFER: Thank you.

Next is Dr. Lewis Coleman.

DR. COLEMAN: I am an anesthesiologist from California. I have no conflict of interest and I am sponsoring my own participation at this

meeting. I believe that emerging theories can serve as guides to evaluate potential anesthetic toxicity in early life.

A "Stress Mechanism" that explains Capillary Gate Theory and Unified Stress Theory has been formulated on the basis of current peer-reviewed literature. It describes how the Coagulation Cascade, the Central Nervous System and the Autonomic Nervous System function together to govern thrombin production to control the cell-based process of tissue repair and development.

All cells respond to thrombin via receptor combinations that are unique to each type of cell.

Thrombin utilizes ATP to energize the cellular and enzymatic actions involved in tissue repair and maintenance. The mechanism explains how thrombin production is affected by stressful stimuli and forces, including drug effects.

Ninety-eight percent of the human genome has no known function in the mature animal, but recent studies indicate that this "excess" DNA

functions as the blueprint for embryological development. It is believed that the embryological development process governs DNA expression and is closely related to a Stress Mechanism that completes the embryological development of organs and tissues.

I hypothesize that embryological development occurs via the generation of thrombin at precise time intervals, locations and quantities to govern three-dimensional embryological tissue proliferation of various cell types, and that additional quantities of thrombin are subsequently generated by the Stress Mechanism to complete the development process.

Human development involves both cell proliferation and apoptosis and remains active for at least a year after birth. During this time there is increased risk that medications such as coumadin, salicylates and thalidomide that interfere with thrombin production and thrombin effects may disrupt the development process.

Thrombin generally promotes cell activity,

vitality and proliferation and inhibits apoptosis so that sudden declines in thrombin can cause apoptosis. In contrast, for reasons that are not clear, thrombin elevations may cause apoptosis and other toxic effects in neurons.

Considering both evidence and theory, I believe that the most logical means to assess the potential effects of anesthetics and other drugs on the newborn is to focus on drug-induced perturbations of thrombin production.

The Stress Mechanism also explains Crile's Hypothesis and suggests a simple, safe and practical approach for minimizing toxicity and optimizing stress control to reduce morbidity and mortality in all anesthetized patients.

Synergistic interactions of hypnotic and analgesic agents can be manipulated to minimize toxic hypnotic-agent exposure via greater reliance on benign analgesics plus deliberate mild hypercardia.

Many details of the Stress Mechanism remain unclear, but it can be tested in its present

form. If proven, it would offer wide improvements in medical practice.

I have prepared papers describing the Stress Mechanism, together with proposals for testing it, and have submitted these for review and consideration by the committee. I welcome suggestions or help that might facilitate the empirical testing of the mechanism and would be happy to answer questions.

I thank the FDA for allowing me the opportunity to present these materials.

DR. SHAFER: Thank you, Dr. Coleman. The rules of the open session do not permit us to direct questions to you, but I very much appreciate your presentation.

The next presentation will be Dr. Scott Kelley.

DR. KELLEY: Thank you, Dr. Shafer, members of the Committee, members of the FDA and the audience.

By way of conflict, I am an employee and officer of Aspect Medical Systems. My company

manufactures a brain monitoring technology demonstrated to reduce anesthetic exposure in clinical practice.

I would like to share with you today new concerns regarding the adverse impact of anesthetic exposure. Three specific areas: are there additional concerns in pediatric patients, what about the preclinical and associative clinical evidence of worrisome adverse consequences in adult patients and, finally, some recommendations.

Much of this morning was spent on the issue of neonatal anesthetic exposure and potential consequences in terms of development. Two issues still need to be addressed about the potential harmful effects of anesthetic exposure in our young patients.

We continue to see a high incidence of epileptiform EEG changes in the presence of sevoflurane anesthesia in a dose related manner. In addition, we continue to see a clinical scenario of emergence agitation and delirium associated with volatile anesthetic administration. The long-term

consequences of these two events remains to be demonstrated.

In addition, I believe the growing body of evidence suggests there may be hidden harm from anesthetic exposure to other patients specifically in the area of late outcomes. I will touch on three areas: Alzheimer's disease, cancer, and complications and consequences in the forms of morbidity and mortality.

An important consideration for all of us to consider is that anesthetic exposure may influence patient comorbidities and thus their long-term outcome.

As Dr. Eckenhoff just reviewed, and from the other laboratory from Mass General, there is preclinical evidence that neurodegeneration and acceleration of the mechanisms for Alzheimer's disease may be occurring with administration of volatile anesthetics.

Are we comfortable telling our patients there is no clinical association with anesthetic exposure and subsequent development of Alzheimer's?

In the study referenced by Dr. Eckenhoff from Bohnen's group at the Mayo Clinic, 10 years ago we were told that it is unlikely that multiple exposures to general anesthesia increased the risk of Alzheimer's. However, this relative dose-response curve showing increasing risk in the small case-control series puts me at concern, specifically when we look at an adequate power size would involve 6 to 10 times as many patients.

If we turn now to cancer, there is both preclinical evidence and clinical association published in the last six months in Anesthesiology.

If we use a mouse model with lymphoma cells and an outcome of liver metastases, again we see an interaction about volatile anesthetics, a combination of volatile anesthetics and surgery, and potential modulation by the addition of a regional anesthetic.

Interestingly, in a clinical study we see again a nonrandomized study, but a combination of a paravertebral regional block seems to modulate the effects of general anesthesia when looking at

recurrence of breast cancer approximately 2 to 3 years following initial surgery.

Finally, anesthetic exposure and outcome.

We see now associations with morbidity and mortality. Nearly 30 years ago, John Tinker's group found a striking correlation between time under general anesthesia and the incidence of myocardial reinfarction. A recent publication from the NSQIP database involving 15,000 patients--not randomized, but a large number of patients with 3 anesthetic techniques, when its veteran patients were administered general anesthesia as compared to spinal anesthesia, significant increased risk for cardiac events, postoperative pneumonia, graft failure, as well as need to return to surgery for surgical indications.

In a study from Terri Monk's group conducted at the University of Florida, an association was found between anesthetic exposure as measured by deep hypnotic time in terms of also increase in the risk of 1-year mortality. It is important to note that in this study, patient

comorbidity was the most important risk factor.

These concepts of anesthetic exposure and outcome are gaining increasing attention in the anesthesia community, but I am not seeing that attention for adult patients from the FDA.

Terri Monk's study was accompanied by an editorial by Neal Cohen from the University of California, San Francisco. In addition, the APSF had initiated a project to explore the anesthetic responses that may influence long-term outcome.

In summary, preclinical and associative evidence of adverse effects continue to accumulate with anesthetic agent exposure. I believe it is important for this committee to inform the FDA that broad populations are at risk. We have seen data today regarding developing neonates and pediatric patients. If we look closely, elderly patients with comorbidity are also at substantial risk.

My recommendation would be to include and request preclinical and clinical safety studies to determine the impact of anesthetic exposure on late outcomes.

A pediatric anesthesiologist, Peter Davis, wrote very eloquently that all of us as anesthesiologists need to add new dimensions to considering the Goldilocks conundrum: how much of our drugs and techniques is too much, how much is too little, and how much is just right.

I believe it is important to engage the APSF to alert the anesthesia professionals to these new concerns and to explore clinical options for improving patient care and finding that "just right" dose.

DR. SHAFER: Thank you, Dr. Kelley.

Our next speaker is Dr. Reid Rubsamén.

DR. RUBSAMEN: Thanks for having me. I am Reid Rubsamén. I am an anesthesiologist in private practice at John Muir Medical Center in Walnut Creek, California. We are the trauma center for Contra Costa County and Marin County, which is just north of the Golden Gate bridge .

We do about 7,000 cases per year in our main operating room. I also serve as the Medical Director and we all in our practice do pediatric as

well as adult anesthesia.

I will be talking about topics related to the induction and maintenance of anesthesia and awareness monitoring. I bought my own tickets and I don't have any financial ties to any relevant companies today.

You know, gosh, ever since I was a resident in Boston, you know, you become rather struck by the fact that these inhalational agents are basically organic solvents that have kind of a troubling aroma when the patients are in PACU recovering from anesthesia.

I think all of us, even in the absence of scholarly peer review articles that we are hearing about today, are motivated to use less of these drugs. I think that is exactly what is happening.

People are using more intravenous drugs and adjunctive ways to inhalational anesthesia, propofol in particular.

And I just sort of throw up here I think you don't even need an organic chemistry degree to notice that chemical formula of sevoflurane,

isoflurane, the oldest drug in the series, and here is 3M dry cleaning fluid, they have a similar chemical structure. They are all halogenated ether, so obviously, aesthetically, there would be kind of a motivation to use less of this stuff.

I think that we are in a position now with drugs like propofol to really consider doing total intravenous anesthesia for all of our patients.

I became aware of a surgery center near us where the vaporizers were removed from the anesthesia machines by two anesthesiologists who ran the surgery center to facilitate wake-up in the PACU, so I decided to do a couple thousand cases essentially consecutively without nitrous oxide or vapor. I have not been using ondansetron, which is our primary anti-emetic agent, and I have been impressed with the short-term benefits of this approach.

Obviously, I don't know much about the long-term benefits of this approach we have been talking about today but what is interesting here is that gosh, you really don't see nausea and vomiting

when you don't turn the vaporizers on. There are some papers that you are probably aware of that are talking about this, as well.

And people don't wake up with that weird kind of decerebrate posturing that they have with the vapors. And I really can't make patients get laryngospastic when they wake up and this is especially important in kids.

You can yank that tube out, you know, fairly deep with the patient coughing a bit with TIVA, and they just don't go into laryngospasm, and that, of course, can be a fatal complication in kids and something that we don't ever want to see.

So, what is interesting is if you just use oxygen, air, propofol, opiates with or without muscle relaxation in intubated patients--you know, I can do cases with laryngeal mask airways with intubated patients using a propofol pump, and I use a BIS monitor.

I think it is important to do some monitoring here, because we don't have the end-tidal agent information that we have with

inhalational anesthesia and awareness is something to be concerned about if a pump should fail or the IV should fail.

I think we still need sevoflurane to induce anesthesia in children without an intravenous line, but I think it becomes possible to pretty much eliminate vapor from the practice.

Now, am I a popular guy at the group for taking this position? Well, yes and no. Your vapor is the easy chair. You get muscle relaxation for free with that. You are not going to have awareness if you have any end-tidal agent at all on-board, so you don't need to worry too much about BIS monitoring. To the extent that healthy patients get hypertensive, that gets smoothed out automatically and, as I mentioned, you have got this internal check that the patient is anesthetized in tidal CO<sub>2</sub> in the operating room.

If you are doing TIVA, you are going to work harder, because you are going to have an extra monitor, you are going to be getting more muscle relaxation, you are going to be managing blood

pressure a-la-carte with various drugs, and I think, you know, my colleagues had a hell of a time turning the vapor off.

I had this nightmare that I had to have surgery and I went to the head of my group in the dream and I said, "Will you please do me without vapor," and he said, "No."

So, I went to him in real life and I said, "Hey, Dave, I had this nightmare I had to have surgery, would you turn" -- he said, "Of course, I am not turning the vapor off." I said, "Why?" "I don't want you to be awake."

I think that really is kind of a psychological hurdle that people have to overcome, and I think that, you know, hopefully, the various level of consciousness monitors will get people over that hump. But I think it is pretty compelling that the short-term benefits of not using vapor are real. I think you can use TIVA widely including for spontaneously breathing patients.

I think the question we were talking about

today is a fascinating one. I am suggesting using TIVA, what the heck is the long-term effect of that on children or adults, and I think prospective trials are critical here, and I would love to see them done.

I think also I would love to see workload-reducing activities to make TIVA easier to do, perhaps even something like closed loop administration of propofol or other technologies that might make it easier for more people to do this.

So, my conclusions. I really think you can use TIVA to essentially replace vapor, I think that you really want to have a drug like sevo to induce anesthesia in pediatric patients when you don't have an intravenous line in place for comfort of the patient and ease of conduct of the anesthetic.

I am very moved by the short-term benefits of not using vapor, so I try to avoid it where possible. And my theory is that if I use TIVA and I don't use too much, I use the BIS monitor to dial

in a dose that seems rational, that I won't see long-term issues associated with total intravenous anesthesia, but I think studies have to be done to verify that, and I think it's great that you guys are considering these issues.

Thank you very much.

DR. SHAFER: Thank you, Dr. Rubsamen.

Our next speaker is Dr. Wei.

DR. WEI: First, thank you for having me.

I have no financial relationship with any company but this project is supported by the March of Dimes Birth Defects Foundation, a NIH NIGMS KO8 grant and the Department of Anesthesiology and Critical Care E and D Fund.

So we and Dr. Slikker's lab and others have demonstrated that isoflurane-induced apoptosis dose and time dependently in different neuronal cultures. Dr. Todorovic and Dr. Olney also showed that isoflurane-induced apoptosis in the developing brain of 7-day-old rat pups and subsequent memory and learning disability.

Also we have studies to show that

isoflurane causes permanent memory loss, learning disability in aged rats from Dr. Cross's group at Harvard University. So we hypothesized that isoflurane inhaled to pregnant rats will induce apoptosis in the fetus developing brain and possibly subsequent post-natal memory and learning disability.

To test this hypothesis, we first did a pilot study to determine the highest dose of isoflurane we can use without affecting arterial blood gas, mean arterial blood pressure and glucose for 6 hours. The concentration in pregnant mother rats is 1.3 percent.

So we treated the pregnant rat with 1.3 percent for 6 hours. Then, at 2 hours, 18 hours or post-Day 5 after isoflurane treatment, we obtained the fetus brain, or newborn brain, and determined apoptosis by evaluating the density of caspase 3 and maternal-positive neurons.

Contrary to our hypothesis, isoflurane at 1.3 percent for 6 hours did not induce apoptosis in any of the time points either at the hippocampus

CA1 or at the retrosplenial cortex. Isoflurane significantly inhibited spontaneous apoptosis determined at 2 hours but not at 18 hours at post-natal day 5 isoflurane treatment. This is determined by the density of caspase-3. The result for maternal is basically the same as that as in caspase 3.

So, in separate groups, we let the rat deliver and tested the learning and memory ability of the post-natal rats with the Morris Water Maze.

I don't have luxury of the time to explain all the Morris Water Maze protocol, but it is similar to what Dr. Todorovic explained this morning.

So we determined the learning ability by the place trial and learning to reach criteria trial in either juvenile age at P-36 and at adult age at around P-120. We didn't find any significant difference. So that means that isoflurane does not significantly affect learning ability in the post-natal rat.

However, isoflurane significantly improved the spatial-reference memory in juveniles at P-36

but not at P-119, not at adults, suggesting this improvement of the memory did not last into the adult age.

So, in summary, 1.3 percent isoflurane for 6 hours in pregnant rats inhibits the spontaneous apoptosis in the fetal brain at 2 hours but not at 18 hours or post-natal-day-5 after treatment and 1.3 percent isoflurane for 6 hours in pregnant rats improved spatial-reference memory in post-natal juvenile rats but not in adult rats. 1.3 percent isoflurane for 6 hours in pregnant rats did not significantly affect the learning ability in post-natal rats.

The isoflurane has been considered a neurotoxic-protective agent and neuroprotective agent for ischemia for a long time. But right now, increased evidence suggests that it is neurotoxic.

Is isoflurane neuroprotective or neurotoxic? We believe that it depends on the dose and the duration of use. If you use short duration and low dose, that will precondition the neuron-induced and that is a neuroprotective agent, that will be

neuroprotective.

If you use it long high dose, long duration, that will induce neurotoxicity by its inherent neurotoxic ability. This is similar to cerebral ischemia. If we have 2 minutes cerebral ischemia, that will induce a neuroprotective agent genes that are providing neuroprotection against cerebral neuroischemia. That is similar. I think, in the future, our focus is that what is the dose or duration, the stress code, that will change the neuroprotective ability of isoflurane into the neurotoxicity and also what is the population like, what kind of neuronal would be vulnerable to isoflurane neurotoxicity.

Thank you very much.

DR. SHAFER: Thank you very much.

Our last presenter is Dr. Crosby.

DR. CROSBY: Good afternoon, everybody. I am Greg Crosby. I am an anesthesiologist by trade and a laboratory investigator by choice.

I have been interested in the notion of long-lasting effects of general anesthetics for

some time.

My purpose here today is relatively straightforward. It is to, number one, thank the committee for considering this topic because I think it is an important one but to, perhaps, broaden it, and you have already heard some of that, by suggesting that the focus on juvenile brain is really too narrow.

I hope to convince you that the data are at least as good for potential long-lasting effects in the old brain, and then finally to urge some restraint in what we do with this information at the present time.

The first thing is I want to point out that the clinical population for whom there is evidence of long-lasting effects after general anesthesia and surgery, long-lasting cognitive effects, are the old.

That is the only group for whom this has been well demonstrated. The issue here is whether this is related to anesthesia per se or might be related to the surgical procedure. There is some

debate about that because other studies that look at the patients who are randomized to regional anesthesia versus general anesthesia, in fact, find no difference between the incidence of cognitive impairment, which tends to be about 10 percent about 3 months after surgery and anesthesia.

We are not sure if this is drug related, but it may be. In the laboratory, we have some evidence that old animals that are subjected just to anesthesia, no surgery, in fact, have some cognitive impairment lasting, in this case, at least a couple weeks.

As you can see here, here is the control group learns over time, as do the previously anesthetized animals. They just don't learn so well, so they are slow learners like Vesna was talking about earlier.

You have already heard some of this information. These are a variety of selected papers showing that, in fact, in cells largely with the exception of the bottom one, and that is in vivo. But the others are cell-culture systems showing

that, in fact, isoflurane can have an effect of increasing A-beta, one of the presumed pathogenic molecules in production of Alzheimer's disease, and the combination of A-beta and anesthesia seems to be more toxic in cells than either one alone.

So, there is evidence here that anesthetics may have long-lasting effect in old brain. I put up this little score card for you to tally up where we stand with old and young, and I think you can see that it is relatively similar.

There is evidence for toxicity at the cell culture level both in old and young brain. There is evidence for in vivo toxicity in the old and young. There is even evidence for learning deficits in the old and young brain after general anesthesia alone.

What we are lacking, though, is down here, toxicity data in primates, we have heard some of that today, so I may have to change that to a check, and the big weakness thus far is lack of evidence that any of this, as Vesna said earlier, matters in terms of cognitive performance.

I want to emphasize to you that if you are looking to go where the money is on this topic, you may want to look at the old brain and consider it, because here is number of procedures, as you can see, in thousands. Here is a group less than 15 years of age, 3 million.

To go over here, in patients over the age of 64, you are talking about several fold higher and, if you normalize this by numbers of patients in these groups, you see that, in fact, the incidence of elderly folks having surgery and anesthesia is roughly 10 times that of younger children. So, this may be, as I said, where the money is.

So, in conclusion, I want to point out again that elders are the only age group for whom there is kind of prospective randomized data using neuropsych tests who are demonstrated to have long-lasting cognitive impairment after anesthesia and surgery. As I said, that may or may not be due to the anesthetics, we don't know that for sure yet.

There is evidence from cells and aged animal studies that show that anesthetics can produce some neurodegeneration and indeed can produce persistent learning impairment.

As I just pointed out, elders are the largest consumer of surgical and anesthesia resources, so if we are looking at public health issues, then, if these things turn out to be real in the long run, this could be a major public health problem.

Having said that, though, I want to emphasize that, as an anesthesiologist and somebody who is interested in this, while I personally believe the evidence both in developing brain and older brain are pretty compelling for some sort of toxic or long-lasting effects, the data do not, at this point in time, in my mind, warrant any kind of restrictions or whatever on usage in any patient population or modification of our anesthetic techniques at this point in time largely because you have heard very little in the way of evidence that there is long-lasting cognitive impairment.

I think that has got to be one of things we emphasize in the future in order to determine, as Vesna said earlier, whether this matters. We need to show that there are lasting cognitive deficits, and we don't have that information yet. Hers was the only study previously that showed cognitive changes.

Therefore, I think we do need some more research, both basic to get at the mechanisms of why this is happening, and also clinical to see whether it is a real phenomenon in patients.

Thank you very much for your attention.

DR. SHAFER: Thank you, Dr. Crosby.

I want to thank all the speakers at the Open Public Hearing. I know that you came here to share your thoughts and your concerns with us, and we appreciate hearing from you.

That brings the Open Public Hearing to a close and now we will turn to a general discussion.

As mentioned, what I would like to start with is to direct questions to Dr. Todorovic, Dr. Slikker, Dr. Soriano relative to their

presentations about the current database and understanding of the risk of apoptosis from the anesthetics in children.

#### **Committee Discussion**

DR. RAJA: This is a question to both of you, Dr. Todorovic and Dr. Slikker, when we think of cognitive dysfunction and memory, we think of areas such as the hippocampus and the limbic system, a lot of the data presented there on the thalamus and the cortical sites, is it because of absence of effects on the other sites, or could you tell us a little bit more on the other sites important for memory?

DR. JEVTOVIC-TODOROVIC: That is an excellent question. Actually, when we started doing these studies when I was a Fellow in Dr. Olney's lab, we found that the damage in hippocampus was not as profound as what we found in thalamus and cortex.

There was damage, but it wasn't quite as substantial. So, in designing these studies further, we actually started doing hippocampal

slices to assess that issue because you have an issue of morphological change. But you also have an issue of functional change.

We know very often that, even if you cannot see the morphological change, you may see functional change. Thus, we did very extensive LTP studies with the help of Dr. Zorumski and Dr. Zuppa.

So, for these experiments we exposed animals for the same triple combination. Actually, we had single anesthetics, as well, and they collected--they actually took hippocampal slices from these animals from the age of 28 to 33 days, which I think is optimal for LTP studies, and they found significant changes in LTP.

So, that was one of the reasons why we moved on to do behavioral studies, not based on our initial histological findings. However, if you look at our initial study from 2003, we do have quite a few fold increase in damage in hippocampus, as well.

DR. ANAND: One other question I had was

it was pointed out in your talk, and Dr. Olney's talk, that about half to 1 percent of neurons in the rat brain undergo apoptosis, physiological cell death.

In the human brain, Rabinowitz and Chahal, two separate papers, have shown that more than 60 percent of cortical neurons will undergo apoptosis after 28 weeks of gestation. That seems the peak cellularity period.

Can you comment on how that would impact this model?

DR. JEVTOVIC-TODOROVIC: This is actually interesting, and Dr. Wei brought up that issue by looking at fetal and pregnant mothers. I think that if you are talking about neurogenesis, and I think that is about the time for neurogenesis, from what I remember in humans that is the second trimester of pregnancy, there is a massive dilution of neurons. In some areas, it is more than 50 percent.

So, I am not exactly sure how that type of cell death relates to cell death that we study in

synaptogenesis, so I cannot really comment as far as what is the morphological basis for cell death during neurogenesis as opposed to synaptogenesis.

But once neurogenesis is over, the assumption by the physiological system is made that what we have left has to live somehow.

DR. ZUPPA: I actually have two questions, the first of which is for Dr. Slikker. You had mentioned in your questions that remain, I guess for future studies, microPET, I guess would be a surrogate for apoptosis.

Has that been explored, is there other data out there that would support PET scans as a surrogate for that?

DR. SLIKKER: What this would require would be the development of a ligand that would be specific for apoptosis, and we are moving toward trying to develop such a ligand, and then to identify that ligand with a PET tag, for example.

This is a work in progress, but what I wanted to sort of emphasize is that I think people generally feel the need to try to find a

translational biomarker and this is going to be one of the directions that we pursue in order to try to produce such a biomarker to get to use across species and including into the clinical setting.

DR. ZUPPA: That would be fundamental to doing, yes, the work in the clinical setting.

Just the other point that I really wanted to make, and it is mostly to Dr. Soriano's presentation. I am not an anesthesiologist. I am an intensivist. And I just want to point out that, in the ICU, we have children on ketamine infusions, receiving ketamine infusions, for days for treatment of reactive airways disease, midazolam infusions for weeks, neonates with RSV who are intubated. So I think the notion that duration may not be as applicable should be considered because these children are receiving it for a very long time.

DR. SORIANO: I was speaking more for the intraoperative management of these patients. But certainly there is a group at risk, the premature neonate who is in ICU for weeks to a month at a

time.

I know Dr. Anand has looked at some of this in his collaborations with other institutions.

Perhaps he may want to address this issue, the neonates on prolonged ventilation and who have been sedated with midazolam, morphine in some of your studies, and ketamine.

DR. ANAND: There is significant concern, no question, because the duration turns out to be not hours for an operative procedure, but several weeks and during a very active period of brain development in these pre-term babies who are in the NICU.

We had done an initial randomized clinical trial, which was published in I believe '99, where we compared placebo, morphine, and midazolam infusions and we found a fairly high incidence of, I think it was 7 out of 21 patients--I am not exactly sure of the numbers, but a very high incidence of white matter damage in the babies who had received the midazolam infusions, so much so that when we went from that pilot study to a

definitive trial, we actually dropped midazolam. We just did a head-to-head comparison of placebo versus morphine.

Our hypothesis was that morphine analgesia, preemptive morphine analgesia, will reduce intraventricular hemorrhage and white matter damage and neonatal death, but we found no differences in the definitive trial.

DR. SORIANO: But keep in mind that these neonates are severely compromised anyway, and whether or not to tease out the natural history of the disease process versus similar to our own interventions, mechanical ventilation, sedative agents still needs to be distinguished.

DR. ZUPPA: My concern is for the pre-term infant, but also the healthy 28-day-old who comes in with croup and requires intubation and mechanical ventilation for thoracic air obstruction and is intubated for five days requiring high doses of sedation.

DR. ARMSTRONG: The point you made about white matter change has sort of stimulated a

question and you had talked a little bit about the imaging plans in your group.

When we look at neurotoxicities that are associated with other agents, chemotherapy agents in cancer, and things of that nature, one of the things that has happened in that pattern has been that there have been measures of acute neurotoxicity, and the apoptosis that we have looked at today really is that sort of measure of processes occurring acutely.

Do we have any information in any of the studies that has linked that apoptotic process to then long-term volumetric decrease, white-matter anisotropy that is showing white matter changes and decreases?

The model really suggests it is going to be white matter more than gray, but do we have any kind of links between the acute event and any long-term permanent changes on imaging that we could then associate with the neurobehavioral changes?

I would open that up to anybody who has

presented today.

DR. ANAND: I would be happy to respond.

Brad Peterson from Yale University had published a comparative study of X pre-term and X full-term children who were imaged at 8 years of age. They did volumetric MRIs and they found marked reductions in gray matter, and so on, and those were correlated with cognitive, as well as emotional and other outcomes, at 8 years of age. But that was not studying anesthetic drugs. That was simply the effects of prematurity.

DR. ARMSTRONG: I will follow that up.

That is an important issue in terms of the neurobehavioral developmental component that we are looking at.

I think that in a lot of areas we have seen a window of opportunity for potential recovery, but also for potential damage, but as we have looked at late cognitive effects, the issue that comes up is that the acute event may have very few cognitive behavioral manifestations.

It is not until the brain has developed to

the point where the child--and I will talk about a human model at this point--that the child is unable to do something developmentally that other children would do at the time that skill would emerge, that we are actually able to tell the difference.

So, when we are thinking about--a lot of what I have seen today has been discussion of thalamus and frontal cortex, visual pathway. And most of the studies that we have suggest that the long-term outcomes are not picked up for 2 to 3 years after exposure and they tend to be in very subtle areas like reduced processing speed, very specific memory function, problems with visual-motor integration, and the like.

So, I think that is one of the concerns is that any questions that we would ask may wind up taking many years. When we look at other models, HIV, we are seeing onset dementia of perinatally infected children when they are 18 or 19 years old.

The outcomes in children who were cocaine-exposed during pregnancy, these subtle kind of problems are showing up when they are 12 and 13

and 14 years old. We are seeing long-term complications of infarctive, minor infarctive, processes in sickle cell now in young adults in their 20s and 30s.

So, I think there is a challenge for us here. I would be interested in the animal models, whether there are some suggestions of interim evaluations that would help us to be able to answer this question without having to wait 20 or 30 years, or even 12 to 15 to get the answer.

DR. SHAFER: Dr. Olney, would you be motivated to respond to it or not? No need to if you don't wish.

DR. OLNEY: I thoroughly agree with the need. I was suggesting when I presented that the neuroapoptosis model in rodents is suitable for studying some of those things because we can delete different populations of neurons at different times during the development and then let them grow up and study them at intervals as they are growing up.

So I think these models are relevant to addressing that question.

DR. SHAFER: Before you leave, Dr. Olney, while you are there, I have two questions for you.

Are there studies looking at the same kind of phenomena in the spinal cord? Is the spinal cord at risk?

DR. OLNEY: We have shown in the alcohol model that the degeneration occurs from the frontal cortex to the caudal region of the spinal cord. The lumbosacral and cervical expansions of the cord are most sensitive and both dorsal and ventral portions of the cord are affected.

DR. SHAFER: One other question for you while we have you here. There seems to be a fairly clear phenotype for anti-epileptics in the sense that there is known damage. We know that there is a phenotype associated with neurodegeneration of women who are anti-epileptics.

Does that show a no-effect level? Is there a dose that is thought to be safe and you get to a certain point that you kind of hit a hockey stick, if you will, in your exposure-response relationship, and then it becomes dangerous? Do we

know that?

DR. OLNEY: I don't think we know that in detail. But I think the main issue here is that for anti-epileptic therapy, it requires chronic application and that is a little similar to the ethanol issue. A fetus of an ethanol addict mother who is addicted is likely to get exposed many times during pregnancy and, of course, we know what the fetal alcohol outcome is.

I think if an infant has to be exposed to anti-epileptic drugs chronically, then, that is certainly going to compound the type of damage that would occur from only a single exposure.

DR. SHAFER: Other questions?

DR. EISENACH: You mentioned in passing that sodium channel blockers were positive in your studies. There are two circumstances where sodium channel blockers are used clinically in these infants. It has been suggested that regional anesthesia is an alternative we might consider.

With peripheral regional nerve block, the brain is exposed to low concentrations of local

anesthetics, non-selective sodium channel blockers, and, with spinal administration, the spinal cord is exposed to, I won't say extraordinary, but clearly, pharmacologically active concentrations of drug for a period of time.

Is that something we should be discussing, as well?

DR. OLNEY: Well, I guess it would be relevant for discussion. I don't think we have any data that could address that. We haven't approached it from the regional-anesthesia standpoint; that is, our mode of delivery has not been that, so we really haven't tested that.

DR. EISENACH: But you have some data with sodium channel blockers? Did I hear that correctly?

DR. OLNEY: We do have some data for sodium channel blockers. I don't believe that we have looked at the spinal cord following sodium channel blockers so I can't specifically say that they affect the spinal cord.

DR. EISENACH: But these were probably

local anesthetics you were looking at?

DR. OLNEY: Well, anti-epileptic drugs is the context in which we were using sodium channel blockers. Dilantin is a sodium channel blocker.

DR. EISENACH: I am thinking of the more selective ones. Those drugs do block a variety of channels and work in a variety of mechanisms and the local anesthetics may also. But they are predominantly sodium channel blockers. So, that didn't relate to local anesthetics in your previous studies?

DR. OLNEY: Yes, but I think the sodium channel blockers are the least well studied of all of these agents so far. We just haven't really done a thorough study of them.

DR. EISENACH: I raised it since it was proposed as an alternative.

DR. SORIANO: In response to Dr. Eisenach's comment, Dr. Kirsch and I chaired a session at NST Society meeting last fall where a couple of abstracters showed that long-acting local anesthetics administered to a neonatal lab pup had

more neuroapoptosis than short-acting agents.

So, they compared bipivacaine versus tetracaine and found that the tetracaine did indeed have some detrimental effects as far as behavioral testing, as well as counting apoptotic neurons. However, that is just an abstract, so it's preliminary data and the journals will still have to read it out to make sure it's true or not.

DR. DESHPANDE: I have a comment and a question for the speakers. One comment is really from the discussion today, that along with the pre-human primate studies, as well as animal studies, looking at mechanism, concurrent long-term human studies similar to the Framingham for the cardiovascular data really are called for, it sounds like, to look at neonatal and developmental brain outcomes.

For the speakers, the question that comes to mind for me is that differentiating between mechanisms of specific agents versus sensory deprivation in the developing brain, we know that sensory deprivation in the developing brain can

create developmental delay and some long-term effects, as well.

Although today was focused on mechanisms, particularly the NMDA receptor actions, how do we differentiate between the sensory deprivation caused by prolonged sedation versus the specific actions of agents that we are considering as potential poisons?

DR. SHAFER: Any of the speakers care to respond?

DR. JEVTOVIC-TODOROVIC: I can try.

DR. SHAFER: Thank you.

DR. JEVTOVIC-TODOROVIC: When we studied animals using our behavioral paradigms, we made sure, first of all, that an important element--it's a very good point, because what you have to do is you have to take these infants away, these baby rats away, from their mom for, in our case, it was 6 hours. So it's lack of nutritional support and it's lack of mom's presence and stimulation that they get constantly by being licked and pushed around and whatever mother does with them, and some

mothers are better than others. But, overall, they all try to do that, and they feed it seems almost constantly although it is not quite that much.

So, what we did for our control animals is to inject them with, in this case, it was saline and DMSO, so we simulate at least that portion of what we did to experimental animals by injecting them with midazolam. But then also we took them away from their moms for the same duration of time and they were kept again a box that was the same as the one that was used for anesthesia, the same chamber. They were kept warm, but they did not have any kind of nutritional or sensory support from their mom.

The only problem that you cannot sometimes--and it's an excellent point actually that was brought up--is that when you bring experimental animals back to their moms after being away for 6 hours, they do smell of isoflurane. It's a smell of gas, smell of vapor, so for some moms it takes a while to take them in, to actually recognize them and take care of them.

Some moms were pretty bad about it and these animals obviously, if you notice that, you really should not use them for your experiment because they don't develop quite as well if you were to follow them over a course of time.

So, we were particularly sensitive about these issues and we followed the behavioral mom very closely the whole day after they were exposed to anesthesia, measured daily weights in babies every day to make sure that nutritionally they were pretty much developing, the amount of growing that was happening, their look. It was all very important.

We started testing them on these very simple behavioral tests that would indicate problems with attention and sensory motor development such as the scent test, for example, which is all you can do in a blind animal. They are blind at day 7. But we started at day 10, which is 10 days of age, and they performed exactly the same way as experimental animals.

So, to answer your question, it is not a

perfect setup and we cannot eliminate it completely, but I do believe that we tried to minimize the difference between controls and experimentals when it came to actually getting them ready post-anesthesia to go back to the every-day world. That's the best we could do.

DR. SHAFER: Dr. Pollock.

DR. POLLOCK: I have a clinical question for Dr. Soriano. I recognize that it's probably very simplistic and very premature, but do you think that we could say that a healthy 3-year-old coming for a tonsil or an inguinal hernia repair, or some anesthetic that is going to last less than 3 hours, is that a different category of risk than that chronically or critically ill patient that is going to have repeated anesthetics or long-term ICU exposure?

DR. SORIANO: Well, certainly the 3-year-old is taking the most risk because there is nothing wrong with him or her when she shows up for surgery. So, if anything is going to happen in the worst way it would be in that child.

That is why, in this GAS study, we chose healthy infants who will be having inguinal herniorrhaphies to try and limit the confounding variables.

It becomes more difficult with sicker neonates, because they need surgery no matter what, and they cannot tolerate the surgery without any interventions as intensivists as well as anesthesiologists. So, they also are at risk. I mean, there is no doubt. So until we can really demonstrate a phenotype, we still should continue what we are doing, but be cognizant. That's why the work that was presented this morning is important because it makes us think about this in the back of our minds and try 4 million ways to study this.

I just want to add some other items to what Dr. Todorovic said in response to the sensory deprivation.

If you recall the cell-culture work and some of the organohippocampus slices that are being done in Dr. Henthorn's lab, that is supposed to

nullify any other confounding variables. And we do see the same process, as well. In that model, we are not sure that truly is the same mechanism as we see in the in vivo models, but it is fairly convincing in the in vitro models that are being used.

DR. SHAFER: Raja.

DR. RAJA: A general question for those who are working with preclinical models, particularly as it relates to ketamine because I think it offers a potential opportunity to understand mechanisms, as people looked at the racemic mixtures and differentiated the effects of the enantiomers in this effect.

DR. SLIKKER: As far as I am aware, the racemic mixture idea has not been fully addressed.

It is an interesting concept, but I am not aware of data sets that have really looked at that in terms of the models that we discussed here today.

DR. HENTHORN: I have a question for Dr. Slikker.

I am really intrigued with what Dr. Zuppa

brought up with the microPET, but also about how your exploration for biomarkers is coming, say, in the protein area.

It would seem like if you could find signature proteins that might relate to mechanism, it would be interesting and I don't know if it's fair in this session to talk about what was in the open session, but it seems like that might be a link to some other cognitive-function issues with these agents in the elderly.

DR. SLIKKER: Well, yes, we do see that there can be certain advantages of using proteomics, metabonomics and other kinds of approaches to look for biomarkers. It would also be useful where one is trying to focus on the numerous agents that are out there that may need evaluation and, if you could find out certain biomarkers, it could be helpful.

In one case, you could compare and contrast them perhaps rapidly, before you did subsequent studies. That may help prioritize what lists that you would develop to look at more fully.

So, we really think that is a good way to go.

In terms of the other kinds of indicators, we have already discussed about the possibility of imaging perhaps being helpful in identifying markers that could be used in a longitudinal design, which would be much more powerful than having to do more incremental type approaches.

Then, you get into the behavioral endpoints. We really feel that it is important to use behavioral assessment tools that have been well defined and validated in a variety of species, and the approach that we have been using there at the NCTR is a battery approach that includes a number of cognitive function tasks.

These include short-term memory, new learning, discrimination properties, timing behavior, and motivation and, by looking at that in a battery approach that has now been developed in monkeys and applied both to rodents and to children, we feel pretty confident that these approaches can uncover subtle behavioral deficits.

It has been very fortunate, over the last

15 years, Merle Paule and others at NCTR have developed databases using nickel reinforcement for children to operate these operant behavioral tests, and banana-flavored pellets for monkeys.

Therefore, we are using similar kinds of reinforcement tools to motivate both children and animals to play these games, if you will, that allow us to assess the cognitive function in the multiple species. And so these validative tools will be the ones that we are going to apply in the animals that we are currently loading into these studies.

DR. ZUPPA: This is for all the preclinical investigators but, in the pediatric intensive care unit, we are starting to institute therapy with hypothermia for neuroprotection after cardiac arrest.

I was just wondering if any of the preclinical studies have been done under mild hypothermic or hypothermic conditions, and whether or not influence neurotoxicity.

DR. SLIKKER: Certainly, you are

absolutely right that temperature regulation is critical both in terms of perhaps providing some protection under certain scenarios and also in complicating your experiment if you do not control it for these studies.

So, I think in our cases, I know Vesna and I have spoken about this, we control temperature very, very carefully, and maintain it throughout, so that it is not a variable that has to be considered.

Now, on the other hand, you know, with the interest in cooling, and head cooling in particular, that has been very popular for the post-natal models in humans. I think there certainly is some possibility here.

We have not particularly looked at that under our conditions as of yet, but it certainly is one of those variables that could be very interesting to pursue.

DR. SNODGRASS: I have a comment about biomarkers and future studies in this era of personalized medicine, whether it would be

worthwhile doing some genomic array, perhaps investigations like single nuclear di- and polymorphisms or haplotypes, that there may be some association that would become helpful in the future to predict risk.

DR. SLIKKER: Well, we are certainly thinking that if we could understand the changes that occur under these conditions that we have described, and understand that for proteins and the genome, and even at the level, as you mentioned, of polymorphisms, that it could be very useful in extrapolating and also trying to understand the difference between subsets of these particular agents.

So, we certainly plan to pursue that. That is the next level of work that we are doing. We already have some data from the rodent which has been helpful in sort of guiding the idea that oxidative stress seems to be playing a role, and that may also be useful in defining potential mechanisms for protection.

DR. ZUPPA: I think from a genetic point

of view, the influence of genetics on drug disposition and drug metabolism should also be explored in context with the neuronal mechanisms.

DR. SLIKKER: That is a very good idea. Of course, in rodent models, we might not expect quite so much variation, but certainly in the human situation, you might expect variation. We know something about the pathways for metabolism of some of these agents. In some cases, it could be something that could be under a polymorphism type control, so I certainly understand your interest in that area of pursuit.

DR. SHAFER: I would like to address a question to Dr. Todorovic. Xenon is an interesting drug, and xenon we think primarily is having an MDA mechanism of action. But there are data and most recently data in Anesthesiology from the March issue by Dr. Mayes and Franks and their group, suggesting that Xenon, in fact, is neuroprotective and, in the specific model of the apoptosis from isoflurane, Xenon actually protects the brain to some extent from that.

Could you comment both on Xenon in general, and does it make sense that a GABA-ergic effect would be reversed by the NMDA effects of I think it is about a fifth of a MAC of Xenon, as I recall, something like that?

DR. JEVTOVIC-TODOROVIC: I think this is a study also when they show nitrous oxide to be toxic, is that right? Is that the same study?

DR. SHAFER: I have it here. Let me see.

DR. JEVTOVIC-TODOROVIC: I think that is the study where they also looked at the effects of nitrous oxide.

DR. SHAFER: That's correct.

DR. JEVTOVIC-TODOROVIC: I don't know what to make of it because we studied, for example, nitrous oxide extensively. And when I was in Dr. Olney's lab, we actually applied four different concentrations of nitrous using our 7-day animal protocol and we went as high as 180 volume percent of nitrous oxide--that is certainly MAC for these animals--and used 2 atmospheres of pressure to achieve that. And we could not see any signs of

apoptosis with any of the concentrations of nitrous oxide.

So, that is one thing that quite differs from what Dr. Mayes is showing so I am not exactly sure how their assay with what we have done compares and what Xenon does in the whole thing and it may all go back to what Dr. Akenhart said to begin with. It would be overly simplistic to lump them into an NMDA-antagonist category and GABA-ergic-agonist category.

It is possible that what we are looking at is a combination of factors. You know, why do we see difference in effect of sevoflurane versus isoflurane if mechanistically they do the same?

Dr. Wei just showed us that isoflurane exposure in utero doesn't affect learning and memory. There was a study done 30 years ago using halothane where they showed massive learning and memory deficits in rats for many months post-birth and post-anesthesia exposure. Well, how do we compare that?

It is very difficult. I am not exactly

sure whether Xenon does exactly what nitrous oxide does in regards to other receptor systems and neurotransmitters, and although it seems to be doing the same thing to the NMDA receptors, it doesn't exclude effects on other.

So, it gets a little messy if we try to box them into a specific box and walk away thinking that we drove our message home. It is more important to look at the whole picture.

I don't think the nitrous alone causes the same type of apoptotic damage that you see with, let's say, isoflurane alone. So, I am not sure how then their neurotoxic findings compare.

DR. SHAFER: Is it fair to say from what you just said in part is saying we have NMDA antagonists, we have GABA agonists, but that is a fairly crude classification, and ultimately, we have to consider each of these agents individually?

DR. JEVTOVIC-TODOROVIC: That is exactly right, they all have to--we cannot make any assumptions whatsoever and, if we are going to do clinical studies, we really have to base them on

well done preclinical studies. We cannot make any assumptions that, because we have effect with isoflurane, we will have it with sevoflurane, we will have it with propofol. It may be the case, but it may not.

DR. SORIANO: Well, this is certainly the Holy Grail of basic anesthesia research. As Dr. Eckenhoff has mentioned, we don't even know yet how this stuff works, how this stuff works.

Certainly, if you look at some of the work by Dr. Kelley and Crosby looking at microarray analysis in their model in the old rats and some of our own preliminary data looking at microarray analysis of the rat pups exposed to ketamine, everything seems to be affected up and down and just choosing which pathway to pick is difficult enough.

So, I think there is a lot to be learned from this model and certainly how we can use it for our clinical care.

DR. ANAND: Dr. Olney, you had suggested that it is the intrinsic pathway for apoptosis that

seems to trigger off widespread cell death. Have any of the groups looked at cytokine expression or other biomarkers that may activate the extrinsic pathway, as well, in this setting?

DR. JEVTOVIC-TODOROVIC: That is the one actually we did when we looked at changes in FAB and caspase-8 activation, which is, to a certain degree, external way of activating apoptosis.

We also published a study where we looked at the effects of neurotrophic factors, BDNF in particular, in the developing brain, and we found that anesthetics--that our combination affects BDNF quite a bit, and it actually does it in a region-specific fashion where we see changes in cortex indicative of downregulation of BDNF and downregulation of activated or phosphorylated AKT that leads to caspase-9 and caspase-3 activation.

However, when we studied thalamus, we found that BDNF is not decreased. It is actually upregulated, which was puzzling for a while until we looked at other ways of downregulation of phosphorylated AKT.

It turns out that in thalamus, anesthetics actually activate P-75 anti-P cascade in going through ceramide activation in order to decrease the level of phosphorylated AKT.

So, we know, we have a pretty good idea that anesthetics can activate the mitochondria-dependent pathway. They can also activate the extrinsic pathway and they can also activate at least BDNF-dependent pathways.

DR. ANAND: How about the non-caspase mediated pathways for apoptosis? Have we looked at it--because Bob Clark is coming up with these new pathways that are apoptotic morphologically and electron microscopically but don't have any caspase activation.

DR. JEVTOVIC-TODOROVIC: I haven't done any of that.

DR. SNODGRASS: I would like to emphasize the point that was just made, that each agent needs to be looked at individually. There is clear precedent for this. For example, in the area of the organophosphates where there is longer term

low-level exposure occupationally and cognitive deficits in adults years later, and also the primate data done with the military years ago, the cardiovascular effects are very different agent to agent. And yet they are all classified the same.

Then, the recent neurologic data, the genomic array data, it is different for each agent.

DR. SHAFER: I would like to take just a moment to interrogate two of our speakers to see how they have integrated their findings into their own practice, so I sort of get the sense of how do they interpret it.

Do you take care of children?

DR. JEVTOVIC-TODOROVIC: [Shakes head.]

DR. SHAFER: If you were taking care of children--I don't do kids either, but I am always terrified when I see them because I am concerned about controlling the airway, being sure they don't get hypoxic. You know, will I smile enough.

My question for you, if you looked at the OR schedule in the next day in the operating room, and you saw that you had a baby to take care of,

and, in fact, you didn't have a colleague that you could exchange this case with, how much would your care be affected by these concerns about neuronal damage?

DR. JEVTOVIC-TODOROVIC: I kind of agree with Dr. Soriano--he is truly an expert in dealing with children--that the main concern is just take good care of that child, not to do anything anesthesia-wise that can harm them.

If you have concern about the airway, you should choose your anesthetic based on that, what is the safest thing to do, because hypoxia and hypercarbia are serious issues. They are certainly going to cause damage more so than some animal studies that really, as of now, do not have a clear phenotype saying one way or another.

So, at this point in time, I really think that the most important factor is to look at the child and look at the previous medical and surgical history, and anesthesia history, if you have got that, and base your anesthetics on what you think is the safest thing to do, knowing what you know

about anesthesia.

Then, if and when many years from now, and Dr. Soriano has mentioned 2014 as a possible timeline, we know more about the specific phenotype, then, I would be willing to reconsider all of that.

But as of now, for example, that question we always ask--let's say we are dealing with kids that have severe burns and we do get them occasionally--I am sure that Boston takes more care of these kids, and I did a lot of these patients when I was at Washington University--they are very ill kids that come back over and over for burn dressing changes.

This is very painful, very uncomfortable, and they have to be put in a tub each time, so you can take care of that tissue and re-dress everything.

Well, what do you do then? Well, we used to do ketamine anesthesia for all of these kids, combined with midazolam, and, yes, there were some additional concerns. But we never had problems

with airway, with hypoxia, with hypercarbia. They were fairly comfortable, as comfortable as you can be under the circumstances.

The question is what is the ideal anesthetic for these kids. Yes, I am concerned about ketamine, but I am more concerned about pressing issues, pain control, comfort, amnesia, safety, airway protection.

Right now I don't think that anything that I am doing is changing. If I can choose alternative route, sure, I may not use nitrous oxide or I may not use midazolam. I may use propofol although some of the studies that John Olney has shown us, propofol is bad in that sense, preclinical sense.

But you simply do what you have to do to take the best possible care using state-of-the-art anesthesia knowledge we have got at this point. If we were to have a phenotype, then, we would have to know which one.

The only thing I would say, though, that has changed my way to looking at these things is I

truly believe that right now the most important element is the timing of exposure. Dr. Soriano said you don't have a lottery sometimes to decide that.

But if and when you can, I think that is the most important thing to do right now is look at it and say if this is relatively elective--I do get these calls all the time from parents--and if it's a relatively elective case that could be put off, it may actually be beneficial not to do it during the neonatal period, maybe wait 5, 6, 7 months, because based on animal studies, that seems to really matter.

The very same anesthesia protocol does not cause nearly as much damage if it's done later when brain development is at the tail end of it. That's the only difference.

DR. SHAFER: Dr. Soriano, the same question. You do take care of kids. How has this changed your practice?

DR. SORIANO: I take care of kids fairly on a daily basis, and the only thing that has

changed in my practice--and Dr. Frank McGowan, one of my colleagues from Children's Hospital--having to take care of the parent that is armed with the Science article from 1999--and spending the time to explain to the parents that this is an observation done in a laboratory, we are cognizant of this, that it can potentially occur, but there is no phenotype, as we have mentioned here.

But it takes a lot of time and patience to explain to the MIT professor that comes in with this paper, or the one from Journal of Neuroscience, and that is very compelling data, very compelling data. But, as part of our practice, we also have to take care of the parents, just reassure them that we are doing the best we can, we are not there to harm their kid.

DR. SHAFER: I don't know who the pediatric anesthesiologists are around the table. Can you raise your hand, so I can just identify those of you who consider yourself a pediatric anesthesiologist?

Yes. So, let's go right here and then

here.

From what you have heard, and in thinking about your own practice, I am interested in how you would incorporate all of this clinically.

DR. ZUPPA: I think unfortunately, there are not many alternatives, and we have to deliver care, but I guess to state the obvious, more is not always more, and it can be too much, and once you give it, you give it, so try and get away with as much as you can.

DR. DESHPANDE: I think the lessons learned have been--like Sul, as well--that cases that need to be done have to be done in the neonatal period even with preemies and those cases are not scheduled. They are not elective. And therefore a majority, if not 100 percent, of our practice in pediatric anesthesia dealing with infants is urgent or emergent surgery, or non-delayable surgery.

Where one can, one should postpone the surgery to an older age, and that is actually safer for the child from an airway hemodynamic standpoint

anyway. And that is the practice we would have even before this information.

It is important to be cognizant of it. And I think Sul brought up a good point that there are parents who are quite aware of the preclinical data and we should be able to have an appropriate response for that.

The other challenge is the ICU, particularly the neonatal ICU, where there are children who are suffering for prolonged periods of time and need sedation and need analgesia, and moderation I guess is the key. But the other alternative to these agents really is children who are suffering in the nursery.

It took us, in the specialty of pediatric anesthesia, almost 20 years to make people cognizant that children do hurt. Sunny took a big lead on it, Sunny Amand, and now people understand that babies can hurt, do hurt, and therefore need treatment.

Going forward based on the information I have heard today, I would not eliminate pain

management from those patients.

### Questions

DR. SHAFER: I am going to start moving through our questions here as long as there aren't other sort of questions that people have about the scientific database. Are there other questions that people would like to ask the speakers about the general database that we are working with?

The first question does not appear on the list, but I am going to read you a statement from the abstract of the article that was distributed, and I am actually going to ask people for a show of hands on whether they agree or disagree with the statement because I think it is an important statement.

The statement is: the lack of information to date precludes the ability to designate any one anesthetic agent or regimen as safer than any other.

In other words, we don't have the data to try to direct practitioners towards any one technique versus another technique.

By a show of hands, how many would agree with that?

[Show of hands.]

DR. SHAFER: Who would disagree with that?

[No response.]

DR. SHAFER: Okay. I think there is some guidance there that might be helpful to you. So, our discussion points.

The first one is: please discuss whether there are sufficient data to determine the applicability of the findings for anesthetics in nonclinical models to humans.

Based upon the vote we just had, we have heard from the anesthesiologists, particularly those actually practicing and taking care of kids, who have done the research, I am not seeing a lot of support for saying that we have enough data to make extrapolations to human patients.

I said we weren't going to have formal votes, but I do want to have a formal vote. Who would support the statement that there are not adequate data to extrapolate the animal findings to

humans?

Bob, is it okay if I take a vote on it as I phrased the question?

DR. RAPPAPORT: Okay. Only voting members.

DR. SHAFER: Actually, everybody could raise your hand. I can't prevent that. But it is only going to be counted if you are a voting member.

So, we will repeat the first vote, just so you can get a count.

The lack of information to date precludes the ability to designate any one anesthetic agent or regimen as safer than any other.

I think we are going to have to go through and you are actually going to have to push a button and just say agree or disagree. I will start with Dr. Kirsch.

DR. KIRSCH: Agree.

DR. MATTISON: Agree.

DR. SNODGRASS: Agree.

DR. ARMSTRONG: Agree.

DR. POLLOCK: Agree.

DR. ZELTERMAN: Agree.

DR. ZUPPA: Agree.

DR. SORIANO: Agree.

DR. WLODY: Agree.

DR. DESHPANDE: Agree.

DR. SHAFER: Agree.

DR. JEVTOVIC-TODOROVIC: Agree.

DR. ANAND: Agree.

DR. EISENACH: Agree.

DR. HENTHORN: Agree.

DR. RAJA: Agree.

DR. SHAFER: Thank you.

The next discussion point, just to make it clear.

Would you discuss whether there are sufficient data--and we have been discussing that--and the question that I would like to put forward is: are there sufficient data to apply the findings in animals to humans?

Again, Dr. Kirsch, do you agree, are there sufficient data, yes or no?

DR. KIRSCH: No.

DR. MATTISON: I think there are data that are worrisome and in other exposure settings have been extrapolated to humans to raise concerns about risk. So, I believe that there are sufficient data and that additional research on periods of vulnerability and mechanism of action are needed to understand how to extrapolate those more clearly to humans. So, that is neither a yes nor a no.

DR. SNODGRASS: No, there are not sufficient data to change clinical practice.

DR. ARMSTRONG: No.

DR. POLLOCK: No.

DR. ZELTERMAN: No.

DR. ZUPPA: No.

DR. SORIANO: No.

DR. WLODY: No.

DR. DESHPANDE: No.

DR. SHAFER: No.

DR. JEVTOVIC-TODOROVIC: No.

DR. ANAND: I would like to add some discussion to this. I think clearly data at one

time point on P-7, post-natal-day 7 in the rat. If you look at the microarray data that Huda Akil's lab has published recently in the Journal of Neuroscience, I think it came out last year, on that day P-7, there is a major switchover of, you know, thousands of genes that were upregulated, get downregulated, and those that were downregulation get upregulated, and it all happens on P-7, so I would be interested in finding out whether this is just P-7, or does P-6 and P-8, or P-5 and P-9 carry the same vulnerability. I think that is a question that still needs to be answered.

The other question that is key is how do you extrapolate this point of time in development to the human brain. So I think one has to discard the old rules of thumb, you know, P-7 is equivalent to day of birth in the human. That clearly does not work.

There is a bioinformatics approach that our group has published--this will be coming out in Neurotoxicology this month--where we use a bioinformatics approach looking at 102 different

developmental events across mammalian species and, across 10 different mammalian species, come up with an equation that can match up or help translate developmental time across these.

So, before we apply rodent or guinea pig data to the human, we really need to be sure as to which window of development we are talking about.

DR. SHAFER: I don't know if you do this in the voting booth. Is that yes or a no?

DR. ANAND: The question say "Please discuss whether" --

DR. SHAFER: We won't do that. But, if you do this in the voting booth, they are going to wonder why it is taking so long to cast a ballot. So, I need a yes or a no.

DR. ANAND: No, they are not.

DR. SHAFER: We will return to the discussion.

DR. EISENACH: No.

DR. HENTHORN: No.

DR. RAJA: No.

DR. SHAFER: Okay. Thank you. So, now

continue.

DR. ANAND: The flip side, what sort of practicing anesthesiologists and committee members need to consider, is what is the impact of unrelieved pain and that, clearly, is, from a human and animal study point of view, quite major.

If you go back to some of the early studies that were done on follow up of children who had surgery during infancy. There is one study by Professor Stevenson and L. Aynsley Green from England.

They looked at the British Twin Registry and they isolated 92 pairs of twins that were discordant only for one factor, that one member of the twin pair had surgery in the first year of life.

They studied these children at 79 years of age, and they found that the twins who had undergone surgery early in life had much greater incidence of ADHD, impulsivity, and a few other phobias and things like that, so there is a psychological impact.

There is also an emerging literature from Ruth Grunau, who has looked at pre-term babies and specifically counted the number of skin-breaking procedures that these pre-term babies underwent in the neonatal ICU.

When these babies are 8 months or 18 months old, the ones that had undergone greater degrees of unrelieved pain have a completely abnormal HBA axis. They are unable to turn off in response to a psychological stressor. They are unable to turn off the ACTH and cortisol response in their follow-up clinic.

This is simply to bolster what Dr. Soriano and the points that others have made, is that we really need to consider very carefully what is the potential harmful effect of the anesthetic per se, recognizing the anesthetics were given in the absence of any surgical stimulation or ongoing pain.

Then, what are the detrimental effects of unrelieved pain, particularly occurring during these developmental windows.

DR. SHAFER: Continuing with that line of discussion, the FDA is interested in guidance specifically on the kind of preclinical data to help to complete the database. The things which I have noted so far, I have summarized as the time course of gene expression as being very important.

We have talked about needing to calibrate the concentrations in animals against endpoints, so that we understand how they relate to concentrations in humans. The concentrations, for example, in monkeys for ketamine are very, very high compared to human concentrations, but it may just be that it takes more time to put down a monkey. And a slightly awake child is not much of a threat. I suspect that a partially anesthetized monkey is actually somewhat dangerous.

We have talked about the need to look at multiple inhaled anesthetics, not just isoflurane, but to consider sevoflurane. An inhaled anesthetic is not an inhaled anesthetic is not an inhaled anesthetic,

Similarly, in the NMDA antagonists,

nitrous oxide, Xenon may have different profiles from ketamine and should be considered.

So, these are the points that I have drawn, but I have not kept an extensive list. If people can at this point in time speak up as to the additional preclinical data that would help the FDA to sort through this problem.

DR. ARMSTRONG: Effects of repeated exposure.

DR. SHAFER: So, did I understand that time-course data--is it area under the curve or is it concentration?

DR. ZUPPA: We also didn't talk about barbiturates at all, pentobarbital, biopental, and I haven't seen any preclinical data with regards to those drugs, as well. But I think it would be important to explore drugs, as well, as alternatives to the current therapies that we are using.

DR. SORIANO: We should really focus on the drugs that we are currently using now and perceive we will be using in the future. In my

practice, ketamine isn't really a big part of my armamentarium, isoflurane, halothane, probably less so.

So, we are primarily using propofol, I think, by and large, if you can poll some of the practicing anesthesiologists here. Sevoflurane certainly has become the major inhaled anesthetic that we use in normal practice and then, looking to the future, Xenon, as Dr. Mayes group probably is going to try to push through, dexmetatomidine, which seems to be getting a lot of press lately in clinical applications.

So, if you want a plan for the future, I would say that these are the drugs that we should focus on rather than the old ones. I know Dr. Todorovic mentioned that isoflurane is toxic, and in this GAS study, perhaps we won't be addressing that issue, because we are using sevoflurane. Again, we should think about the drugs that we will be using, not the ones that we have used in the past.

DR. WLODY: Another drug class that hasn't

come up, I don't think today, is the opioids, and I think that we need to look at the extent to which the concomitant use of opioids can decrease the doses of the putative neurotoxins. I think that clinically would be extremely helpful.

DR. SHAFER: Although there actually was a signal for fentanyl in one of the studies, to my surprise.

DR. JEVTOVIC-TODOROVIC: We have to come up with a way to anesthetize sham animals, and we use the fentanyl as a neuro-opioid agonist thinking we are staying away from this boxing GABA versus NMDA.

I don't know whether you can recall. We didn't really didn't see a significant decrease in neuronal density at 5 days of age in guinea pigs after the brain was fully developed, but we did see an increase in caspase-3 signal after fentanyl, and that was actually a fairly low infusion of fentanyl for guinea pigs.

DR. SHAFER: And then we have the local anesthetics, as well, that we talked about.

DR. DESHPANDE: I would like to keep ketamine on the list. I think as part of the international, as well as the national, armamentarium for taking care of children, ketamine is a drug--as Dr. Todorovic pointed out, has a safety history from the clinical side and I think it is important to keep that on the list for discussion.

Actually, methadone, in particular, is an opiate that was listed in the summary table in terms of having NMDA actions, and this is a class of agents, particularly methadone, that we ought to keep on the list for study, as well.

DR. SORIANO: I stand corrected. I agree with Dr. Deshpande, because ketamine is now being used by the emergency-room physicians now as the mainstay of sedation for reduction of long-bone fractures at 1 mg/kg. So more and more of our pediatric colleagues are using it.

DR. SHAFER: Dr. Snodgrass.

DR. SNODGRASS: Just a point about the opioids. This gets back to the individual agents.

Methadone and fentanyl are synthetic. Morphine is a true opioid. There is at least one--besides the signal issue with fentanyl, I don't think there was a signal with morphine--there is at least one paper, I can't recall well now--a few years ago on neonates where morphine versus fentanyl was compared for later subtle, just behavioral, and fentanyl was picked up as having some adverse effect, morphine was not.

DR. ZUPPA: Along those lines, remifentanyl is being used I think more and more. Etomidate is being used quite frequently in the emergency-room setting and the ICU setting even though there is some debate about its immunosuppression.

DR. SHAFER: Dr. Kirsch.

DR. KIRSCH: I would like to bring up three areas of concern. One is the area of gender. In none of what we have discussed today has anyone discussed the possibility that there might be gender differences in how the brain responds to these agents.

Clearly, in other paradigms of brain injury, in particular ischemia, there are dramatic differences in how the brain responds even at the neonatal period of life. So that is something that needs to be looked at carefully.

The second is something that was brought up by one of the other presentations, which is other extremes of life. I think that it is critical that we not ignore the adult brain, particularly the aging brain. I think that would be error.

Last, no one that I heard today discussed the issue of neurogenesis in the older brain and clearly, inhaled anesthetics, in particular, are strong promoters of neurogenesis in the adult brain in the paradigm of ischemia in particular.

It is likely, in my opinion, that there is probably a balance between what these agents do as far as neurodegeneration and their effects on neurogenesis even in the adult brain.

DR. SHAFER: Dr. Eisenach.

DR. EISENACH: I have a couple things to

say. First of all, it seems that what we need is a clinical signal. We are not in an ethically difficult position in being convinced that we are giving neurotoxins. So we are giving these drugs, and there is the ability to get a clinical signal.

And, without a clinical signal, I think it is very difficult for us to move forward.

The second general issue is that I feel extremely uncomfortable suggesting that a branch of the Federal Government identify all the important research related to this area. As we went around the table, you got more and more dumped on you of practical drugs that need to be studied and mechanisms that need to be investigated.

This is why we have investigators throughout the United States funded on a competitive basis through the NIH and I would think it would make a lot more sense to get Congress to earmark some money through the NIH or for FDA or CDER to earmark some efforts and open it up to the academic community to determine what is the most appropriate way to look at both mechanisms and

practical importance.

It is not to say that I don't think wonderful work is being done here, but this is a big issue that I think deserves some investigation around the country.

DR. SHAFER: Before we take our break, I will ask the question, how high a priority is this, because I think that's right. The FDA has limited resources and my guess is you are kind of near what you can actually do within the agency. But if we, as a group, feel that this is a priority, and we can express that, that does become part of the record to say this is something that this committee feels is a priority that needs to be looked at.

DR. EISENACH: Right, within the world of anesthesia. I don't think we can comment really within the world of pediatrics how this fits in.

DR. JEVTOVIC-TODOROVIC: If you go diligently through the list of medications that we use for induction and maintenance of anesthesia, the list is not that horribly long. It is manageable as long as we would have some resources

to do that. It is unlikely that one would cover for that, but there are ways of going down the list and actually going through a list of medications that we do use on a daily basis and study them.

DR. SHAFER: Any other comments on preclinical models?

DR. RAJA: I was just concerned with the issues raised about sodium channel blockers. I think it is going to be an important area to look at, compare the sodium channel blockers especially the local anesthetic with the general anesthetics.

DR. ARMSTRONG: One other thing that is needed at the preclinical level when we applied it to children is potential synergistic effects with other common medications that are used with kids that have an effect on CNS.

I am thinking about all the kids who will come in who may be on stimulants at older ages, other children who are receiving primary therapies where there are known neurotoxicities associated with those drugs, and finding out whether there are special subclasses that need to be looked at.

DR. SNODGRASS: This is about the importance issue that was brought up. In the BPCA Act, Best Practices for Children's Act, there were drugs, and the first 10 that were listed included drugs for pain and drugs for sedation. And that was all of pediatrics, that wasn't just anesthesia.

DR. SHAFER: I have a list. For purposes of the record, going around the table, data that would help the preclinical evaluation, genomic data, the gene expression time-course, calibration of concentrations, response in the preclinical model against measures that we might see in humans.

I don't know, by the way, if electroencephalographic analysis could help that. That has been done in a lot of other domains, we look at electroencephalographic response in an animal and try to get dose-response curves of that and compare it to electroencephalographic response in humans.

Among the agents looking at multiple different inhaled anesthetics, nitrous oxide and Xenon, as well, etomidate, barbiturates, opioids,

local anesthetics, methadone. The need to look at different time courses, repeated exposures, long-term exposures.

The examination of gender, and also I added here, because we discussed it earlier, understanding the apoptotic risk in the spinal cord. Well, it has been looked at, but we haven't really seen much data and certainly, for local anesthetics, that would be a very relevant consideration.

DR. HENTHORN: You might add magnesium to your list.

DR. SHAFER: Yes, I would agree, adding the risk of magnesium.

DR. ZUPPA: Concomitant therapies like hypothermia.

DR. SHAFER: Okay. Concomitant therapies like hypothermia, and, in fact, concurrent drug therapy, as well, that was mentioned.

Is this a funding priority? Is this something that we should say we believe is a high priority? I would appreciate people just

expressing their thoughts on the record.

DR. EISENACH: I go back to the clinical signal. We do have a clinical signal, as Dr. Eckenhoff suggested, in postoperative cognitive dysfunction in the elderly, and it would seem to me that would have a higher priority where we have basic science and clinical medicine both suggesting there is a problem. So, I would place that above this issue at the moment.

DR. SHAFER: Dr. Anand.

DR. ANAND: Yes, it is a high priority.

DR. DESHPANDE: I will say from the pediatric standpoint, because I think that the earlier prematures, the more extreme preemies, that we are seeing in the nursery and the need to bring these patients comfort in the nursery, as well as bring them to the operating room for multiple surgical procedures, puts these children at risk for the types of things that we have discussed here today.

In addition, more children are being brought in for critical care and prolonged support

where earlier they would have died. With that, pain management has come to the forefront and is one of the metrics by which JCAH and others gauge quality of care. Therefore, this is a critical situation for pediatric patients and therefore, I think it needs to be on the forefront.

DR. ZUPPA: The other population that we have not talked about is the congenital heart-defect population, and they are at significant risk in the neonatal and infant period from bypass and circ. arrest. I just think they are a very vulnerable population, as well.

DR. ANAND: One other comment. There is some preclinical data to suggest this, but it has not been very well characterized, that early exposure to anesthetic agents or the data that is available is mostly related to opiates, produces a long-term, life-long tolerance to these drugs.

So, I think the issue of tolerance and decreased pharmacological effectiveness of the whole class of drugs is an important issue, pretty clearly because many premature babies are on

infusions of midazolam for weeks, and they do develop tolerance. They end up on very high doses of these drugs.

So, I think the issue of early exposure leading to life-long tolerance. We see that in the congenital heart disease population, kids who have had their surgical repair within two weeks of birth.

When they come back for their second stage of Norwood or of Fontan later on, they require three- or four-fold increases in anesthetic drug even though during the intervening period there has been on continued exposure.

DR. SHAFER: Thank you. That is fascinating.

I don't know a way of phrasing this to get a formal, around-the-table vote, but certainly the consensus of the committee is that this is a priority.

I am just stating that, not so much for the Agency but just for the record, that this is something that the committee finds is an important

research question that is worthy of investment.

With that, that brings us to our break. We will take a 15-minute break, reconvene here at 3:30. We are actually moving fairly quickly through the final portion of our day.

[Break.]

DR. SHAFER: Let's reconvene.

So, we have discussed Question No. 1 and we now turn to Question No. 2.

To what extent are the doses and durations of exposure to the anesthetics used in nonclinical studies relevant to the clinical use of these drugs?

In my view, we have actually addressed this question. But if anybody would like to further expound, that's fine, or we will go on to the next question.

Are there any additional comments? Yes.

DR. DESHPANDE: I just an additional comment. In the list that you read out earlier, I would like to make sure that we add that the surgical stimulus as part of the study is an

important--or a stimulus in the ICU or in the operating room, there is an additional stimulus that is going on.

DR. SHAFER: So, in the preclinical studies, you are recommending there also be--in the presence of surgery.

DR. DESHPANDE: That's correct.

DR. SHAFER: Not seeing anybody wishing to further comment on Point 2, we will turn to Point 3, Discussion Point 3.

3. Combinations of anesthetic drug products are frequently used in the setting of pediatric anesthesia. I believe, Dr. Soriano, you said they were virtually always used in your practice. Most of the preclinical data are derived from studies of drugs examined in isolation.

Does the committee have any advice on how FDA may best approach the issue of neurological toxicity of combination use?

There is, of course, the obvious answer of studying the drugs in combination. Is there any more specific guidance than that, that the

committee would like to offer?

DR. EISENACH: I think Dave Wlody and someone else brought up the issue of combining these drugs in a way that makes sense to clinical practice so that it wouldn't be all of one and all of another, but it would be some lesser amount.

DR. ZUPPA: There are also some drugs that can offer neuroprotection, such as dexmetatomidine, and maybe studying these drugs concomitantly with other medications and seeing if there is any type of synergistic effect in terms of limiting neurotoxicity.

DR. SHAFER: I would like to add one other comment to that, which is for some of these drugs, like for isoflurane, we saw evidence that at low doses it might be protective, and at high doses it is toxic.

In fact, one might suspect that individual drugs we should really have a full concentration response curve, because, in fact, there might be a neuroprotective dose and a neurotoxic dose, and if one is studying drugs in combination--this is a

personal preference, but I personally like to see response surfaces where each drug is studied in and of itself, and then the combination drugs are studied from low doses of the combination right up to high doses so you actually have a response surface rather than just single point sort of supermaximal dose studies that tailor themselves down.

It's another methodology, but I would support studying these and looking at response surface methodologies and later drug interactions.

DR. ARMSTRONG: I would agree with that, but I think that the other thing to consider is to study them at different ages. The developmental issue could interact so much with that point that you just made.

DR. HENTHORN: I would think just as a general comment that this is going to get extremely empiric unless we have mechanisms for each of the drugs. I think once you have a mechanistic approach, you could do this. But otherwise you are just going to be studying gazillion different

combinations.

DR. RAJA: Just a question for Jim Eisenach. In studying drug combinations, the isobolographic analysis, is such an analysis relevant to studying toxicities as well?

DR. EISENACH: I think Steve's suggestion of response surface is probably more useful. The isobole requires that you look at a fixed level of effect or a defined fixed level of effect which may or may not be what you want, particularly if you have biphasic responses that are protective. But that would be an approach for two or even three drugs if you wanted to look at their interaction.

DR. SNODGRASS: I just want to comment about the mechanism. I think it would be very useful, maybe not in the immediate future, but to look to the mechanism.

Let's say you pick sevoflurane and ketamine, maybe two ends of the spectrum perhaps, and try to figure that mechanism because I was impressed, in the ketamine data, if you look at the 3-hour--and I think there was either a 24-hour kind

of data there at one point--and it leaves the serum fairly quickly, it leaves the brain fairly quickly, but there is a time later for the expression of the effect.

Well, what is going on inside the cell is obviously the key issue. And the fact you can get an antisense response from one subunit, what is ketamine doing? It is probably not hanging onto that receptor per se. It is probably something at the nuclear level or someplace in the cell signaling pathway. So to really look at that would be perhaps quite useful for that agent, and maybe, maybe not, something similar for sevoflurane as an example.

Once you get mechanism, then, you will be in a position to really ferret out other issues including dose-response and susceptible time points.

DR. JEVTOVIC-TODOROVIC: It is not unlikely, though, that you are going to have a single mechanism for deactivation of apoptosis. At least in our hands we find three or four mechanisms

to be affected, and actually the whole point of non-caspase-dependent hasn't even been touched. It gets a little more complicated than that.

DR. SNODGRASS: You are correct. You have to consider effect size, magnitude of effect, so if you have three or four processes going on, which one or two are contributing the most to the overall effect, and then within each one of those, where is a step or two within the process. For example, what is the rate-limiting process in some cell signaling pathway.

Your point is well taken, but I think there are ways to try to approach it to get at least some much deeper level of mechanism than we have currently and that will lead, not always, but sometimes, to other therapeutic approaches to kind of allow us to use an agent in a broader way than we have before. We are less concerned about risk if we get, not an antidote, but some other agent to affect the pathologic path.

DR. SHAFER: Dr. Anand.

DR. ANAND: I Just want to get back to

your point about the fact that ketamine leaves the brain and the plasma very quickly. The graphs that Bill Slikker had presented were measurements of ketamine after the last injection, so there were 6 injections given of 20 mg/kg. During the injections, there were no concentrations. It is after the last, that's when they have their 5 minute, 1 hour, 2 hours.

The area under the curve is, you know, the preceding period is not even on the graph.

DR. SHAFER: Yes, I was aware of that, yes, that is a good point to reemphasize.

DR. SNODGRASS: Actually, I would say that at least I think what speaks for anesthesiologists is less repeated injections and continuous infusions. Our patients usually require a sustained level of drug effect for a period of time and, in a lot of these studies, the continuous infusion probably is a more--actually, it is more continuous infusion, of course. But that is a more representative model of what we do than the repeated bolus injections.

DR. SHAFER: Other comments?

Okay. So, let's turn now to Question No. 4.

DR. MELLON: If I could ask for a little bit of clarification regarding the approach to the combinations, the approach to date has really been looking at primarily ketamine as a way of trying to characterize the timing, the exposure durations, the vulnerability period, the doses that may produce these responses versus the doses that do not produce these responses.

Could I get some input regarding is this approach a reasonable approach with an isolated compound at this point in time, and would you recommend that we continue along those lines given the various differences between these compounds prior to starting combination studies, or would it be more advantageous to you to have information on the combinations prior to trying to carefully delineate what is taking place with individual compounds in terms of priorities?

DR. SHAFER: I would be happy to answer,

but do others want to respond?

From a personal perspective, I would always start with the isolated agents. If you start with compounds, you just have no idea which one is actually contributing, so you first need to understand the isolated agents, dose-response curves, and then explore the combinations. Otherwise, I don't know how to interpret the data.

DR. ZUPPA: I just want to state the obvious. I think we are saying here dose-response curves, but in some of the preclinical models, we are giving the dose IP or sub-Q, and at 30 mg/kg, which is not the dose that we would give in the clinical setting.

So, I really think that we should be talking about exposure-response relationships as opposed to dose-response relationships.

DR. SHAFER: I agree completely. I am using dose as sort of an encompassing term, but you are absolutely right. Measuring concentrations is key.

So, let's turn to Question No. 4, quite a

different topic.

Are there feasible clinical or other study designs to assess the potential neurological toxicities of exposing pediatric patients to anesthetic agents?

How do we find the phenotype that we are looking for? In what populations could we actually try to assess whether there are changes that are occurring consistent with the animal findings, consistent with the behavioral-affected animals?

DR. SORIANO: I could respond to that. Certainly, there is quite a bit of data showing that cohort studies, that there is indeed a difference in the outcome. The recent TIP study, where they compared medical versus surgical treatment of ductus arteriosus in premature neonates showed that the surgically treated group of cohort did much poorly in the neurodevelopment outcome.

But a lot of cohort studies, I think what is really needed is a randomized controlled trial.

This first attempt at it is this GAS study that I

was speaking of by the Australian, U.S. and UK group looking at a group of patients with very little confounding variables, such as, in this case, we are using healthy neonates and infants who need hernia surgery.

So, that way we will be able to delineate what the effect of anesthetic is as the causative agent, if indeed there is a neurological difference, developmental difference.

But again the patients at most risk for this are the sickest patients that we have, the premature neonate who is developing necrotizing enterocolitis with all these compounding variables.

They are a more difficult group, and they are the ones with the highest vulnerability on this issue.

DR. RAPPAPORT: Perhaps there is no other way to do that but, as I recall, that GAS study is an equivalence trial?

DR. SORIANO: Yes.

DR. RAPPAPORT: Could you comment on how you are going to establish what the level of equivalence has to be, how you are going to

establish your delta for that type of a trial without really having a historical basis to do so?

DR. SORIANO: I don't have quite the details, because I am not a primary investigator. But they actually did a power analysis and they needed about 600 patients for the type trial to provide enough power.

Maybe Dr. McGowan has more details on that.

DR. MCGOWAN: [Off microphone.]

DR. SHAFER: I am sorry, I will have to cut you off because we are supposed to keep the conversation around the table. My apologies.

DR. SHAFER: Can you tell us just a little bit about what is the phenotype, what is your endpoint, what are you looking for and are you confident you are looking for the right thing?

DR. SORIANO: You are never confident that you are looking for the right thing. With this study, I think Andrew and Mary Ann are looking at the Bailey score at 2 years of age and Wechsler Preschool Intelligence Score at 5. Jane Neuberger,

the cardiologist in our group, used the WPSSI score for a lot of the neurobehavioral studies. And these are NIH-funded studies looking at the neurodevelopmental outcome in neonates undergoing cardiac--deep circulatory arrest, the whole shebang.

When I showed that, there wasn't much deviation from normal. There are some kids that did poorly, there is no doubt about that. But you would expect a 50 percent decrement in their behavioral outcome if you draw any parallels between this and the preclinical models. But you just don't see it.

Again, we still have to identify a phenotype and I don't think our measuring ability is keen enough to do that.

DR. SHAFER: I would like to pose a question to Dr. Zuppa. Certainly, in the ICU, children are exposed to drugs for long periods of time where, in fact, there might be very substantial exposure, and even though these children have multiple risk factors, in theory, if

it's a double-blind, randomized trial, those risk factors are divided between the two groups. So that you can make some sort of causal inference.

Are there populations in the ICU where you could randomize children to--when you talk about morphine versus midazolam randomization, and use that to (a) try to see what does the phenotype look like, essentially, the first time, go on a fishing expedition, see what signal comes up, and then potentially do a more definitive trial based upon identifying a phenotype in the first trial?

DR. ZUPPA: There are definitely populations in the ICU that have somewhat of a uniform diagnosis, so the ENT cases for airway reconstruction usually have that as an isolated defect. The neonate or infant that comes in with RSV pneumonia and is intubated for a few days, or proceeds to ARDS, but is otherwise healthy.

So, there are definitely subpopulations that you could take and randomize either continuous infusion of midazolam or intermittent diazepam, or fentanyl, or morphine, and do the study that way.

But then that would also require long-term follow-up, and again what is the phenotype, but it definitely be done.

DR. ANAND: A comment on that. I think those are all important study designs. What we would need to do after these children have left the ICU or the hospital is to do a developmental follow-up, and it is really important as to what we look at.

Yes, looking at, you know, IQ using the Wechsler or the Stanford-Binet, or something like that, is a reasonable outcome. But intelligence is not what all of childhood is about. So, looking at particularly testing methodology that would get us the answers, and personally, I have been sort of researching this for another project that we are currently initiating.

What I have been attracted by is a method called the CANTAB. It's the Cambridge Neuropsychological Test Automated Battery. It has very specific tasks which are related to very specific pathways, the orbitofrontal pathway, you

know, the various different pathways, the frontotemporal pathways, and so on. And these can then be correlated with diffusion-tensor imaging or other neuroimaging to see what the impact is and where the impact is greatest of specific analgesic or sedative regimens.

I think refining our methodology would really help to then be able to propose specific hypotheses, that ketamine alters visual motor integration by affecting such and such pathway, or executive function by affecting such and such pathway.

I think that is the level of finesse that needs to come into these studies.

DR. SHAFER: I have a question for Dr. Eisenach.

The pregnant population, is there anything to be learned by looking at the outcome in neonates if it is delivered under general anesthesia or spinal anesthesia given the fact that those themselves, the use of one of those might in fact be predictive of how emergent the case was and

other potential risk factors?

DR. EISENACH: I don't think so, but I think the comment that Tom made about the importance of magnesium may have relevance to the obstetric population. We have a group of women that are receiving magnesium for more than one indication, some of which could be controlled for actually and are receiving that for many hours.

We know the concentrations that are present on the fetal side of the circulation of that magnesium, and it's in the pharmacologic range. We know that it penetrates at least the maternal CNS quite well, because people have done CSF concentrations of magnesium in those women getting magnesium for failed tocolysis, for example.

I think going to the brief general anesthetic exposure that is present for a cesarean section would be less useful. But I think Tom's suggestion of magnesium is interesting if the animal--and then I think the preclinical studies would guide you.

So, if magnesium at serum concentrations that we know are going to be present in the fetus and in the mother have this apoptotic effect, that might be an interesting clinical trial to do.

I am not sure that it would necessarily require randomization either. You would have to match for a variety of things. But the use of magnesium is--I wouldn't say haphazard, but it is not uniformly administered in some ways. Some indications are somewhat difficult to define, so there is a lot of variability in it.

DR. JEVTOVIC-TODOROVIC: Another patient population, Steve, I am thinking of is a population that actually gets in utero operation done. I don't know how many centers in the United States do that, but there are a few. I know CHOP, for example, does an average of 40 to 50 a year.

Usually, they select the last trimester of pregnancy obviously, because of the size of the fetus and because of our belief that the majority of the development is already done.

So, talking to colleagues, I don't know

how often you do them, a new institution, but it appears that the anesthetic of choice is a very high concentration of vapor anesthetic and hitting 3 to 4 percent throughout, and it can go on for hours.

I wonder whether that would be a good population although these fetuses are ill--that is why this is being done--whether that would be something to consider in the future and how randomized we could have that done, but whether we could attempt different anesthetics and see what the outcome is on these fine cognitive batteries of tests.

DR. EISENACH: If I could just think out loud some more about magnesium, I mean there are women that come in that have cesarean sections for preeclampsia, and they have them at different gestational ages and oftentimes it is not delayed very much.

So, some of these women come in and are sectioned before they are exposed to very much, or delivered before they are exposed to very much

magnesium, whereas, others come in and have magnesium for periods of time up to a couple days actually of therapeutic concentrations of magnesium before the delivery.

So, I think that would be a large population base, common population base, that if you set your entrance criteria, and again if the preclinical studies showed something--would be a very interesting place to go to.

DR. SHAFER: You would, as a natural experiment, get a dose-response curve out.

DR. EISENACH: Well, you would have time also. Women are exposed for different periods of time, but also at different periods of gestation from 24 weeks on probably.

DR. SHAFER: Let me follow up with Dr. Todorovic's comment because I think there are studies that give you asymmetric results. If you looked at those patients, I assume they are given high doses of sevoflurane primarily for tocolysis, just to keep the uterus quiet--

DR. JEVTOVIC-TODOROVIC: That's correct.

DR. SHAFER: But if you looked at those studies where they are exposed to very high levels of sevoflurane for a number of hours, and you did not see a signal in those kids, that would at least be quite reassuring.

DR. JEVTOVIC-TODOROVIC: Yes.

DR. SHAFER: If you saw a signal, you wouldn't know what to make of it, because it's confounded. But if you look at a population that ought to have a problem, and there is no problem--

DR. JEVTOVIC-TODOROVIC: Yes, I agree, and that can actually help you to decide. As Dr. Anand said, it is really important to decide what is the battery of tests that we are going to use so we have adequate measurement of what we are trying to discover because, for example, if you are not using appropriate staining in histology, I don't care how much you do and how often you repeat it, you are simply not going to see it.

So, that is the same thing here. If these cognitive batteries are very complex, and they have to be carefully designed, it almost seems that we

have to put more time into deciding what to use than to use them afterwards.

For these kids that are being exposed to very high concentration of, let's say, sevo in eighth month or ninth month before delivery, that would be very interesting to know and follow them and see how they do.

You can say, well, maybe there is a surgical reason that can cause some of the cognitive problems. But there are quite a few surgical reasons for doing it so we can even look at each and every one of them and see if there are cognitive deficits. Are they all very much similar because they were all exposed to sevo, or are they all very different, which could be because of different surgical problems, or maybe they don't exist at all.

But it may not be a bad population because you really have no choice but to use high concentration of inhaled anesthetic. You are stuck, and if the decision was made that we are going to proceed with the case, you have to have a

general anesthetic on-board, you cannot do that at all.

So, there are a lot of things that are already given. So you don't really have to get much of that approved, and yet you have, not a huge, but a reasonable size population to study. Mothers might be very motivated to see what happens.

DR. SHAFER: Dr. Slikker.

DR. SLIKKER: Just to comment to sort of summarize some of the things I have heard, it seems to me that we have a small amount of data from animal models on this issue having to do with behavior and looking for the phenotypic sort of indicator.

I think certainly when we have some data, we should use it. As we know, that data is not well developed yet, so it can't be as instructive as we hope it can be in the future.

The second thing is that when we develop these kinds of assessment tools, as both Sunny and Vesna have been saying, we have got to make sure

that they are going to be able to see a variety of insults and have outcomes that we can really feel confident in.

That is going to take some investment of time and selecting the tools. But, also, it would be really great if those tools also had the ability to look across species, so that we could back them into animal models and go across species and learn more about other agents in the class or outside the class.

I just put that out there to look at some of these assessment tools and really understand how they could be useful to you and how they could be useful to others once we invest in them.

DR. SHAFER: The neonatal water maze.

DR. ANAND: I wanted to bring up another issue. We have been talking about factors that increase vulnerability and periods of vulnerability, and so on, but I think, at the back of my mind, there is also the question of what are the factors that show resilience because we all have had patients who went through the most

horrendous clinical course and yet came back to the clinic, you know, looking pretty normal.

It is always surprising when that happens.

But I think that is an area that needs to be studied in this context as to what are the protective, the natural protective factors that promote a good outcome.

DR. SHAFER: I agree. Actually, it is amazing both that they look so good and that they came back to the clinic.

DR. ARMSTRONG: Several things. I agree with the discussions about the needs for specific assessment and the cognitive assessment is interesting. It is more difficult than you would expect. I wrote a chapter a few years ago--it is one of my cited anecdotes--the way that a neurobehavioral battery is determined. You put 10 investigators in the room. One of them walks out with a battery. The other 9 are dead.

We have so much discussion about which test to use and which is best. That can be something that takes us off track if we are not

careful. There are some principles to the clinical studies that I think we need to think about.

One is that in order to get the kind of specific cognitive and behavioral outcomes that we would be interested in, in defining a phenotype, based on everything else we have looked at in terms of weight effects, it is going to take a period of time for the brain to develop in an abnormal fashion after the injury to be able to detect those kinds of differences.

So, that becomes a longer-term strategy. It may be that one approach would be think about a carefully designed 1-point cross-sectional study looking retrospectively at chart review about what was exposed in a group of people who are 10 to 15 years of age where we then begin to look at those specific issues that you talked about.

But prospectively, I have heard a couple of things today that really sort of trigger my thinking, and one is not my area. I think if there was a way, and we could find the appropriate imaging, non-invasive imaging, to be able to

determine at the time that a child was exposed to the anesthetic, can we measure in any way if apoptosis is occurring at an abnormal rate or not in the patients who are going to participate in the trial.

That seems to be a critical feature, and whether that could be done with an imaging approach, whether it could be done with an array approach to look at the percentage of genes that are upregulating or downregulating at the point in time, to really be able to use that as a defining factor for entry into the study, and then discriminate on those where there are high levels of activity versus not so high levels of activity, to look at polymorphisms, and then to develop this short term.

I think imaging probably is the right direction with diffusion tensor or even MEG, magnetoencephalography, at some of the selected institutions that have that capacity, to be able to look specifically at the imaging changes over a short period of time.

Those would seem to be robust mechanism-related kinds of studies that might help us answer the question quickly.

DR. SHAFER: Dr. Olney, is there any sort of non-invasive way of assessing apoptosis? Is there any imaging study, any way of imaging this other than removing a brain slice, which would obviously not be possible in a clinical study?

DR. OLNEY: We have certainly asked ourselves that question, and I am in communication with several people in our own Neuroimaging Department who are very interested in developing such technology and tell me that maybe they are getting there. But it's slow progress.

So, we certainly are working on that. I have also considered the other possibility. There are infants who die on the surgical table after 6, 8, 10 hours of anesthesia, and other infants die all the time who weren't exposed to anesthesia.

If you could do autopsies in a timely manner on those infants, and had the appropriate markers for histopathological diagnosis, that might

be a useful approach.

DR. SHAFER: Thank you.

Are there other thoughts about the kinds of clinical studies that would be done to help understand the risk of anesthetics on neuroapoptosis? Yes.

DR. ZELTERMAN: You might want to identify who are the individuals at risk. I mean part of the earlier studies we saw there were the rats at day--or was it the mice at day 7 seemed to be vulnerable, but at other ages not so much.

I think the same issue would be not to identify the individuals once the damage has occurred certainly, but who are the individuals at risk.

DR. SHAFER: Very good.

What I have heard we have a randomized controlled trial, the GAS trial coming up. We have talked about healthy children going for things for hernia repair that can potentially look at different anesthetic techniques, ICU trials. There are some uniform populations that can be done.

There are some very sensitive assessment tools, the CANTAB tools.

The possibility for neuroimaging is thought-provoking, but needs still to be worked out. There is a possibility of studies in pregnant women, particularly looking at magnesium, which would sort of naturally lend itself.

In utero, surgery involves enormous exposures which if you saw something, you wouldn't really know what to make of the difference from some sort of cohort group. But, if there was no signal there, that would be at least be reassuring.

That is a quick summary I think. Earlier, in some of our conversations, there was concern that this was un-studyable, but it looks like there might be some populations that could be looked at.

The major problem I have heard from people from around the room--correct me if I am wrong--is that we don't really know quite what to measure. We don't have this phenotype, we don't have a handle on what this phenotype would look like.

Is that a fair summary? Would anybody

care to add to that?

That brings us to the last question.

The last question is: given the risks associated with delay of surgical intervention or with the use of sub-optimal anesthetic techniques--and we have certainly talked about those risks so far--how does one incorporate the current knowledge base into the practice of pediatric anesthesia?

That is actually pretty close to the question that was posed about two hours ago, and the answer that I heard from the group was the existing and well-understood risks of anesthesia continue to be the overwhelming considerations in designing an anesthetic, and the understood risks of delaying surgery are the primary reasons to determine the timing.

All things being equal, it might make sense to delay surgery, but that is a high hurdle to say all things being equal, because children at this age do not undergo surgery that is truly elective.

Is that a fair summary of the consensus that went around the table? I see nodding heads.

Does anybody care to offer a divergent view?

That being the case, we have answered all five questions.

Bob, is there any other issue you would like us to address at this time?

DR. RAPPAPORT: No, I think we have gotten a lot of good information today, and we are very appreciative for the input we have received from you all.

I would also like to thank the speakers for the excellent presentations.

DR. SHAFER: I would like to also second that.

[Applause.]

DR. SHAFER: Does anyone have any additional comments to make at this time?

In that case, the meeting is adjourned.

[Whereupon, at 4:05 p.m., the meeting was adjourned.]