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REPORT OF THE NEUROTOXICITY RISK ASSESSMENT GUIDELINES PEER REVIEW WORKSHOP

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RISK ASSESSMENT FORUM
U.S. Environmental Protection Agency

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CONTENTS

NOTICE

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This workshop was organized by Eastern Research Group, Inc., Lexington, Massachusetts, for the EPA Risk Assessment Forum. ERG also assembled and produced this workshop report. Sections from individual contributors were edited somewhat for clarity, but contributors were not asked to follow a single format. Relevant portions were reviewed by each workshop chairperson and speaker. Their time and contributions are gratefully acknowledged. The views presented are those of each contributor, not the U.S. Environmental Protection Agency.

PREFACE

On June 2 and 3, 1992, the U.S. Environmental Protection Agency's (EPA's) Risk Assessment Forum sponsored a workshop for peer review of draft EPA guidelines for neurotoxicity risk assessment. The meeting was held in Washington, DC, and was chaired by Dr. William Greenlee of Purdue University (57 *Federal Register* 21086, 18 May 1992). Participants from academia, industry, and state and federal government brought expertise from a wide range of disciplines to the discussion. Members of the public and EPA scientific staff attended the workshop as observers. The Agency is using the peer review comments to help complete a proposal for neurotoxicity risk assessment guidelines that will be published for public comment and reviewed by EPA's Science Advisory Board during the coming year. This workshop report presents information on issues discussed at the workshop, identifies participants, and summarizes workgroup conclusions.

1. INTRODUCTION

1.1 RISK ASSESSMENT GUIDELINES PROGRAM

In its 1983 book *Risk Assessment in the Federal Government: Managing the Process*, the National Research Council recommended that federal regulatory agencies establish "inference guidelines" to promote consistency and technical quality in risk assessment, and to ensure that the risk assessment process is maintained as a scientific effort separate from risk management. A task force within the U.S. Environmental Protection Agency (EPA) accepted that recommendation and EPA embarked on a long-term program to develop such guidelines. The first guidelines were published 3 years later (51 *Federal Register* 33992-34054, 24 September 1986); two of the 1986 guidelines were recently revised (56 *Federal Register* 63798-63826, 5 December 1991; 57 *Federal Register* 22888-22938, 29 May 1992). Currently, six other guidelines are in various stages of development and review.

1.2 NEUROTOXICITY GUIDELINES PEER REVIEW WORKSHOP

An EPA work group, chaired by William Sette and Suzanne McMaster, prepared draft neurotoxicity guidelines for peer review. The purpose of the guidelines is to describe the principles, concepts, and procedures that EPA will follow in evaluating data on potential neurotoxicity associated with exposure to environmental toxicants. Like EPA's other risk assessment guidelines, the draft neurotoxicity guidelines are organized around the National Research Council's paradigm for risk assessment.

On June 2 and 3, the EPA sponsored a workshop to peer review the draft guidelines. The meeting opened with discussion of key features of the draft guidelines, including areas of expected controversy, followed by workshop review of the scientific foundation for each element in the guidelines. Workshop participants from academia, industry, and government (state and federal) brought expertise in a wide range of relevant disciplines to the discussion.

The workshop did not attempt to address all of the principles, concepts, and methods that are important for neurotoxicity risk assessment. Rather, EPA asked for expert opinion on the logic, scientific validity, and utility of the principles proposed in the workshop draft as

general guidance for EPA risk assessors. Workshop participants were asked to review the draft guidance with these objectives in mind. To help focus the review, EPA distributed an issues paper highlighting issues raised during EPA reviews of earlier drafts.

1.3 ORGANIZATION OF THIS REPORT

This workshop report presents the issues paper (Section 2); the overall workshop summary prepared by the chairman, Dr. William Greenlee (Section 3); a summary of the opening presentations (Section 4); and reports of the four workgroups (Section 5), including their conclusions and recommendations to EPA regarding the draft guidelines for neurotoxicity risk assessment. The workshop agenda, list of panel members, participants and observers, and premeeting and postmeeting comments are provided in Appendices.

2. ISSUES PAPER: PEER REVIEW WORKSHOP — DRAFT EPA GUIDELINES FOR NEUROTOXICITY RISK ASSESSMENT

The purpose of this workshop is twofold: (1) to develop expert information and opinions on the risk assessment guidance presented in the draft guidelines, "Proposed Guidelines for Neurotoxicity Risk Assessment," and (2) to ascertain scientific consensus among workshop participants on principles and methods proposed as guidance for EPA Neurotoxicity Risk Assessment. Each Peer Review Panel should examine the conclusions, suppositions, and limitations stated below for their consistency with available data and applicable scientific principles.

2.1 PANEL 1: NEUROTOXICITY AS AN APPROPRIATE ENDPOINT FOR ENVIRONMENTAL RISK ASSESSMENT

The workshop draft concludes that neurotoxicity is an appropriate endpoint for environmental risk assessment.

Conclusions and Suppositions Used in Reaching This Position

- Proper functioning of the nervous system is an essential element of health.
- A wide variety of agents is known to cause neurotoxicity.
- Human exposure to neurotoxic agents may be significant.

Areas of Special Focus

- Inadequate toxicological information is available for a vast majority of chemicals.
- Standards for evaluating neurotoxic potential have not been firmly established.

2.2 PANEL 2: INTERPRETATION OF NEUROTOXICITY DATA WHEN EFFECTS ARE TRANSIENT

The Workshop draft concludes that both reversible and irreversible effects of chemicals on the nervous system should be considered adverse.

Conclusions and Suppositions Used in Reaching This Position

- The nervous system contains billions of cells wired in complex patterns and is known to be resilient to environmental and toxicological insult by a process known as compensation or adaptation.
- Once damaged, nerve cells have limited capacity for regeneration.
- Apparent recovery actually represents activation of reserve capacity, decreasing remaining potential adaptability.

Areas of Special Focus

- Traditionally, effects of toxicants are considered to be persistent or long lasting, while pharmacological effects are considered to be transient or short-acting.
- An effect that appears to be transient in an unchallenged organism may be revealed as long lasting through an environmental or pharmacological challenge.
- It is not known whether transient effects observed following developmental exposures should be evaluated at specific points in the life span.

2.3 PANEL 3: AGENTS ACTING THROUGH INDIRECT, AS WELL AS DIRECT, MEANS CAN BE CONSIDERED NEUROTOXIC

The Workshop draft concludes that chemicals may produce neurotoxic effects by direct and by indirect means.

Conclusions and Suppositions Used in Reaching This Position

- Distinctions between direct and indirect action on the nervous system are the

same as those sometimes referred to as primary and secondary effects, respectively.

- Agents such as glutamate damage specific neurons through direct stimulation of receptors, whereas agents such as carbon monoxide act indirectly to kill neurons by decreasing oxygen availability.
- Effects on endpoints of neurotoxicity produced through direct or indirect means are functionally equivalent.
- It is logically inconsistent to say that a compound produces neurotoxicity but is not a neurotoxicant.
- Any compound delivered in a high enough dose will be lethal, and, by definition, neurotoxic.

Areas of Special Focus

- The kind of information available to risk assessors rarely permits a firm determination of primary versus secondary sites of action.
- Compounds damaging the liver, or producing diabetes, may produce nervous system damage as a secondary consequence of the primary damage.

2.4 PANEL 4: EXTRAPOLATION OF NEUROTOXICITY DATA FROM LABORATORY ANIMALS TO HUMANS

The workshop draft notes that EPA must frequently make risk assessment judgments regarding the potential neurotoxicity of a substance for which the human data base is absent or inadequate. The draft concludes that with an adequate animal data base, as defined in the draft guidelines, such judgments may be scientifically valid.

Conclusions and Suppositions Used in Reaching This Position

- Substances producing neurotoxicity in humans also result in neurotoxicity in other species.
- Compared with human studies, animal studies are more often available and provide more precise dose information and better control for environmental factors.

- Many diagnostic procedures employed to evaluate neurotoxicity in humans have corresponding animal models.

- The range of uncertainty factors used to extrapolate from animal data to human risk for other endpoints of toxicity are applicable for neurotoxicity risk assessment.

Areas of Special Focus

- The full range of human behaviors, for example language, is not present in other species.

  Factors such as differences in metabolism can result in differences among species in sensitivity to a compound.

- The most sensitive species may not be the species affected most like the human.

2.5 PANEL 5: INTERPRETATION OF BEHAVIORAL DATA

The workshop draft concludes that behavioral changes can provide evidence of neurotoxicity in the absence of additional data.

Conclusions and Suppositions Used in Reaching This Position

- Behavior is often regarded as one of the most sensitive indicators of toxicity.

- Evaluations of behavior have played an important role in neuroscience research efforts to understand brain function.

- Most behavioral evaluations conducted in humans have well established animal counterparts.

- Behavioral effects often appear prior to measurable effects on physiological or morphological endpoints.

- The primary effects of developmental exposure to some chemicals may be behavioral.

Areas of Special Focus

- Some behavioral changes may represent non-specific effects such as sickness or malaise.
- It is difficult to identify a maximum tolerated dose for behavioral studies, since most chemicals produce behavioral effects at high doses.
- Toxicant-induced changes in behavior can result from a variety of physiological changes in addition to effects on the nervous system.

3. CHAIRPERSON'S SUMMARY OF THE WORKSHOP

William F. Greenlee, Ph.D., Workshop Chair

3.1 WORKSHOP OVERVIEW

The Environmental Protection Agency (EPA) invited 13 scientists from academia, industry, and government to participate in a workshop convened to review the draft document "Proposed Neurotoxicity Risk Assessment Guidelines." This draft, prepared by an EPA Workgroup headed by Drs. William Sette and Suzanne McMaster, was structured in accordance with the risk assessment paradigm set forth by the National Research Council (NRC) in its 1983 book, *Risk Assessment in the Federal Government: Managing the Process*. The draft guidelines were distributed to participants prior to the workshop, along with a series of issues papers designed to focus participants' attention on topics of particular concern to EPA. Preliminary comments on the guidelines were submitted by each participant and were also distributed prior to the workshop.

The workshop was held at the Omni Georgetown Hotel in Washington, D.C. on June 2 and 3, 1992. To promote discussion of issues appropriate for the peer review process, the Agency designated five working panels to meet during the course of the workshop:

- Panel 1: Neurotoxicity as an Endpoint (Dr. William Greenlee, Chair)
- Panel 2: Transient and Persistent Effects (Dr. John O'Donoghue, Chair)
- Panel 3: Direct and Indirect Effects (Dr. Barry Wilson, Chair)
- Panel 4: Animal-Human Extrapolation (Dr. Shayne Gad, Chair)
- Panel 5: Behavior (Dr. John Orr, Chair)

The infrastructure of the workshop was thus a matrix in which panels formed to consider particular issues were assigned the task of reviewing a document organized around the various stages of the risk assessment process (see Figure 1). Individual panels were asked to review the draft guidelines from the perspective of issues related to endpoint selection (Panels 1 and 5), issues concerning the nature of neural responses to environmental toxicants (Panels 2 and 3), and issues involving the extrapolation of experimental results from one species to another

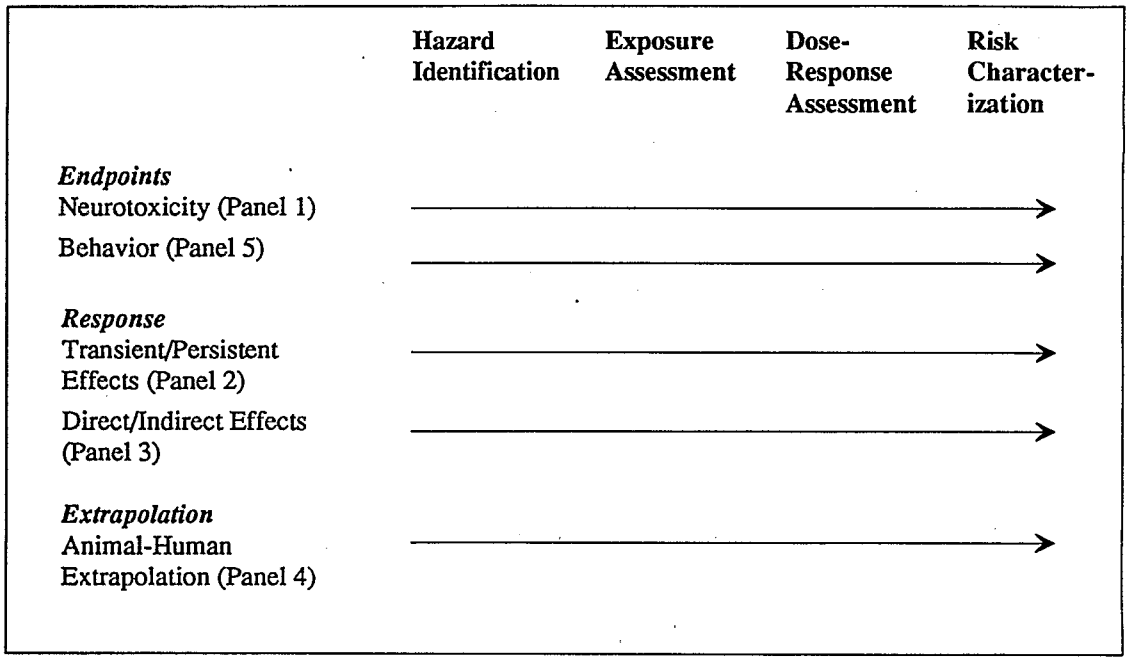

Hazard Identification
Exposure Assessment
Dose-Response Assessment
Risk Character-ization
Endpoints
Neurotoxicity (Panel 1)
Behavior (Panel 5)
Response
Transient/Persistent Effects (Panel 2)
Direct/Indirect Effects (Panel 3)
Extrapolation
Animal-Human Extrapolation (Panel 4)

Figure 1

(Panel 4). Each of these issues, in turn, was to be addressed across all four components of risk assessment defined by the NRC: hazard identification, exposure assessment, dose-response assessment, and risk characterization. At the conclusion of the workshop, panel chairs were asked to prepare summary reports of the discussions that took place in their respective workgroups (see Section 4 of this report).

3.2. NEUROTOXICITY AS AN ENDPOINT

Workshop participants were in general agreement that neurotoxicity is an appropriate endpoint for risk assessment, and that regulatory action may be warranted for substances found to be neurotoxicants under certain conditions of exposure. There was considerable discussion and debate, however, regarding the definition of adversity as it relates to effects of a toxicant on the nervous system. Panels 2 and 3, for example, proposed a three-tiered system for classifying nervous system effects according to their degree of adversity. One goal of this classification scheme was to provide a basis for distinguishing between substances that are neuroactive or behaviorally active and those that are neurotoxic. Panel 5 concluded that behavioral changes could provide evidence of neurotoxicity even in the absence of other data, but the panel did not provide guidance as to how behavioral endpoints might be incorporated into routine chronic toxicity bioassays.

Participants also agreed that is important for the best available data and scientific judgment to be brought to bear on decisions about a substance's potential neurotoxicity. It is important, for example, to be able to distinguish actions of a chemical on the nervous system that are causally linked to a clinically observable effect versus those that are not. Toward this end, participants thought that more explicit guidance may be needed if non-neuroscientists will be expected to use the guidelines to assess the adequacy of available data in establishing whether or not a given substance should be regarded as a neurotoxicant. For example, participants suggested that a short list of quantifiable endpoints be developed in lieu of the "laundry list" of potential endpoints contained in Table 1 of the draft guidelines. This "short list" would be comprised of those effects that are most likely to arise in association with chronic, low-level exposure to environmental agents. One panel recommended that the guidelines also encourage risk assessors who lack expertise in the relevant neurosciences to seek appropriate

outside help if they encounter difficulties in dealing with data or situations that are not clear-cut.

During the final session of the workshop, after all of the panels had met and their conclusions had been discussed by the group as a whole, the Chair asked workshop participants whether there are features of the nervous system that present unique challenges in the development of risk assessment guidelines. A number of features were discussed, including:

- The fact that the normal range of functioning is narrower for the nervous system than for other organ systems.
- The need to consider toxicant levels in extracellular spaces (e.g., at the myoneural junction) as well as within the target tissue.
- The confounding role that conditioning (and/or other aspects of an organism's history of interaction with the environment) can play in the interpretation of behavioral data.
- The greater subjectivity involved in assessing the functional status of the nervous system than in assessing the function of other organ systems.
- The fact that biomarkers of nervous system function are fewer in number and are less well understood than biomarkers of function for other organ systems (e.g., the liver).

Participants agreed that additional research is needed in all of these areas.

3.3 OTHER CONCERNS

In the opening session of the workshop, the Chair raised the question of whether additional sources of information about the effects of chemicals on the nervous system might be useful in assessing the neurotoxic potential of environmental agents. Although the importance of well-controlled epidemiologic studies is widely recognized, for example, it is also possible that data from pharmacologic studies could provide important insights into the neurotoxic potential of certain chemicals or classes of chemicals. Similarly, the veterinary literature could serve as an important adjunct to the types of information currently obtained from animal toxicity studies.

Throughout the workshop, there was a fair amount of discussion regarding the relevance of animal models for predicting neurotoxicity in humans. Panel 4 concluded that appropriate animal models do exist for many if not most human behaviors. Differences in toxicant metabolism, distribution, and other pharmacokinetic properties, however, are important determinants of interspecies differences in sensitivity to potential neurotoxicants. Participants agreed that there are currently major gaps in our understanding of the pharmacokinetic determinants of neurotoxicity, and suggested that additional research is needed, particularly in the area of low-dose exposures to known or suspected neurotoxicants. Advances in the field of pharmacokinetics have significantly improved our ability to study the uptake, distribution, and metabolism of potential neurotoxicants. Based on this new understanding, physiologically-based models of neurotoxicant handling should be developed, incorporating what we now know about the metabolic properties of specific cell populations within the nervous system, factors that control circulating concentrations of a toxicant, and factors related to the uptake and localization of toxicant molecules within the nervous system. These pharmacokinetic data, which are usually generated early in the process of characterizing a compound, should be integrated with available dose-response data throughout the risk assessment process.

While noting that both animal and in vitro models could be useful in this regard, participants were in general agreement that in vitro systems should probably be viewed mainly in terms of their potential to provide supporting information about a toxicant's mechanism of action, rather than as screening tools in a more general sense. At the same time, contemporary cell and molecular biological techniques could be used to gain new types of knowledge about nervous system function that might be relevant to the risk assessment process. As factors that influence the neurotoxic potential of chemicals continue to be identified, comparative pharmacokinetic studies should be conducted in vivo and in vitro to determine the extent to which these factors are or are not species-specific. Hepatic metabolism and elimination, blood flow to target tissues, interaction with the blood-brain barrier and/or brain lipids, and high-affinity binding to target proteins are all examples of pharmacokinetic processes that need to be studied in this way. It is important that differences in toxicant handling by humans and rodents be incorporated into pharmacokinetic models used to predict the effect of a given neurotoxicant on a nervous system target. At the same time, interspecies differences in metabolism and elimination need to be considered in determining appropriate doses for toxicity

testing, particularly in studies that attempt to address the effects of low levels of exposure to a suspected neurotoxicant.

Participants also suggested that it is important to recognize the speed with which advances continue to occur in the field of neuroscience. Because of this, there was general agreement that the guidelines should be structured to facilitate the incorporation of new knowledge into the decision-making process.

3.4 RISK CHARACTERIZATION

As noted previously, discussion during the workshop focused largely on issues related to the definition and classification of adverse effects. These discussions reflected both the inherent complexity of the nervous system and the difficulty that inevitably arises when one attempts to form conclusions about the functioning of this complex system on the basis of isolated observations. The workgroup did not resolve all of the outstanding issues related to hazard identification, but did recognize the need to avoid an inappropriately rigorous classification scheme for known or suspected neurotoxicants. Criteria need to be established to provide guidance in determining the adequacy of results obtained from chronic bioassays for assessing the neurotoxic potential of a given agent.

The group also agreed that the goal of risk characterization in the area of neurotoxicity should be to develop a mechanisms-based approach that incorporates our current understanding of the biology of the nervous system to the greatest extent possible. Given the complexity of this system, it is essential to focus on endpoints that are quantifiable and that are linked to a clinical outcome under conditions of exposure that are likely to occur in the environment. Toward this end, physiologically-based pharmacokinetic models should be used to develop quantitative descriptions of the behavior of neurotoxicants at low doses. At the same time, quantitative descriptions of the molecular and biochemical interactions that occur between a neurotoxicant and its nervous system target need to be fully elaborated and, together with pharmacokinetic models, incorporated into the risk characterization.

4. SUMMARY OF OPENING PRESENTATIONS

4.1 INTRODUCTORY COMMENTS

Dr. William Greenlee, Purdue University

Dr. William Greenlee, Chairman of the Peer Review Workshop, opened the workshop by welcoming participants and observers. Dr. Greenlee noted that the purpose of the workshop was to provide a scientific peer review of the draft Neurotoxicity Risk Assessment Guidelines. While noting that discussion among workshop participants was expected to comprise the bulk of the meeting, Dr. Greenlee also indicated that there would be an opportunity for comments from observers at the conclusion of the Opening Plenary session. Observers wishing to comment at that time were requested to add their names to a sign-up sheet. Following this introductory statement, Dr. Greenlee asked Dr. William Wood to give an overview of the Risk Assessment Forum's goals for the peer review meeting.

4.2 WELCOME AND OBJECTIVES

Dr. William Wood, U.S. EPA Risk Assessment Forum

Dr. Wood began by thanking workshop participants for agreeing to take part in the peer review process. This review represents a first step in the Agency's efforts to develop risk assessment guidelines in the area of neurotoxicity. The purpose of his presentation was to give the group some general background regarding the guidelines development process and to answer any questions participants might have about the objectives or intended audience for the draft guidelines the group had been asked to review.

The Risk Assessment Forum was established in response to a 1983 study by the National Academy of Sciences (NAS). Among other recommendations, the NAS study suggested that each agency develop what were called "inference guidelines" to foster consistency within and among federal agencies in the area of risk assessment. Originally a group of nine scientists drawn from various EPA programs and laboratories, the Risk Assessment Forum has since grown to involve more than 30 members, including specialists in the areas of exposure assessment and ecological risk assessment. The purpose of the Forum, however, has remained

essentially the same: to develop Agency-wide guidance to promote consistency in the application of risk assessment methodologies.

Dr. Wood noted that the 1983 NAS study identified four general elements of the risk assessment process:

- Hazard identification
- Dose-response assessment
- Exposure assessment
- Risk characterization

Although some of the newer guidelines involve slight modifications to this general scheme, the NAS paradigm has largely been preserved in all of the risk assessment guidelines issued to date, including the draft neurotoxicity guidelines.

Dr. Wood also pointed out that the NAS study made a clear distinction between risk assessment and risk management. Risk characterization represents the area of overlap in which the "hand-off" between risk assessment and risk management occurs. As such, much of the work that has taken place in guidelines development over the past several years has been directed toward improving our ability to describe or characterize risk.

The original set of risk assessment guidelines, issued by EPA in 1986, consisted of five guidelines. Three of these (carcinogenicity, mutagenicity, and developmental toxicity) were related to specific endpoints, while two others (exposure assessment and chemical mixtures) cut across multiple endpoints. Within days of their publication, recommendations for revisions to these guidelines began to be considered by working parties within the Risk Assessment Forum. Revised versions of some guidelines have subsequently been published (e.g., developmental toxicity and exposure assessment), while others are at various stages of review and revision (e.g., carcinogenicity and chemical mixtures). The point is that the guidelines development process is a dynamic one, in which revisions and updates to existing guidelines are constantly being considered. Similarly, efforts to develop new guidelines in other areas are ongoing. In addition to the draft neurotoxicity guidelines currently before the group, for example, Dr. Wood

indicated that efforts are also underway to develop risk assessment guidelines in the areas of reproductive effects, quantification of non-cancer effects, immunotoxicity, and ecological risk.

Dr. Wood noted that, in reviewing the proposed neurotoxicity guidelines, it would be important for workshop participants to keep in mind what the guidelines are not intended to be. The guidelines are not intended to serve as a step-by-step "cookbook" in risk assessment methodology, as a rigid and inflexible "rule book," or as a comprehensive "textbook" that reviews all of the relevant literature. Rather, the guidelines are intended to provide a framework that establishes the boundaries of acceptable scientific methodology, while allowing as much flexibility to risk assessors as the state of the science merits. In this way, the guidelines are intended to provide assistance in moving from a body of unanalyzed data toward a meaningful characterization of risk. As such, Dr. Wood requested that reviewers critique the draft guidelines mainly in the following terms:

- Does the document provide guidance on how to think about the types of information that are likely to be available to a risk assessor?
- Does the document provide guidance regarding judgments about the adequacy of available methodologies and the significance of the data various methods produce?
- Does the document provide guidance regarding the appropriate use of available data as the risk assessment process moves from hazard identification toward a more complete characterization of risk?
- What kind of guidance does the document provide for integrating available information into a meaningful characterization of risk?
- What kind of guidance, if any, does the document provide regarding risk communication between the risk assessor and a risk manager?

Dr. Wood pointed out that the primary audience for the final guidelines will be Agency scientists who conduct risk assessments or who review risk assessments submitted to EPA. Although ideally one would prefer to have neuroscientists performing this task, he noted that it is likely that the task will often fall to a team of generalists in toxicology. For this reason, it is important for the guidelines to lay out areas in which there is general scientific agreement about acceptable and/or preferred approaches, areas in which several different approaches may be

equally appropriate, and areas in which the science is simply not advanced enough for a particular type of information to be of predictive value at this time.

Dr. Wood noted that the scientific peer review represents a relatively early stage in the guidelines development process. Based on the peer review group's report, the draft guidelines will be revised before being submitted to internal reviews by the Risk Assessment Forum and the Risk Assessment Council, a senior management group that considers the implications of proposed risk assessment guidelines for program offices within the Agency. Following this internal review, there will be a 90-day period for public comment on the proposed guidelines, as well as a review by the Science Advisory Board. Another round of revisions will then take place, culminating approximately a year later with publication of final guidelines in the *Federal Register*. Altogether, Dr. Wood estimated that it will take another 2 to 3 years for final neurotoxicity guidelines to be published.

Following Dr. Wood's presentation, Dr. Greenlee reviewed the proposed agenda for the two-day peer review meeting. He noted that the workshop was designed to follow an iterative process, in which small-group discussion sessions would alternate with plenary sessions involving all participants. He said that the goal of this process is to assure adequate discussion of specific issues as well as to integrate these issues into more general guidance for the Agency in its continuing development of the proposed guidelines. To begin this process, panel chairs had been requested to provide an opening presentation summarizing the issues and concerns likely to be addressed during each panel's individual deliberations.

4.3 NEUROTOXICITY AS AN ENDPOINT (PANEL 1)

Dr. William Greenlee

Dr. Greenlee began his presentation by noting that the whole process of guidelines development is based on the general agreement that neurotoxicity is, in fact, an appropriate endpoint for environmental risk assessment. The challenge to the peer review workshop, therefore, is to identify ways of bringing the best available science to bear on the risk assessment process. More specifically, the group's task is to conduct a scientific peer review of the draft guidelines in order to provide guidance to the Agency in its continuing effort to develop a

scientifically-based risk assessment process for neurotoxicity. This guidance should include recommendations about how best to use the existing neurobiological knowledge base, as well as identifying areas of research that are likely to fill existing gaps in our ability to assess neurotoxic risks.

Dr. Greenlee noted that the overall purpose of risk assessment guidelines is to reduce the uncertainty in assessing the potential of a physical, chemical, or biological agent to produce an adverse response. He said that this is a particularly challenging task in the area of neurotoxicity, because the extreme complexity of the nervous system poses challenges beyond those typically confronting the toxicologist. Dr. Greenlee suggested that toxicology falls short of its goals when it does not keep pace with the current state of biological understanding. This failure may occur either because toxicologists attempt to extend their conclusions beyond the current state of biological understanding or because their conclusions do not fully incorporate all available data. One task of the workshop, therefore, would be to address both sides of this question by determining areas in which the proposed neurotoxicity guidelines might fall short of our current understanding of neurobiology as well as areas in which the proposed guidelines might extend beyond current neurobiological knowledge.

Dr. Greenlee noted that, in his view, a scientifically-based neurotoxicity risk assessment process is necessarily a biological mechanisms-based approach, couched within the exposure-dose-response paradigm. In this context, he speculated that three types of efforts might be especially likely to improve our understanding of risk assessment in this area:

- Physiologically-based pharmacokinetic studies, particularly those that would improve our ability to predict tissue doses based on exposure to an environmental agent
- Development of pharmacodynamic models, particularly those that would improve our ability to quantify biological processes at both the cellular and the whole-animal level
- Mechanistic studies, particularly those that would increase our understanding of the biological determinants of tissue- or species-specific actions of an environmental agent

Dr. Greenlee suggested that another important topic for the group to consider is the extent to which existing resources are or could be exploited in identifying the neurotoxic potential of an environmental agent. Although we recognize the importance of controlled epidemiologic studies, for example, he wondered whether we are taking full advantage of other types of human studies, such as pharmacologic studies that might offer important insights into the molecular determinants of toxicity. Similarly, while we recognize the importance of well-controlled animal toxicity studies, we may not be taking full advantage of the wealth of information that is available in the veterinary literature. He cautioned that the results of *in vitro* studies may have to be considered somewhat more rigorously, since *in vitro* systems are clearly more limited in their ability to reflect the complexities of the intact nervous system. *In vitro* studies can play an important role in exploring mechanisms of toxicity, but only when the relationship of the *in vitro* system under study to responses in the whole animal is reasonably well understood. The results of *in vitro* testing should not be over-interpreted.

Given the variety of approaches that can be taken to study nervous system function, Dr. Greenlee suggested that one goal of risk assessment is to incorporate data obtained from human, animal, and *in vitro* studies into an integrated understanding of the biological mechanisms of neurotoxicity. Effective integration of all available data, in turn, is most likely to occur when biologists, mathematicians, and risk assessors are able to work together throughout the risk assessment process. In this way, it might be possible to develop quantitative descriptions of the complex biological processes that are characteristic of the nervous system, including the potential for interactions of this system with environmental agents. Although it is important for the workshop to focus on specific issues of concern, Dr. Greenlee expressed the opinion that it would also be important for the group to keep in mind how its review of the proposed neurotoxicity guidelines could foster this larger, overarching goal. Part of his role as Chairman, he concluded, would be to assure that the group not lose sight of this aspect of its charge.

4.4 TRANSIENT AND PERSISTENT EFFECTS (PANEL 2)

Dr. John O'Donoghue, Eastman Kodak Company

Dr. O'Donoghue's presentation focused on participants' pre-meeting comments on the interpretation of transient and persistent effects of an environmental agent on the nervous system. Dr. O'Donoghue said that identifying areas of agreement and disagreement proved to be somewhat difficult, since there was a fair amount of imprecision and ambiguity in the way similar terms were used by different reviewers. Transient effects, for example, are not equivalent to reversible effects, although these terms were used interchangeably in some reviewers' comments, including his own. Likewise, use of the terms "persistent" and "irreversible" was sometimes confusing, as was use of the terms "effect" and "toxicity."

In an effort to clarify the distinctions among these terms, Dr. O'Donoghue proposed a series of definitions for consideration by the group:

- *Transient effects* are temporary, fleeting in time, or short-lived. These effects can usually be measured in minutes, hours, or at most a few days. Mechanistically, transient effects involve the direct exposure of the target system to a chemical; as a result, they generally persist only as long as the chemical is present in the body. *Persistent effects*, on the other hand, continue beyond the pharmacological life span of the causal agent, even if only for a short period of time. As an example, Dr. O'Donoghue described a temporary impairment of vision by a flash of light as a persistent effect, since it lasts beyond the period of the flash.

- *Reversible effects* are those that can be corrected or rectified by repair processes that enable the body to return to its original state. Although reversibility is usually thought of in both structural and functional terms, it is often difficult to determine that a return to the original state has occurred in both of these areas. *Irreversible effects* are effects that cannot be corrected or repaired. As a result, irreversible effects result in some permanent or long-lasting change in the structure or function of the nervous system.

- *Latent effects* are those effects appearing long after the last contact with the causal agent, usually after the agent is no longer present in the body. *Residual effects*, on the other hand, are a subtype of irreversible effects in which damage is incompletely repaired. These effects may be readily apparent or they may be silent, reappearing only at some later time when the organism is challenged. Structural damage that persists after clinical recovery has occurred is an example of a residual effect, since this type of damage could be expected to reduce the residual capacity of the organism to accommodate subsequent insults.

Dr. O'Donoghue suggested that recognizing the differences among these terms is critical if a consensus is to be reached about how each type of effect should be interpreted and dealt with in the risk assessment process.

There was a general consensus among participants regarding the importance of distinguishing among different levels or degrees of adversity in characterizing the effects of a chemical on the nervous system. In the area of pathology, for example, Dr. O'Donoghue noted that there are many different types of changes that could be observed, not all of which would be considered equally strong evidence of toxicity by most pathologists. Some pathologic changes, for instance, mimic artifactual responses so closely that it may be difficult to tell whether a pathologic change has actually occurred. Because of their ambiguity, these types of effects should be considered relatively weak evidence of toxicity. Quantitative changes in morphology or in the rate or extent of functional processes that normally occur in the organism, on the other hand, might be considered stronger evidence of toxicity. More significant still would be clearly pathologic events involving structural or functional changes that are never seen in a healthy animal. Similarly, in the area of behavior, important distinctions can be made among effects of a chemical that interfere with the animal's ability to survive, effects that perturb the animal's ability to interact with the environment, and behavioral effects that are not specific to the action of the chemical. Dr. O'Donoghue held that qualitative differences in the degree of adversity of various effects should be taken into account when neurotoxicity data are evaluated.

Another general area of concern raised by many participants was the difficulty inherent in attempting to determine whether a chemical is or is not a neurotoxicant, given that toxicity is so intimately related to the conditions of exposure to a chemical. One way of dealing with this problem is to incorporate assumptions about exposure into the classification system. For example, a substance could be classified as an occupational neurotoxicant if it produces adverse effects on the nervous system at exposure levels expected to occur in the workplace. By this definition, acrylamide and n-hexane would both be classified as occupational neurotoxicants. Environmental neurotoxicants, on the other hand, would be those substances that produce adverse effects on the nervous system at exposure levels occurring in the ambient environment. Lead and mercury would fit the definition of environmental neurotoxicants. Dr. O'Donoghue said that focusing on these types of classifications is important, since the guidelines are

ultimately intended to protect people from effects associated with occupational or environmental exposure to the substance under study.

In considering primary versus secondary effects of a chemical on the nervous system, Dr. O'Donoghue suggested that the group adopt the traditional toxicologic distinction between primary and secondary toxicants. Thus, a primary neurotoxicant would be a material such as 2,5-hexanedione that interacts directly with target sites in the nervous system. A secondary neurotoxicant, in contrast, would be a material such as n-hexane, which must be metabolized before it can interact with its nervous system target.

Dr. O'Donoghue then provided a summary of the extent to which pre-meeting comments addressed each of the issues before the Transient and Persistent Effects panel.

Issue #1: The workshop draft concludes that both reversible and irreversible effects of chemicals on the nervous system should be considered adverse.

There was little agreement regarding the extent to which all transient, persistent, and reversible effects of a chemical on the nervous system should be considered adverse. Most commenters agreed that some effects would be of greater concern than others, but opinions diverged about whether all types of effects should be considered adverse. Similarly, there was general agreement that all chemicals could be expected to impact the nervous system at some dose levels, but participants generally did not think that this observation was particularly useful. More important, most agreed, is the likelihood that a substance will produce transient or persistent effects at exposure levels expected to occur in an occupational or environmental setting. If an agent produces adverse effects on the nervous system only at exposure levels significantly higher than those expected to occur in these settings, participants did not agree that the agent should be classified as a neurotoxicant.

Issue #2: The nervous system contains billions of cells wired in complex patterns and is known to be resilient to environmental and toxicological insult by a process known as compensation or adaptation.

Dr. O'Donoghue indicated that this issue seemed to generate a fair amount of confusion, mostly because participants seemed to have some difficulty understanding the significance of the statement. At least some participants held that metabolic or behavioral adaptation should not necessarily be considered adverse, even if the adaptation is in some sense pathological. To resolve this issue, Dr. O'Donoghue suggested that the panel would probably want to try to address where the boundary between adverse and non-adverse forms of adaptation might lie.

Issue #3: Once damaged, nerve cells have limited capacity for regeneration.

There was agreement that once neurons are killed or central neural processes damaged, regeneration is very limited, if indeed it occurs at all. The capacity for repair is, however, much greater following transient effects or effects that do not involve structural damage to central neural processes. On the functional level, in fact, the capacity for repair often is substantial.

Issue #4: Apparent recovery actually represents activation of reserve capacity, decreasing potential adaptability.

There was general agreement that the reversibility of effects involving cell death or destruction may represent an activation of reserve capacity which in turn could decrease future adaptability. There was not, however, a consensus that reserve capacity is diminished by neurotoxicants acting by other mechanisms, such as those producing strictly neurochemical or functional changes.

Issue #5: Traditionally, effects of toxicants are considered to be persistent or long-lasting, while pharmacological effects are considered to be transient or short-acting.

Participants agreed that transient effects occurring at high exposure levels are not necessarily indicative of environmental neurotoxicity. The group also agreed that transient, short-acting, or pharmacologic effects should not be put in the same category as permanent or irreversible effects. In general, commenters recognized a need for more than two categories of classification — i.e., neurotoxicant or non-neurotoxicant. To adequately address all available

information, most participants felt that there would need to be a broader range of possible classifications.

Issue #6: An effect that appears to be transient in an unchallenged organism may be revealed as long-lasting through an environmental or pharmacological challenge.

Issue #7: It is not known whether transient effects observed following developmental exposure should be evaluated at specific points in the life span.

Most workshop participants did not address either of these issues in their pre-meeting comments, so it was not possible to get a sense of the group's thinking about these topics.

Following Dr. O'Donoghue's presentation, there was discussion among workshop participants of several of the issues he raised. One participant suggested that another term that could be added to Dr. O'Donoghue's list of definitions is *progressive effects*, which might be defined as effects that continue to evolve even in the absence of continued exposure to a toxicant. An example of a toxicant producing this type of effect is carbon monoxide, which may continue to produce progressive deterioration in the nervous system, despite an apparent recovery of function after an acute exposure. Progressive deterioration can also occur late in life, when the results of prior exposure to a chemical combine with age-related heuronal attrition to produce an accelerated or progressive loss of function.

The same participant also noted that, in addition to addressing the need to specify whether an effect is primary or secondary, some people had also brought up the need for distinctions between direct and indirect effects. The examples that Dr. O'Donoghue had provided for primary and secondary neurotoxicants were both direct-acting agents, as opposed to agents that exert their effects on the nervous system indirectly — for example, via effects on energy metabolism. The commenter wondered whether it might be useful to consider primary and secondary effects as subcategories of both direct and indirect effects of a chemical on the nervous system.

Third, this participant suggested that it might be useful for the group to think about classifying some substances as neuroactive agents, as distinct from neurotoxicants.

Dr. O'Donoghue indicated that he thought all of these issues would be discussed during the workgroup's deliberations. Noting that a separate panel had been established to consider the question of direct and indirect effects, he predicted that there would be a fair amount of overlap among the issues to be addressed by the various workgroups. He suggested that it would be difficult to divorce transient and persistent effects from direct and indirect effects, for example.

Also important in the consideration of direct and indirect effects is the issue of identifying neurological signs and symptoms that may be incidental to processes occurring in the rest of the body. Citing a passage from an 1897 text on poultry pathology, Dr. O'Donoghue pointed out that it has long been recognized that there are a number of characteristic behaviors that animals exhibit when they are sick, regardless of the nature of the illness, and that these types of effects need to be distinguished from either direct or indirect effects of a chemical on the nervous system. One participant agreed, noting that the definition of neurotoxicity in the draft guidelines might be overly broad. This participant suggested that more categories or levels of concern would probably be necessary to reflect important differences between a chemical that has a short-term, transient effect on nervous system function and one that causes widespread structural damage within the brain. Dr. O'Donoghue wondered whether part of the problem might be the draft guidelines' overwhelming emphasis on hazard identification as opposed to other elements of the risk assessment process, such as dose-response relationships, exposure assessment, and risk characterization.

Another participant suggested that the guidelines might need to be broadened to provide guidance to individuals designing and performing neurotoxicity studies as well as those interpreting the results of these studies. It might be possible to describe a more complete spectrum of possible approaches and possible results in neurotoxicity testing, if in the process it is specified how different types of findings might contribute to an overall assessment of a substance's neurotoxic potential. Dr. O'Donoghue agreed that there might be some merit to such an approach, but wondered whether this type of discussion would be appropriate, given

that the group's assigned task was to review the scientific underpinnings of the existing draft of the risk assessment guidelines.

4.5 DIRECT AND INDIRECT EFFECTS (PANEL 3)

Dr. Barry Wilson, University of California (Davis)

Dr. Wilson began his presentation by summarizing the proposals and statements laid out for consideration by the Direct and Indirect Effects panel. These statements included a proposal that agents acting through indirect as well as direct means could be considered neurotoxic, that direct and indirect effects of a chemical are equivalent to primary and secondary effects, and that effects on the endpoint of neurotoxicity are "functionally equivalent," whether they arise from direct or indirect effects of a chemical on the nervous system. In addition, Dr. Wilson noted that the panel had been asked to concentrate on two areas of special focus: a caveat that information available to risk assessors might not be adequate to distinguish primary from secondary actions of a chemical, and a statement that hepatic toxicity, which could secondarily damage the nervous system, might or might not represent a neurotoxic effect. Finally, Dr. Wilson indicated that the panel had been asked to consider a syllogism in which the logical inconsistency of claiming that a compound can produce neurotoxicity but not be a neurotoxicant is linked to the conclusion that any compound, at a high enough dose, must be a considered a neurotoxicant because of its potential to produce lethal effects.

Participants' pre-meeting comments were in general agreement regarding the need to classify as neurotoxicants those chemicals that directly affect the nervous system. There were, however, a number of conditions that various participants thought should be added into the equation. Most people agreed, for example, that in order to qualify as evidence of neurotoxicity, an effect should occur at low doses of a chemical and should exhibit a dose-response relationship. Similarly, most participants thought that the observed effect should fit a restrictive definition of adversity, although there was a wide range of opinions regarding how restrictive this definition should be. Dr. Wilson pointed out that the definition of adversity proposed in the draft guidelines is any alteration in the structure or function of the central nervous system and/or peripheral nervous system that diminishes the ability to survive, reproduce, or adapt to the environment. Noting that some participants would not consider this

definition restrictive enough, Dr. Wilson suggested that coming up with an acceptable definition of adversity might be one of the most important tasks before the workshop as a whole, since this definition is in a sense where the process of risk assessment begins.

Participants were not in agreement regarding the extent to which chemicals with indirect or secondary effects on the nervous system should be regarded as neurotoxicants. Similarly, no consensus was apparent on the question of whether any chemical-induced change in the structure or function of the nervous system should be considered an adverse effect, regardless of the nature of the change.

In order to sort out these differences, Dr. Wilson speculated that the Direct and Indirect Effects panel would need to address a number of important issues. For one thing, it would probably be important to try to come up with more precise definitions for many of the non-trivial terms used in the guidelines, including "adverse effect" as well as terms such as "direct" and "indirect" or "primary" and "secondary." In this respect, the panel's efforts would complement some aspects of the work that Dr. O'Donoghue had proposed for the Transient and Persistent Effects panel.

Second, Dr. Wilson said that the panel would probably want to consider the dose level at which an assessment of risk should begin. Given the important role of the nervous system in modulating and integrating homeostatic responses, he suggested that it is not immediately obvious whether risk assessment should begin at the dose level where an effect is first detected or some higher dose level where the observed effect exceeds some physiologic limit or limits. In this sense, he suggested that there might be a fair amount of room for a chemical to produce effects on the nervous system that do not represent damage.

Finally, based on a general sense of participants' concerns about the definition in the draft guidelines, Dr. Wilson proposed an operational definition of neurotoxicity in which a chemical would be classified as a neurotoxicant if it had its major action on the nervous system, whether via primary or secondary mechanisms, such that at low dose levels it produces effects that injure the short- and long-term health of the organism. While acknowledging that this definition might not address all of the participants' reservations about the existing definition of

adversity, Dr. Wilson suggested that it might provide a reasonable starting point for further discussion.

Following Dr. Wilson's presentation, other workshop participants offered their views on issues to be considered by the Direct and Indirect Effects panel. One participant suggested that the difficulty people were having with definitions used in the draft guidelines might have to do with the fact that the focus was on trying to label compounds rather than describing observed effects. It is more important that exposure to a toxic compound be limited than whether the compound is classified as a neurotoxicant or a hepatotoxicant. In this sense, he said, it is the effect rather than the underlying mechanism that should form the basis for regulation of a chemical. Dr. Wilson agreed that it is important to keep the focus of the discussion on the ultimate goals of the process, but noted that there is a great potential for misunderstanding if one group of people uses the term "indirect" to refer to substances that must be metabolically activated while another group uses the same term to refer to substances that act mainly on non-neural systems.

Another participant asked representatives from EPA to clarify the extent to which the guidelines were or were not intended for the protection of species other than humans, and the extent to which the neurotoxic potential of biological agents should be taken into account. An EPA representative indicated that the guidelines are intended to address risks to humans. Although attention in this area has traditionally focused on chemical and physical agents, it is increasingly the case that the Agency is being asked to evaluate the risks associated with biological agents.

The same participant also asked for clarification regarding the breadth of the Agency's definition of neurotoxicity. He wondered, for example, whether the neuroendocrine system should be considered part of the nervous system. Similarly, he wondered whether agents that act principally on muscle should be considered potential neurotoxicants, since many agents that act in this way are known to produce behavioral and neurologic effects in humans. Dr. Wilson echoed this concern, noting that damage to target organs of the nervous system often results in damage to the nerve or nerves innervating that organ.

An EPA representative responded that the Agency had not directly considered effects on muscle or on the neuroendocrine system, and would leave it to the expert reviewers to recommend whether these types of effects should be incorporated into the definition of neurotoxicity. The participant who had raised the question indicated that he thought that effects on the neuroendocrine system and on muscle should be included within the definition of neurotoxicity. Dr. Wilson wondered whether there might not be a property, such as excitability, that would link all of the various systems that had been being discussed. Another participant argued that it did not seem productive to attempt to look at the nervous system in a vacuum. This person suggested that the point should be to identify the lowest-dose effect of a chemical and regulate it on that basis. It doesn't matter whether the basis of the regulation is one effect or the other, so much as it matters that exposure to the toxicant be limited. It is important to keep in mind that neurotoxicity will not be the only basis upon which exposure to a chemical can be or should be regulated.

Dr. Greenlee said that this issue will need to be addressed by one or more of the individual workgroups, probably in the context of how specific endpoints do or do not reflect complex interrelationships between the nervous system and other organ systems. Another participant observed that this problem is not unique to the nervous system. Noting that in a biological system everything is related to everything else, this commenter pointed out that it is equally difficult to determine, for example, whether hypertension should be considered a disease of the cardiovascular system or the central nervous system. In this person's view, the problem may be that the draft guidelines are attempting to fit too many different types of effects into the definition of neurotoxicity.

Another participant suggested that the whole process of risk assessment and regulation could be thought of as a diagnostic exercise, in the sense that it is sometimes possible to treat a disease specifically and sometimes possible only to treat it symptomatically. The parallel to specific treatment is that it will sometimes be possible to regulate a chemical because its role in producing signs or symptoms of neurotoxicity is well understood. In other cases, however, it may be necessary to make a judgment about which "symptoms" are important enough to trigger regulation of the chemical, even though the mechanism by which the chemical causes those symptoms may not be well understood. Dr. Wilson expressed some reservations about this

analogy, noting that it probably would not be desirable to have one way of handling risk assessment for compounds with known mechanisms of action and another way for compounds that are not as well studied.

4.6 ANIMAL-HUMAN EXTRAPOLATION (PANEL 4)

Dr. Shayne Gad, Becton Dickinson Research Center

Dr. Gad began his presentation by noting that the Animal-Human Extrapolation panel had been asked to consider the conclusion that, if an animal data base exists and is adequate according to the definition provided in the guidelines, it is possible to make judgments about the potential toxicity of an agent for which no or inadequate human neurotoxicity data exists. To set up the discussion of this and other issues expected to arise during the workgroup's deliberations, Dr. Gad provided a summary of pre-meeting comments related to each of the conclusions and suppositions that had been used by the authors of the guidelines to reach this general conclusion.

Statement #1: Substances producing neurotoxicity in humans also result in neurotoxicity in other species.

The importance of this statement is that in most cases the overwhelming majority of toxicity data available in assessing a chemical's potential toxicity will have been gathered in animals. Because hazards are almost always identified first in animals, the relevance of animal data in assessing a chemical's potential toxicity to humans is a question of pivotal importance. Dr. Gad noted that participants were divided in their opinions about the validity of this statement. Although no one disagreed that animal data could be used to identify neurotoxic agents, there was a considerable disagreement regarding appropriate ways of addressing differences between animals and humans that might limit the direct applicability of animal data to an assessment of a chemical's potential risk to humans. Concerns about differences in routes of exposure, metabolism, and the time scale of effects were prominent in this regard.

Statement #2: Compared with human studies, animal studies are more often available and provide more precise dose information and better control for environmental factors.

Dr. Gad reported that there was broad, substantial agreement with this statement among workshop participants. Virtually all participants recognized animal models as a primary tool for hazard identification in neurotoxicology as in other areas of toxicology. The problem, however, is that limitations of individual animal models may sometimes make it difficult to distinguish neurologic from other target organ effects. This may be especially difficult in the sense that risk assessors are typically asked to assess toxicity on the basis of data that were generated by someone else. Although it is true that precise control of dosing is usually possible in animal studies, the trade-off is that our understanding of pharmacokinetic or metabolic differences between the test animal and humans may be rudimentary, at best. These gaps in our understanding, in turn, sometimes make it very difficult to determine whether an observed effect is a nervous system effect or an effect on some other system.

Statement #3: Many diagnostic procedures employed to evaluate neurotoxicity in humans have corresponding animal models.

There was some divergence of opinion among participants regarding the validity of using the term "many" or "most" in this statement. Participants generally agreed, for example, that diagnostic procedures requiring cognitive interactions with a patient do not have corresponding animal models. Another issue that arose in connection with this statement was the caution that laboratory animals are not and should not be considered "little humans." The fact that many human diagnostic tools have corresponding animal models does not mean, for example, that it is possible to use most of these tools in any one animal model. It might be easiest to assess a compound's effects on cognitive function by testing it in pigeons, for example; it would be difficult to assess the compound's more general toxicity in this system, however, since clinical chemistries and many of the other parameters one might wish to consider are not as well characterized in pigeons as they are in rats and mice.

Statement #4: The range of uncertainty factors used to extrapolate animal data to human risks for other endpoints of toxicity are applicable for neurotoxicity risk assessment.

While noting that participants were in substantial agreement with this statement, Dr. Gad indicated that there also was a strong sentiment that efforts should continue to improve

precision in this area. In general, participants agreed that the more we know about the biological basis of an observed effect, the more likely we are to understand the relevance of the effect across species. With this increased understanding, in turn, our assessments of risk are likely to become significantly more precise and, as a result, more effective in achieving the goal of appropriate regulation.

Dr. Gad then turned to the three areas of special focus assigned to the Animal-Human Extrapolation panel. This task was more difficult, he said, since he was not sure that the areas of focus identified in the Issues Paper were in fact the areas that he would consider key to the panel's deliberations. Nevertheless, he had attempted to summarize participants' views on the areas of special focus identified by the authors of the draft guidelines.

Focus Area #1: The full range of human behaviors, for example language, is not present in other species.

This statement raised again the issue of cognitive endpoints, which most participants agreed did not have corresponding animal models — at least not in any one non-human species. On the other hand, participants agreed that it is possible to model most human behaviors by looking broadly across a variety of animal models. For this reason, and because animal data are generally what we have to work with, it was not clear to some other participants what the significance of this statement might be.

Focus Area #2: Factors such as differences in metabolism can result in differences among species in sensitivity to a compound.

Participants were generally in agreement with this point, although Dr. Gad cautioned that the statement should be read very carefully. It should not be interpreted to mean that metabolism is the only or even the most important difference between species. He emphasized that it is also important to recognize the many other factors that can lead to differences in sensitivity to a chemical among different species as well as among individuals of the same species, including differences in age, nutritional status, gender, race, health status, and other characteristics.

Focus Area #3: The most sensitive species may not be the species most like the human.

It is generally accepted that, in the absence of other information, the species that is phylogenetically closest to humans should be expected to predict human responses best. This assumption may be in conflict with the approach to risk assessment in which the lowest observed effect level is modified by application of a safety factor to determine acceptable levels of human exposure to a chemical. Humans are not necessarily the most sensitive species, and the species that best predicts human responses may not be the most sensitive species. This is another area in which our ability to assess the relevance of animal data is directly related to our understanding of the biology underlying an observed effect. Dr. Gad emphasized that only by understanding why an effect occurs in an animal can we truly understand whether and how the effect might be relevant to humans. We should also be clear about which segment of the population we are talking about in assessing the relevance of animal data, given the broad range of sensitivities to a chemical that can be expected to occur in the human population.

In addition to the issues and areas of focus raised by the authors of the guidelines, Dr. Gad also brought up other issues that the Animal-Human Extrapolation panel might address. First, Dr. Gad suggested that it might be important for the guidelines to offer some guidance regarding the selection of animal models. Identifying species that are most likely to provide useful data might be one way of doing this. Although model selection is an important concern in any area of toxicology, Dr. Gad suggested that it is particularly important in neurotoxicology, which attempts to examine very complicated systems and behaviors. In his view, even the statement that a broad range of animal models may be needed to assess certain types of nervous system effects might be a useful addition to the draft guidelines.

Second, Dr. Gad raised a concern about the treatment of *in vitro* models in the draft guidelines, a topic that a number of other participants had also addressed in their pre-meeting comments. As currently written, some people thought that the draft guidelines were very confusing in their treatment of *in vitro* systems, suggesting both that the results of *in vitro* tests are of little predictive value and that these results could be suggestive of neurotoxicity. Workshop participants seemed to be divided in their opinions about the relevance and utility of

in vitro models per se, but most seemed to agree that this type of discussion added little of scientific value to the draft guidelines.

Following Dr. Gad's presentation, workshop participants engaged in a discussion of issues related to topics before the Animal-Human Extrapolation panel. The Chair asked whether there might be prototype animal models that could be recommended for use in studying the neurotoxic potential of specific classes of chemicals. Dr. Gad responded that there are good models for several classes of known toxicants; however, in the larger sphere of hazard identification, one is usually dealing with classes of chemicals or biological agents about which much less is known. In commenting on Dr. Gad's observation that humans are not always the most sensitive species, the Chair noted that much potentially useful information can be lost if the focus is exclusively on finding the most sensitive animal model or, in fact, on finding a response to a chemical. In many cases, asking why a given species does not exhibit a particular response can also be an important way of investigating mechanisms of chemical toxicity.

Another participant recommended that the Animal-Human Extrapolation panel spend some time defining more clearly those areas in which animal models of human behavior are and are not thought to exist. From a behavioral perspective, for example, language is a form of social behavior, and animal models for social behavior do exist. This participant suggested that considering language in terms of the class of behavior in which it falls would probably be more useful than focusing on aspects of language that are related mainly to the characteristics of human vocal cords. Another participant suggested that the symbolic representation of events and objects in verbal behavior may be peculiar to humans, but the first participant thought that even this type of behavior had been shown to occur in non-human primates.

Another workshop participant expressed the view that it would also be important for the Animal-Human Extrapolation panel to consider in more detail the advantages and limitations of *in vitro* tests, both as a screening tool and as a way of investigating mechanisms of toxicity. This person predicted that more and more *in vitro* data will be coming in to regulators over the next few years, and risk assessors will probably need guidance on how to use this information. Dr. Gad agreed, noting that *in vitro* models are widely used in the pharmaceutical industry and elsewhere to try to understand what worked and what didn't. He said that the problem is

slightly more complicated with respect to *in vitro* tests of neurotoxicity, however, since most of the systems proposed as *in vitro* models of neurotoxicity are actually models of more general cytotoxicity.

Another participant pointed out that there is at least one tissue culture system that reproduces the anatomy, ultrastructure, physiology, and pharmacology of the nervous system well enough to reproduce *in vitro* the precise pattern of changes that one sees in humans and animals exposed to a neurotoxicant. This system has been used for more than 30 years, both as a screening device and as a tool for investigating mechanisms of neurotoxicity. A third participant noted that he, too, has been working with tissue culture systems throughout his career. In this person's view, *in vitro* systems are particularly useful in studying direct actions of a toxicant on a living system, independent of metabolic transformations and other detoxification mechanisms that might come into play in the whole animal. Then, by working backward, it is often possible to gain some insight into why a particular effect that was observed in culture did or did not take place *in vivo*. Dr. Gad pointed out that the regulator will be asked to make judgments on the basis of the available data and will usually not have the luxury of going back to ask additional questions of the *in vitro* system.

4.7 BEHAVIOR (PANEL 5)

Dr. John Orr, Southwest Research Institute

To begin his presentation, Dr. Orr observed that the Behavior panel was the only panel charged to look at an endpoint that is unique to neurotoxicity. In this sense, he said, the Behavior panel will be considering an endpoint that is in some sense the fodder from which other panels will start their deliberation.

Noting that the definition of adverse effects had been near the top of the other panel chairs' lists of issues, Dr. Orr indicated that this definition is one that the Behavior panel would also need to consider carefully. A discussion of how various endpoints relate to the notion of adversity could easily fill the whole of the panel's allotted time. In this regard, he predicted that the sensitivity and specificity of behavioral endpoints might be a particularly important area for discussion during the Behavior panel's deliberations.

While noting that most participants' pre-meeting comments focused on topics other than behavior, Dr. Orr indicated that a few issues of central importance to the Behavior panel had been raised. One such issue was the question of how to evaluate the whole body of behavioral data that might be available for a chemical under study. Particularly important in this regard is the relative weighting of different types of information that comprise the "input" side of the risk assessment process. How this hypothetical weighting should be accomplished — for example, whether data should be evaluated in terms of clusters or functional domains of behavior — would probably be an important topic for the Behavior panel to consider.

Echoing earlier concerns about the difficulty of determining whether a substance is or is not a neurotoxicant, Dr. Orr concluded his presentation by suggesting a model that he thought could be used to address this dichotomy. In this model, there would be one threshold above which a chemical would be classified as a neurotoxicant and an opposing threshold below which one could be relatively confident that a chemical would not exhibit significant neurotoxic effects. Beginning at a point somewhere between these two extremes, each piece of data could be evaluated in terms of the size of the step it would warrant toward one or the other threshold. A change in a single endpoint out of a functional observation battery, for example, might move the overall assessment only slightly closer to the threshold for neurotoxicity. A finding of frank neuropathology, on the other hand, would merit a much larger step, in most cases one that would push the assessment over the toxicity threshold. Using this model, some lines of evidence might point toward a finding of neurotoxicity, whereas others might lead to a conclusion that no significant risk of neurotoxicity exists for a particular chemical under specified exposure conditions. While acknowledging that he had not worked out the details of this model, Dr. Orr suggested that it might provide a useful basis from which the panel's discussion of behavioral endpoints could proceed.

Following Dr. Orr's presentation, participants discussed issues related to topics that the Behavior panel would address. One participant asked whether and to what extent epidemiologic approaches to risk assessment have begun to supplant the types of animal behavior studies that the group had thus far been discussing. Another participant observed that the guidelines as written place a great deal of emphasis on epidemiologic evidence of neurotoxicity. In this person's view, in fact, the guidelines place too much emphasis on this type of evidence, given

that epidemiology as a science cannot prove that a causal relationship exists between a chemical and its effects. He suggested that much more valuable information could be obtained from reports of side effects in the therapeutic drug literature, for example, since these studies usually relate adverse effects to exposure to a specified dose of an agent for a known period of time. Uncontrolled human studies can also be very useful; evidence of the neurotoxic potential of methyl phenyl tetrahydropyridine (MPTP), for example, initially came from clinical neurology studies performed at two centers. For hazard identification purposes, this person argued that it might be useful to provide a weighting system for the various types of studies that might have been performed to assess the toxicity of a chemical. If such a system were adopted, he recommended that the greatest weight be given to controlled human studies and controlled animal studies, and the lowest weight be given to tissue culture studies and uncontrolled epidemiologic observations.

Another participant asked for guidance from EPA representatives regarding the intended outcome of the hazard identification process in the area of neurotoxicity. Looking at Table 7A, for example, it seemed that the guidelines were moving toward a multi-category approach similar to that used to classify carcinogens, whereas earlier in the document it had seemed that calculation of a reference dose would be the ultimate goal of the risk assessment process. The Chair agreed that this is an important question, noting that hazard classification schemes had been proposed by a number of workshop participants as a way of getting around the difficulty of stating unequivocally that a particular agent is or is not a neurotoxicant. An EPA representative indicated that it had been the intention of the authors to avoid a classification system like that used for carcinogens, because of the many problems that system has historically engendered. Rather, like proposed guidelines for other non-cancer endpoints, the neurotoxicity guidelines were premised on an assumption that available data would usually be adequate to support calculation of a meaningful reference dose. Although it is appropriate for the group to consider whether this assumption is warranted, it would probably be more useful for the Agency for the workshop to focus on ways of evaluating the value of specific types of data rather than attempting to rank the relative usefulness of different types of data. This distinction is important, since the task of the risk assessor will be to evaluate available data, rather than to determine whether other types of data might be more useful.

Another workshop participant suggested that part of the problem might be that there are many different types of data that might go into the risk assessment process, and the process might produce many different outcomes. The real goal is to find a way of expressing confidence both in the quality of the data going in and in the quality of the judgment coming out of the risk assessment process. The point should not be whether the data are sufficient or insufficient, adequate or inadequate in supporting a particular conclusion — i.e., that a substance is or is not a neurotoxicant — but rather to clearly identify the level of confidence we have in whatever conclusions we can draw from the data. A substance that is not problematic under one set of conditions may become problematic if the conditions change. This participant maintained that attempts to justify a black-and-white conclusion that a substance is or is not neurotoxic are likely to obscure this very important truth.

Another participant suggested that an important part of this debate is who the guidelines are intended to assist. If, as Dr. Wood had suggested, many non-neuroscientists will be using the guidelines, this person thought it very important to provide more specific guidance about how to interpret different types of data. When an effect becomes adverse, when it becomes neurotoxic, and how either adversity or neurotoxicity relate to exposure scenarios are all questions that need to be addressed in more detail if non-neuroscientists are the main audience for these guidelines.

At the conclusion of the panel's discussion, the Chair recognized an observer who had requested the opportunity to comment on the draft guidelines. This individual recommended that the labeling of any substance as a neurotoxicant be approached very judiciously, since this label is likely to be taken very seriously by the general public. In this regard, the observer recommended that the panel confine its discussion to effects of a chemical on nervous system endpoints, which she distinguished from indirect effects of a chemical on behavior or other more functional endpoints. She maintained that subchronic and chronic general toxicity studies should be adequate to protect endpoints involving secondary or indirect effects of a chemical on the nervous system. She predicted that an overly broad definition of neurotoxicity will produce a climate in which everything is seen as potentially neurotoxic. If this occurs, industry will be less likely to explore the interaction of a chemical with the nervous system, since even a small effect would cause the compound to be classified as a neurotoxicant.

After thanking the observer for her comments, the Chair reminded participants that the main purpose of the workshop is to provide a scientific peer review of the draft neurotoxicity guidelines. Toward this end, he suggested that a number of important issues had been raised during the morning's discussion that could be addressed in more detail in each panel's individual deliberations. At the same time, it would be important for the group to maintain a sense of its overall mission, particularly during the plenary sessions that would serve to summarize the conclusions and recommendations emerging from each panel's efforts. Following several procedural questions from workshop participants, he announced the time and place for each of the afternoon panel meetings, and the Opening Plenary Session was adjourned.

5. WORKGROUP REPORTS

5.1 TRANSIENT AND PERSISTENT EFFECTS PANEL

John L. O'Donoghue, V.M.D., Ph.D., Workgroup Chair

The Workgroup met on June 2 to discuss the Draft Guidelines, focusing mainly on issues related to transient and persistent effects of substances that act on the nervous system. On the following day, the Direct and Indirect Workgroup met and continued the discussion of topics relating to both areas.

Overall, it was apparent that the Agency had done a considerable amount of work in putting the Draft together and had carefully considered the scientific issues relevant to establishing risk assessment guidelines for neurotoxicity. The Workgroup was in agreement that neurotoxicity is an important endpoint for health effects evaluation and that development of risk assessment guidelines is appropriate.

The Workgroup was of the opinion that some of the terminology used in the Draft could be clarified by the addition of a lexicon of terms. This lexicon might reduce or eliminate the ambiguity sometimes encountered in discussions about neurotoxicity risk assessment. The Workgroup has provided some definitions for the Agency's consideration in Table 1.

The Workgroup was concerned that the purpose of the guidelines and how they will be used were not explicitly stated in the Draft. Discussions about neurotoxicity frequently involve issues requiring expert judgment. The Workgroup recommends that the Draft indicate that experts in the field of neurotoxicology should be involved in making decisions about the potential neurotoxicity of agents. If the guidelines are to be used by individuals who are not expert in the relevant neurosciences, the Draft should recommend that the risk assessor consult with an expert when dealing with situations that become ambiguous.

The Workgroup recognized that while the Draft followed the basic NRC approach to risk assessment, it focused almost exclusively on the Hazard Identification Step (Section 3, 38 pages) and provided less guidance on Dose Response Assessment (Section 4, 4 pages), Exposure

Table 1. Terms Used to Describe Neurotoxicants or Their Effects

Transient effects are temporary, fleeting in time, or short-lived. Their existence is measured in minutes, hours, or perhaps a few days. Their duration is frequently related to the pharmacokinetics of the causal agent and its presence in the body.

Persistent effects continue for a period of time which exceeds the pharmacological life span of the causal agent.

Reversible effects are those that can be corrected, allowing the organism to return to its original state.

Irreversible effects are those that cannot be corrected, resulting in a permanent change in the organism.

Latent effects are those that occur at a time distant from the last contact with the causal agent.

Progressive effects are those that continue to worsen even after the causal agent has been removed.

Residual effects are those that persist beyond a recovery period. These effects may range from obvious functional or structural deficits to subtle changes that may become evident only at a later stage of life or when the individual is further challenged.

Occupational neurotoxicants are those agents that produce adverse effects on the nervous system under conditions of exposure which occur in the workplace.

Environmental neurotoxicants are those agents that produce adverse effects on the nervous system under conditions of exposure which occur in the ambient environment.

Primary neurotoxicants are those agents that do not require metabolism prior to interacting with their target sites in the nervous system.

Secondary neurotoxicants are those agents that require metabolism prior to interacting with their target sites in the nervous system.

Direct neurotoxicants are those agents or their metabolites that produce their effects primarily by interacting directly with target sites in the nervous system.

Indirect neurotoxicants are those agents or their metabolites that produce their effects primarily by interacting with target sites outside of the nervous system. This interaction then secondarily results in damage to the nervous system. Indirect effects should be differentiated from remote effects on the nervous system, which occur when the effects of a chemical on tissues outside of the nervous system have their principal expression through the nervous system.

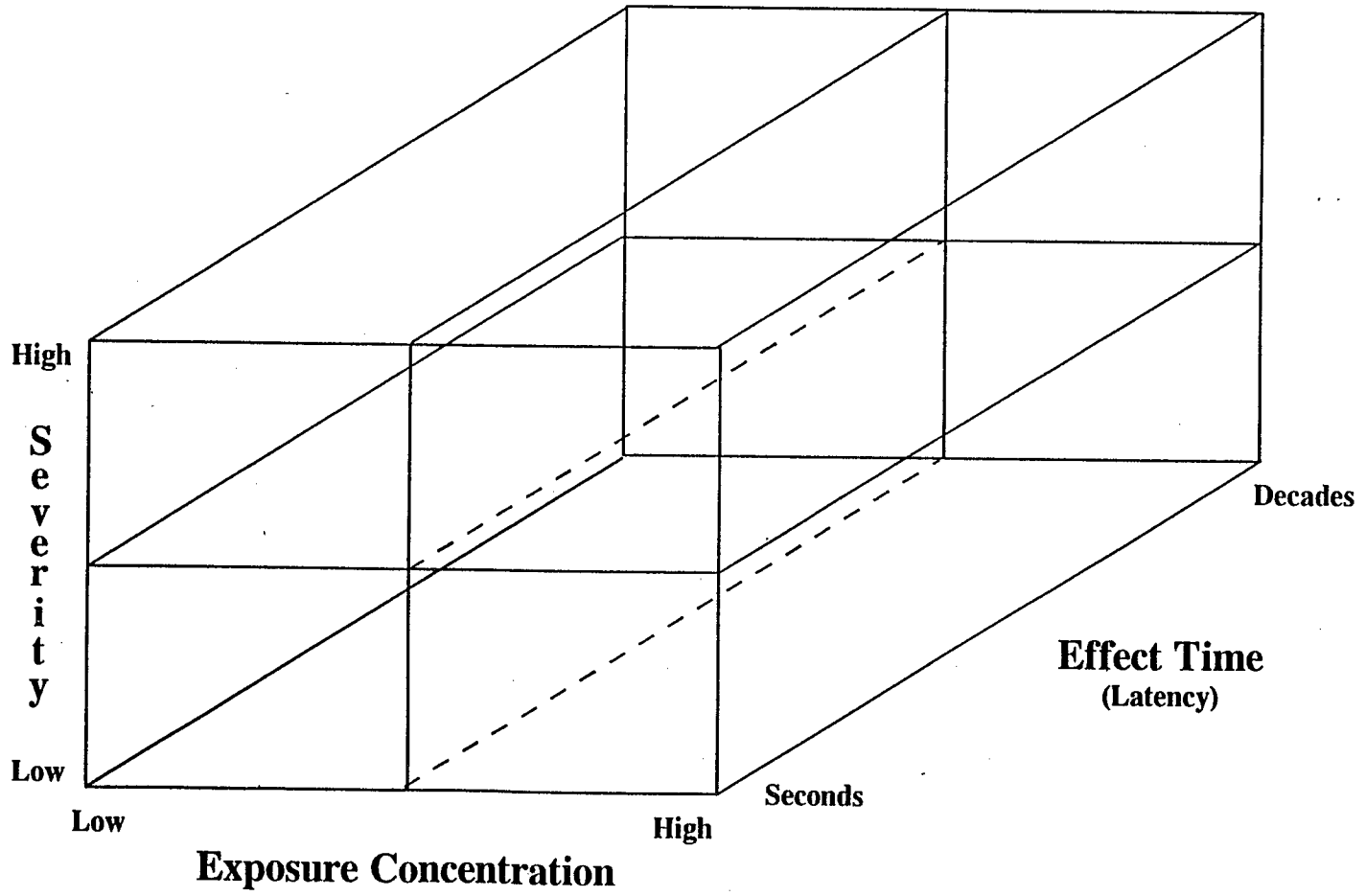
Assessment (Section 6, 2 pages), and Risk Characterization (Section 7, 2 pages). Expansion of the Draft to include more guidance in these other areas should be seriously considered.

The Workgroup was concerned about Section 5 of the guidelines, which considers the adequacy of available information for hazard assessment and dose-response assessment. The categorization scheme presented in the Draft was seen as inappropriately two-dimensional, allowing only a yes or no answer (neurotoxic or not neurotoxic). The particular concern was that, while there are some agents that are clearly neurotoxic, there are also many agents that produce some perturbation in the function or structure of the nervous system that should not be considered neurotoxicants. The range of classifications needs to be enlarged to reflect the fact that there may be adverse functional consequences of exposure to an agent that should be dealt with by the Agency outside of these guidelines, and that there may be some functional consequences of exposure to an agent that should not be considered adverse at all. Also, the category of agents for which there is sufficient evidence to conclude that they are <u>not</u> neurotoxic should be more explicitly identified.

The group discussed the importance of exposure level in determining whether an effect is neurotoxic. For example, one participant pointed out that he would be concerned about a work environment that contained 100 ppm of n-hexane for a year, but he would be less concerned about a work environment that contained 1000 ppm for a brief period (minutes). The Draft should discuss the importance of distinguishing between effects seen at high and low exposure levels. There should also be some discussion of the need for caution in trying to extrapolate effects seen at high exposure levels to real world exposure scenarios. The opinion was voiced that, rather than considering chemicals <u>neurotoxic</u>, we should consider chemicals to have the <u>potential</u> for neurotoxicity at certain dose levels and under certain exposure conditions.

The Workgroup discussed at length the time course of effects associated with exposure to environmental agents. The group concluded that, in determining whether or not the consequences of an exposure constitute neurotoxicity, the first consideration is whether or not the effect is adverse in a dose-dependent manner. The group agreed that consideration of an observed effect should include a multifactorial analysis of features that contribute to adversity (Figure 1). The group decided that the severity of an effect, the length of the exposure

Figure 1
Factors Involved in Determining Levels of Adversity



necessary to produce an effect, and, importantly, the exposure concentration all need to be considered in determining whether an effect should be considered adverse.

Based on its discussion of degrees of adversity, the Workgroup developed levels of concern for classifying effects observed in health effects studies. The highest level of concern was based on two criteria: the effect must be irreversible; and the effect must involve a clear or demonstrable change in either the structure or function of the nervous system at some time during the life span of the species under consideration. The second level of concern included effects that are slowly or incompletely reversible and that clearly or demonstrably impair the structure or function of the nervous system at some time during the life span. The third level of concern included effects that are rapidly reversible and that clearly or demonstrably impair the structure or function of the nervous system at some time during the life span. Structural effects were defined as morphological changes occurring at any level of nervous system organization. Functional changes were defined to include both electrophysiological and behavioral effects.

While the group agreed that only an irreversible "change" had to be demonstrated at the highest level of concern, the majority of the group thought that some "impairment" of structure or function should be demonstrated at the lower levels of concern. There was a minority opinion that considered any "change" in structure or function, particularly if non-volitional, sufficient for an effect to be considered adverse at the lower levels of concern.

The group recommended that dose-response relationships and exposure conditions be evaluated before deciding whether the evidence is sufficient to consider an agent a neurotoxicant.

Based on these considerations, the group decided that reversible effects could be considered adverse if they clearly or demonstrably impair the structure or function of the nervous system in a dose-dependent manner and if they occur at relevant exposure levels.

Issue: The Workshop Draft concludes that both reversible and irreversible effects of chemicals on the nervous system should be considered adverse.

The Workgroup agreed that irreversible effects should be considered adverse, if they involve a clear or demonstrable change in either the structure or function of the nervous system. The group also agreed that reversible effects could be considered adverse if they clearly or demonstrably impair the structure or function of the nervous system. There was a minority opinion that any "change" in the structure or function of the nervous system could also be considered "adverse." For an adverse effect to be considered evidence of neurotoxicity, further considerations such as dose-response relationships and exposure conditions need to be taken into account.

Issue: The nervous system contains billions of cells wired in complex patterns and is known to be resilient to environmental and toxicological insult by a process known as compensation or adaptation.

There was agreement that healing in the nervous system can occur to varying degrees depending on the seriousness of the impairment. When structural damage is incompletely reversed, functional changes that allow the individual to adapt to its environment or compensate for the residual damage may or may not occur.

Issue: Once damaged, nerve cells have limited capacity for regeneration.

There was agreement that once neurons are killed or central neural processes are severely damaged, regeneration of the cell bodies is ruled out and therefore repair is likely to be incomplete. There was also agreement that the capacity for repair is not as limited (and can, in fact, be complete) if effects do not involve the destruction of neurons or central neural processes.

Issue: Apparent recovery actually represents activation of reserve capacity, decreasing remaining potential adaptability.

There was agreement that the reversibility of effects resulting from cell death or from the destruction of cell processes may represent an activation of repair capacity, decreasing

future potential adaptability, but that this is not necessarily true for agents that operate by other mechanisms of action.

Issue: Traditionally, effects of toxicants are considered to be persistent or long-lasting, while pharmacological effects are considered to be transient or short-acting.

The Workgroup recognized that there should be different levels of concern for different types of adverse effects. There was agreement that it is important to distinguish between agents that clearly damage the nervous system and those that do not. The group also agreed that adverse effects that might not be considered indicative of neurotoxicity should not be ignored, but rather that these effects should be considered under a different heading and not regulated as neurotoxicants. In some cases these effects have been referred to as "pharmacological effects" to distinguish them from neurotoxic effects. There was no agreement on what to call these other effects, but a clear concern was expressed about lumping large numbers of chemicals under the term "neurotoxicant," especially when the evidence of neurotoxicity is obtained in experimental situations that involve exposure to high concentrations of the toxicant.

Issue: An effect that appears to be transient in an unchallenged organism may be revealed as long-lasting through an environmental or pharmacological challenge.

This issue was only briefly discussed. There was a consensus that residual effects of a chemical may appear to have resolved during a recovery period yet re-emerge as significant when the individual ages or is later challenged. Transient effects that are recoverable and that do not result in residual lesions would not be expected to be revealed as long-lasting through either an environmental or pharmacological challenge.

Issue: It is not known whether transient effects observed following developmental exposure should be evaluated at specific points in the life span.

This issue was discussed only briefly because the Workgroup was not aware of any data that could be used to identify specific time points in the life span that should be examined. This issue probably needs additional research before it can be adequately addressed.

5.2 DIRECT AND INDIRECT EFFECTS PANEL

Barry Wilson, Ph.D., Workgroup Chair

The panel reaffirmed that chemicals that directly affect the nervous system should be subject to the proposed guidelines. Generally, it was accepted that the data submitted to the Agency would usually consist of multiple tests at more than one level of the nervous system, often including behavioral, biochemical and histopathological studies of one or more animal species. Human studies were not discussed in detail. Even so, several scientists did not agree with the idea that epidemiological studies by themselves are sufficient for risk assessment purposes.

The recommendations of the panel were based upon an appreciation of the difficulties facing a risk assessor who may not have had extensive training in neurotoxicology or neurobiology. The nervous system is extraordinarily complex; on the one hand it exhibits a remarkable plasticity in its ability to adapt to many stimuli while, on the other hand, it may be extremely sensitive to damage by a number of chemicals. Integration of the nervous system with the rest of the body makes it difficult to sort out specific from non-specific effects, and the role of the nervous system in maintaining homeostasis makes it hard to decide when responses have exceeded normal limits. The panel cautions the risk assessor that simple litmus tests usually cannot be used either to classify a chemical as "neurotoxic" or to dismiss a compound as harmless.

5.2.1 Indirect Effects

Although the premeeting comments revealed little agreement as to whether chemicals that do not act directly on the nervous system could be considered neurotoxic, the panel agreed that some could. A number of examples were provided by panel members. These included spasms in blood vessels (such as those caused by cadmium) and other effects on the neurovascular system; disruption of the oxygen-carrying capacity of the blood (such as that caused by carbon monoxide); perturbation of metabolic pathways (such as those caused by

dichloroacetate); rhabdomyolysis (such as that induced by naphthalene), peripheral neuropathy (such as that induced by cyanide), and encephalopathy (such as that occurring secondary to alcoholic damage to the liver). Other agents may exert indirect effects on the nervous system by affecting membranes, causing post-synaptic or sarcolemmal blockade, producing functional denervation of a target organ, or causing damage to the vertebral column.

In general, the panel agreed that agents affecting target organs of the nervous system (e.g. muscle), should be of serious concern, as should systemic toxicants to which the nervous system is peculiarly sensitive (e.g., metabolic poisons). Since their effects are often expressed through the nervous system, these substances should be subject to the proposed guidelines. However, the panel warned that the search for indirect effects can lead to a long list of possible hazards, exposing the assessor to the risk of "analysis paralysis." The panel noted that one criterion for accepting an indirect effect as "neurotoxic" is whether the chemical produces a special effect on the nervous system, regardless of its other effects on the rest of the body. In many cases, however, the chain of events leading to toxicity may not be fully understood, as in the Spanish rapeseed oil incident.

5.2.2 Adverse Effects

The discussion of what constitutes an adverse effect vis-a-vis the nervous system, which began in Panel 2, continued in Panel 3. The final proposal categorized adverse effects on the nervous system into a three-tiered hierarchy encompassing both direct and indirect effects. The effects may be either irreversible or reversible (i.e., recoverable) at the cell and organ levels.

For the purposes of risk assessment, an adverse effect was operationally defined as "a demonstrably recognizable and dose-dependent impairment in the structure or function of the nervous system arising at any stage in the life history of the organism." Effects in Category I deviated slightly from this definition, however, since irreversible changes in structure or function were considered adverse whether or not they could be established as injurious to the animal in other respects.

Category I: irreversible effects on the nervous system, whether or not identified as impairments (e.g., as caused by methyl mercury).

Category II: slowly or incompletely reversible effects on the nervous system (e.g., as seen following exposure to certain organophosphate esters).

Category III: rapidly reversible effects on the nervous system (e.g. as seen following exposure to neuroactive solvents).

The term "reversible" refers to situations in which full recovery of form and function occurs. Effects in all three categories must be dose-related to be considered evidence of neurotoxicity.

The panel emphasized that chemicals *per se* are not neurotoxic; it is the action of a chemical at a particular concentration in the nervous system or in other parts of the body that ultimately results in neurotoxicity. Some panelists did not accept restricting "adverse effects" to impairments; they argued that any significant change in the nervous system is an "adverse effect." The panel advises risk assessors to avoid being too exclusive by narrowly defining "impairment" and to avoid being too inclusive by failing to recognize that some "changes" fall within the normal physiological range.

The panel assigned relative levels of concern to the three levels of adverse effects. They were:

- Category I: ****
- Category II: **to***
- Category III: *

These weightings were not equivalent to uncertainty factors; instead, they were intended to express the relative importance that the panel attached to irreversible versus recoverable changes in the nervous system.

The panel thought of the hierarchy of adverse effects as a first step in assessing potential neurotoxicity. The goal was to help the risk assessor separate long-term damage to the nervous system from short-term, readily recoverable effects. Although the initial level of concern is indicated by the position of an effect in the tier, the relative severity of any given effect was to be assessed later in the process, when dose-response curves, pharmacokinetic data and structure/activity relationships are specifically considered.

The panel also discussed issues related to hazard characterization, in which "real world" considerations play a role. For example, there was some discussion of a compound that causes Organophosphate-Induced Delayed Neuropathy (OPIDN), but only at doses above the lethal level. To see these delayed effects at all, the animal must be protected from the acute toxicity of the compound by pre-treatment with atropine. On the basis of these data, the chemical would be considered to fall within Category I, since the effects were irreversible. However, the assessor might not consider it to be a serious risk after subjecting the data to hazard characterization. Knowledge of the potential for neurotoxicity at very high doses might, however, prompt the risk assessor to request additional study of this compound in order to determine whether chronic exposures at sublethal levels might also produce damage to the nervous system.

The statement in the document that any compound will be neurotoxic at a high enough dose because it is lethal to the organism was not considered germane, and was set aside during the considerations. Some panelists did not agree with this statement regardless of the context in which it would appear. The panel was more interested in directing a risk assessor to events that occur at relatively low doses than in focusing attention on those effects that occur at lethal levels.

The panel chair briefly discoursed on Neuropathy Target Esterase (NTE), both to set the record straight in the premeeting comments and to suggest OPIDN as one of the possible models for risk assessment that the Agency could use to illustrate the panel's proposals. Irreversible inhibition of NTE is accepted as a biomarker of exposure to organophosphate agents that may cause OPIDN. Recent findings suggest that inhibitions of NTE as low as 40% may be associated with lesions indicative of OPIDN in the spinal cords of hens. Indeed, lesions have been detected even after diisopropyl fluorophosphate (DFP) treatment at doses that do not produce ataxia.

5.2.3 Unresolved Issues

During the panel session and the plenary meeting that followed, several additional issues were raised. Although time constraints precluded resolution of these issues, they are important for the Agency to consider. One was concern about the role envisioned for behavioral tests in the risk assessment process. Behaviorists are well aware that not all behavioral changes are evidence of neurotoxicity. For example, they are very careful not to use unhealthy animals in neurobehavioral studies, recognizing that the responses of these subjects will be abnormal. At the least, the risk assessor should be aware of the limitations of behavioral tests and be chary about labeling a compound as neurotoxic based on behavioral evidence alone, especially if the evidence is obtained from only a few tests. Some panelists felt that a second category such as "behavioral toxicant" might be needed to handle compounds with behavioral effects that are thought to stem from systemic or, at any rate, non-neural events. Others felt strongly that this category would not be useful.

Another category that was discussed but not adopted was one that would indicate suspected but unproven neurotoxicants; this category was discussed as being analogous to the Scottish verdict of "Not Proven." A majority of the panel felt this category to be an unnecessary addition, arguing that such chemicals would be addressed somewhere else in the risk assessment process.

The importance of factors such as tolerance, repeated and chronic exposures and differential sensitivity during different stages in the life history of an animal were raised but were not discussed in any detail. An animal may respond to a chemical in different ways depending on the stage of its life history, from embryonic life, through neonatal growth, adolescence, maturity, and senescence. Risk assessors need to be aware of the special sensitivities that may be present at different stages of life. Similarly, the issue of the effects of mixtures was raised, but not discussed in detail. "Real world" scenarios frequently involve more than one agent, and it is important to consider synergisms, especially if one of the agents is an inducer of liver enzymes. Exposure to a chlorinated hydrocarbon that increases liver P450 and monoamine oxidase (MAO) activities could affect the toxicity of other chemicals that require bioactivation. For example, the neurological problems found among workers in a plant synthesizing the neurotoxic agent leptothos were never satisfactorily attributed to exposure to leptothos, to the solvents used during the synthetic process, or to a combination of leptothos and the solvents.

5.2.4 Closing

Agency personnel familiar with the carcinogenicity/mutagenicity risk assessment process may be struck by the apparent looseness of the guidelines recommended here. This is more due to the nature of the nervous system, rather than to the inability of neuroscientists to agree. What constitutes the normal limits of response to a chemical is different for different parts of the nervous system; for example, the resting potential of a nerve is regulated closely, while the avoidance response of an animal to a stimulus may be more variable. Labeling a compound as a potential "neurotoxicant" is likely to have a dramatic effect on potential consumers, and the risk assessor needs to obtain the best data and background information possible before drawing a conclusion.

5.3 ANIMAL-HUMAN EXTRAPOLATION PANEL

Shayne C. Gad, Ph.D., D.A.B.T, Workgroup Chair

The panel charged with reviewing and commenting on the animal-human extrapolation aspects of the EPA proposed guidelines for neurotoxicity risk assessment used as its working matrix the statements presented in the issues paper provided before the review session. The panel's comments and suggestions on the points contained in the issues paper were as follows:

5.3.1 Conclusion: With an adequate animal database, as defined in the draft guidelines, risk assessment judgments, even in the absence of human data, may be scientifically valid.

Conclusions and Suppositions Used in Reaching This Position

- Substances producing neurotoxicity in humans also result in neurotoxicity in other species.

Panel members generally agreed with this statement, but only with some qualifications related to the importance of differences in routes of exposure and times to effect. There are appropriate animal models for neurotoxicity, but not all species will be similarly affected all the time. Currently, animal models and studies constitute the best and most likely form of hazard identification available.

- Compared with human studies, animal studies are more readily available and can provide more precise dose information and better control of environmental factors.

There was substantial agreement on this point. However, limitations of individual models may make it difficult to differentiate neurotoxicity due to direct effects on the nervous system from neurotoxicity secondary to other target organ effects.

- Many diagnostic procedures employed to evaluate neurotoxicity in humans have corresponding animals models.

There is a divergence of opinion on the meaning of "many" in this statement. Diagnostic procedures requiring verbal or written interaction with a "patient" may not universally fit this description. In addition, it must be noted that rats are not little humans with tails. Also, selection of the appropriate species must be carefully considered. Finally, it should not necessarily be expected that there will be a one-to-one concordance between endpoints observed in animals and diagnostic endpoints that are relevant to humans.

- The range of uncertainty factors used to extrapolate risk from animal data to humans for other endpoints of toxicity are applicable to neurotoxicity risk assessment.

There is substantial agreement with this contention from the point of view of categories of factors. At the same time, it is clearly hoped that more precision might be achieved. Also, there is a notable lack of comfort with any set magnitude for these uncertainty factors. The more completely understood the biological basis of observed effects, the more likely it is that data may be made more precise and its relevance to human risks may be clarified. This will lead to refinement of uncertainty factors, which under no circumstances should be arbitrary numbers that fail to take all available information into account.

Areas of Special Focus

- The behavioral domains for animals and humans are similar. However, the complexity of behavior may vary precluding an exact concordance of effects.

- Factors such as differences in metabolism can result in differences among species in sensitivity to a compound.

There was substantial agreement on this point. The statement, however, should not be read as focusing solely on metabolism. A lot of other factors not only lead to differences in sensitivity among species but also among various human "populations" (based on age, nutrition, race, health status, sex, etc.).

- The most sensitive species may not be the species most relevant to predicting risks in humans. In the absence of information to the contrary, however, the use of the most sensitive species is warranted.

There was substantial agreement on this point but its relevance is unclear. The issue of which human population we are "modeling" or concerned about protecting was also raised.

Other Issues

No general guidance is offered on the determination that findings from animal models are relevant for the risk assessment process. Such factors as sensitivity, stability of the model, availability of techniques and baseline data, and overall relevance of the model for the endpoint of interest should be considered.

Substantial disagreement exists on the relevance and utility of data obtained from *in vitro* models in the human risk assessment process. Findings from adequate *in vivo* studies should take precedence over *in vitro* findings. In the face of mixed *in vitro* and *in vivo* findings, the generally accepted relative strengths, as summarized in Table 2, should be considered.

The value of an integrated, full range of data (i.e., neurochemical, pathology, behavioral and physiological data) needs to be explicitly recognized in the guidelines.

The guidelines should also address where the risk assessment process should begin in terms of whether uncertainty factors should be applied to the ED_{10} or NOAEL for the endpoint of concern.

Table 2. Potential Advantages and Disadvantages of *In Vitro* Toxicity Tests

Advantages

1. Avoid complications and potential confounding or "masking" findings of *in vivo* studies.
2. Exposure levels and conditions at target sites can be better controlled.
3. Test condition standardization can be better than for *in vivo* studies.
4. Reduction in animal usage and/or in pain to experimental animals.
5. Ability to directly study some target tissue effects on a real time basis.
6. Reduced requirements for text agents.

Disadvantages

1. Lack of ability to evaluate longer term effects.
2. Limited ability to simulate and evaluate integrated organismic level effects.
3. May not reflect influence of agent absorption, distribution, metabolism, and excretion effects.

5.4 BEHAVIOR PANEL

John L. Orr, Ph.D., D.A.B.T., Workgroup Chair

Unlike the other panels, which were charged with issues that are important in many areas of toxicology (animal-to-human extrapolation, direct and indirect effects, and transient and persistent effects), the focus of the behavior panel was on the set of functional endpoints that reflect the status of the nervous system. The conclusions, recommendations, and statements of the panel touch on issues involving the definition of adversity, the importance of transient effects, the utility of behavioral data, and interpretation of behavioral data in the presence of other evidence of toxicity.

5.4.1 Data Submission Scenario

To focus the panel's discussion, data scenario was envisioned in which a set of data arising from a Toxic Substances Control Act (TSCA) neurotoxicity test rule was submitted. The data package was defined to contain a report with behavioral data arising from the functional observation battery (FOB), motor activity tests (MA), and neuropathology. This scenario served as a useful device to frame the situation and focus discussion, since it was assumed to represent a minimal data scenario in which the risk assessment guidelines might be used. The use of this scenario to focus the discussion does not imply that the panel felt the scene-setting scenario to be optimal for establishing NOAELs or conducting risk assessment.

5.4.2 Issues Paper Conclusions

The panel worked through the items in the Issues Paper and reached consensus with the conclusion that ". . . . behavioral changes can provide evidence of neurotoxicity in the absence of additional data."

The panel had some recommendations to fine-tune the conclusions and suppositions listed in the Issues Paper. The panel concluded that:

- Behavior can be a sensitive indicator of toxicity

- Behavioral evaluations have played an important role in efforts to understand brain function
- The behavioral domains for animals and humans are similar, but the complexity of behaviors may preclude an exact concordance of effects
- Data from measures of behavior can be available prior to data from physiological or morphological studies
- The above four conclusions apply to both adult and developing organisms.

5.4.3 Response to Areas of Special Focus

The panel discussed the three areas of special focus in the Issues Paper: non-specific effects, high-dose effects, and the possibility of indirect effects. The panel endorsed the following paragraph in the specific context of the Draft Neurotoxicity Risk Assessment Guidelines:

> Behavior is an indication of the well-being of an organism. Changes can arise from a direct effect on the nervous system or indirectly from effects on other physiological systems. It is appropriate to use behavioral changes for the determination of reference doses, but such changes may not be sufficient to establish an agent as a neurotoxicant. Our understanding of the interrelationship between systemic toxicity and behavioral changes is limited, (e.g., the relationship between changes in body weight and activity). Interpretation of such relationships should include consideration of factors including experimental design, dose-effect information, chemical class, and other relevant toxicological information. The presence of systemic toxicity complicate, but does not preclude interpretation of behavioral changes as evidence of neurotoxicity.

The panel felt that the draft guidelines language should be changed on pages 27 and 32 (and elsewhere as necessary) to reflect the conclusions expressed in the above paragraph. This could be accomplished by inserting the word "necessarily," for example:

- On page 27, line 17: ". . . data are not <u>necessarily</u> interpreted. . ."
- On page 32, line 13: ". . . not <u>necessarily</u> evidence. . ."

5.4.4 Data Interpretation

With respect to the interpretation of data, the panel concluded that interpretation of FOB data should include an evaluation of the pattern of effects, consistency within functional domains, degree of replication, severity of effects, and statistical considerations of multiple statistical comparisons. Some panel members felt that these factors were reasonable considerations for any neurotoxicology data set, but others felt that more discussion would be required to reach consensus.

5.4.5 Conclusion

The panel considered behavior a major dimension comparable to the physiological or morphologic dimensions. The feeling was not that one dimension was "better" or more important, but that any could serve as an appropriate basis for categorization. The panel agreed that behavior is a reflection of the status of the underlying physiology and morphology, but that behavioral studies can reveal pathology that does not happen to be sampled by the other types of studies in a given data set. An example would be an alteration of neurotransmitter levels that is reflected in behavior but could be difficult to capture in non-behavioral studies (e.g., using the TSCA neurotoxicity battery).

5.4.6 Categories for Classification as a Neurotoxicant

Some members of the panel had a concern about the possibility of overlabeling and calling everything a neurotoxicant. Other panel members felt this was a consideration more appropriate to a discussion of risk characterization than a discussion of hazard identification.

Panel members felt that there were a number of kinds of toxicologic information that would help one decide whether a compound is neurotoxic at certain doses. The panel members felt that the problem of categorization is difficult but not impossible in the presence of systemic effects.

As with other non-cancer endpoints (e.g., the EPA Guidelines for Developmental Risk Assessment), there is difficulty in separating the hazard identification stage from the dose-response assessment stage in neurotoxicity risk assessment. This occurs in part because evidence of a dose-effect relationship may be part of the evidence that the effect is compound-related. A second, perhaps more important reason is that the toxicity profiles of neuroactive agents may be different at different dose levels or chronicities.

The panel emphatically did not agree with the concept that one should have morphologic or physiological "confirmation" before categorizing an agent as a neurotoxicant. On the other hand, they did not feel that any behavioral change is sufficient to necessarily categorize an agent as a neurotoxicant. The opinion was that any behavioral change increases the suspicion of potential neurotoxicity and that evaluations should be made in the light of all available data.

5.4.7 Tangibility of Behavioral Data

In the plenary session, one observer complained that behavior was not tangible and that he could not "touch" it. Another panel member asked if this individual would similarly question the concept of blood pressure. This illuminates an important point. In casual and even learned discussion "behavioral changes" are discussed as if changes in behavior are the object of attention. These are indeed, if not directly observed by the speaker, intangible. However, the phrase "behavioral changes" or "changes in behavior" is really a short form of saying "differences in behavioral data. "The behavior may be ephemeral, but the data is as tangible as any other data. A graph of behavioral performance is as real as any photomicrograph from a histopathologic examination. Behavioral data from an appropriately designed experiment is analogous to a well-prepared anatomic specimen and is just as tangible. The problem is not with behavioral changes, per se, but with the assessment of their adversity.

APPENDIX A

AGENDA

U.S. Environmental Protection Agency

NEUROTOXICITY RISK ASSESSMENT GUIDELINES
PEER REVIEW WORKSHOP

June 2-3, 1992
Omni Georgetown Hotel
Washington, DC

FINAL AGENDA

Monday, June 1, 1992

7:30-9:00PM	Early Registration/Check-In

Tuesday, June 2, 1992

7:30-8:30AM	**Registration**
	Opening Plenary Session: Preliminary Comments/ Definition Of Issues *Dr. William Greenlee, Purdue University*
8:30AM	Welcome and Objectives *Dr. William Wood, USEPA, Risk Assessment Forum*
8:45AM	Neurotoxicity as an Endpoint (Panel 1) *Dr. William Greenlee*
9:15AM	Transient and Persistent Effects (Panel 2) *Dr. John O'Donoghue, Eastman Kodak Company*
9:45AM	Direct and Indirect Effects (Panel 3) *Dr. Barry Wilson, University of California*
10:15AM	**Break**
10:30AM	Animal-Human Extrapolation (Panel 4) *Dr. Shayne Gad, Becton Dickinson Research Center*
11:00AM	Behavior (Panel 5) *Dr. John Orr, Southwest Research Institute*
11:30AM	Observer Comments *Dr. William Greenlee*
12:00-1:15PM	**Lunch**

(over)

Tuesday, June 2, 1992 (continued)

Work Group Break-Out Sessions

1:15PM	Discussion Panels 2 and 4
	Transient and Persistent Effects *Dr. John O'Donoghue*
	Animal-Human Extrapolation *Dr. Shayne Gad*
3:00PM	**Break**
3:15PM	Panels 2 and 4 (continued)
4:30PM	Plenary Reports and Discussion
	Transient and Persistent Effects *Dr. John O'Donoghue*
	Animal-Human Extrapolation *Dr. Shayne Gad*
5:30PM	**Adjourn**

Wednesday, June 3, 1992

Work Group Break-Out Sessions

8:00AM	Discussion Panels 3 and 5
	Direct and Indirect *Dr. Barry Wilson*
	Behavior *Dr. John Orr*
10:00AM	**Break**
10:15AM	Panels 3 and 5 (continued)
11:15AM	Plenary Reports and Discussion
	Direct and Indirect *Dr. Barry Wilson*
	Behavior *Dr. John Orr*
12:15PM	**Lunch**

(continued)

Wednesday, June 3, 1992 (continued)

1:30PM	**Closing Plenary**
	Final Reports
	Recommendations to EPA
3:00PM	**Adjourn**

APPENDIX B

LISTS OF PARTICIPANTS AND OBSERVERS

U.S. Environmental Protection Agency

NEUROTOXICITY RISK ASSESSMENT GUIDELINES
PEER REVIEW WORKSHOP

June 2-3, 1992
Omni Georgetown Hotel
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NEUROTOXICITY RISK ASSESSMENT GUIDELINES
PEER REVIEW WORKSHOP

June 2-3, 1992
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APPENDIX C

PREMEETING COMMENTS

U.S. Environmental Protection Agency
Office of Research and Development
Risk Assessment Forum

PREMEETING COMMENTS FOR

PEER REVIEW OF NEUROTOXICITY

RISK ASSESSMENT GUIDELINES WORKSHOP

June 2-3, 1992

Omni Georgetown Hotel
Washington, DC

TABLE OF CONTENTS

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
Washington, DC
June 2-3, 1992

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Comments

pp. 4-5. Although this may have been discussed in the context of defining the terms neurotoxicity and neurotoxicant, I wonder if inclusion of the term "non-volitional" before the term "exposure" would assist in differentiating neurotoxicity resulting from exposures to chemicals, etc. from the side effects of non-therapeutic compounds taken by choice for their beneficial effects upon the user. In the case of these chemical, physical, or biological agents regulated by EPA, the human population is not the intended population for use or exposure, another clear distinction from therapeutic compounds. Recognizing that the side effects of therapeutic compounds could certainly be neurotoxic, the phrase "non-volitional exposure" carries the connotation of environmental, occupational, etc., type exposures that are essentially unintended and not by choice.

Some aspects of the definition of neurotoxicity itself are rather vague, in particular, the phrase "ability to adapt to the environment." When paired with the other descriptors included in the definition of neurotoxicity, i.e., ability to survive and reproduce, "adapt to the environment" carries the connotation that fairly serious consequences would be warranted to constitute adverse effects. My own perception of the phrase in this context is that it relates again to survival, since failure to adapt to the environment would mean failure to survive, particularly from an ecological or ethological point of view. Does this mean that subtle changes in behavior, which might not be of sufficient magnitude to result in failure to survive, are not considered adverse enough to warrant the term neurotoxicity? This would seem to be at odds

with the conclusions to be discussed by Panel 2 members of the Workshop. If my ability to reach my full potential is diminished but not completely decimated, is that neurotoxicity? Moreover, adaptation to the environment is a long-term process, and this would further suggest that adverse effects that may be relatively transient are therefore not problematic.

p. 5. There are additional reasons for considering reversible effects to be neurotoxic when one considers behavioral changes. First, it has been demonstrated in the behavioral pharmacology literature that past behavioral history is an important determinant of future behavior. This is probably best exemplified by the numerous studies that have shown that responding can be established and maintained by response-produced shock in animals with prior training under shock-postponement or shock avoidance schedules (see Barrett, 1986). In addition, past behavioral history can substantially alter the effects of drugs on behavior, even when the effects of the different behavioral history are not evident in the current or ongoing performance. Thus, even reversible or transient changes in behavior do become part of our behavioral history. As such, these changes can subsequently impact our future behavior and modify the nature of interactions with other chemical agents, since many of the compounds of concern for risk assessment may be compounds which, like drugs, act on the central nervous system.

p. 5. The third paragraph on p. 5 states that there are five principal questions that should be addressed, but only four (content validity, construct validity, concurrent validity, and predictive validity) are listed.

p. 7. It would seem that case studies, since they tend to focus on a relatively few severely affected individuals, should be able to provide a rather good description of the signs and symptoms of toxicity, rather than a poor characterization indicated by the text.

p. 8. The sentence indicating that positive epidemiological data are generally regarded as the most convincing evidence of the potential neurotoxicity of a chemical seems overstated, since these are correlational and not cause-effect studies. If this statement is based on the fact that such studies involve human subjects, then perhaps the statement should be so qualified, since it otherwise suggests that these studies constitute the strongest science.

p. 9. Numerous questions still exist with regard to the issue of the sensitivity of the test batteries being used to examine neurobehavioral toxicity in human populations. In particular, questions remain as to whether the effect level, as determined from the tests represents the lower limits of sensitivity of the test itself, or the actual LOAEL of the toxicant. As stated by Guillon and Eckerman (1986), a neuropsychological or cognitive-abilities test may be reliable and valid over a wide range of levels of the trait measured and yet not be sufficiently sensitive to variation within the restricted range of subclinical effects to be useful for monitoring or screening program. The data indicating the level of sensitivity of these tests are still by and large not available. In fact, the sensitivity issue may be far more important than that of validity, since the levels at which any effects are observed in these tests are very likely to receive primary consideration in the determination of risk assessment. In such a case, levels of exposure may then be based on the outcome of tests for which the sensitivities are unknown. In other words, if the test is insufficiently sensitive, then effects are only detectable above a certain exposure level, even though adverse effects actually may occur at still lower exposure levels. If the assumption that the exposure level rather than test sensitivity is the determinant of the LOAEL of the study, then we end up setting exposure levels too high. This entire issue deserves further consideration.

The reason for suggesting that the sensitivity issue is in some ways more important than the issue of validity is that it is clear that some behavioral function is being tapped by the tests, and the exact nature of that function may be less important to EPA than the level of exposure associated with that effect. Granted, one must also strive to find the specific types of behavioral functions most impacted by the toxicant in order to fully delineate adverse effect levels. However, different toxicants affect different behavioral functions to a greater or lesser degree, inevitably requiring the use of a test battery that crosses functions and includes components with documented sensitivity levels.

Recent studies also raise numerous questions about the reliability of many of the tests typically included in these batteries (Arcia and Otto, 1992). In particular, tests measuring learning/memory were found to be of low reliability. It may be premature to use these types of studies at the current time as a basis of risk assessment.

p. 15 and Table 2. These descriptions leave the misleading impression that lead (Pb) causes its effects via myelinopathy. The table should be better qualified, particularly the title of the table, which indicates "with specific neuronal targets," since this is not the sole target for lead, nor presumably the most sensitive one.

p. 17. Is it really the case that glial fibrillary acidic protein increase actually represents a uniform response to central nervous system (CNS) injury? The list of known neurotoxic compounds or classes of compounds associated with such effects appears to be somewhat restricted, and exhibits some rather notable exceptions, such as lead, carbon disulfides, and pesticides as examples (O'Callaghan, 1992). Have these agents been examined with respect to their impact on glial fibrillary acidic protein levels, or do they simply not induce any changes? While it may be fine to conclude that changes in this protein are indicative of cell injury and hence neurotoxicity, the converse, i.e., lack of effect on this protein indicates no neurotoxicity, should not be assumed and should be clearly stated.

p. 23. Section 3.2.3. This section discusses the difficulty of deciding whether a biochemical or neurochemical change is one of neurotoxicological significance. This, of course, is where behavioral measures become extremely useful, since if such a change is of sufficient biological magnitude and clinical relevance, then it should manifest itself in behavior, and behavioral processes linked to that neurotransmitter system can be examined. Perhaps mention of such possibilities could be made here.

pp. 30-31. The discussion of motor activity seems largely insufficient. For example, it fails to point out how the difference devices used to measure motor activity (a global measure of behavior) can actually measure quite different aspects of motor function. For example, some may primarily measure ambulation, while others may also include measurements of grooming, rearing, etc. This is bound to lead to confusion in the literature, as different investigators note different effects on motor activity since they may actually be measuring different aspects of motor function. Also, in the testing phases for new compounds, how can one ensure that an appropriate device was used, i.e., one that measures "critical" aspects of motor activity, whatever those may be for that compound. Perhaps it would be more appropriate to differentiate the specific dependent variables and their operational definitions

rather than to simply refer to this as motor activity. It seems to refer to ambulation as a subset of motor activity. This should also provide clarity with respect to any noted differences in results across laboratories.

p. 31. The rationale for including the statement that most neurotoxicants decrease rates of schedule-controlled operant responding at some doses is not clear. The reason this occurs is artificial in that one necessarily wants to include a dose that produces overt toxicity or gross behavioral manifestations to ensure that an adequate dose-range has been covered in a study. But, aside from that, different toxicants have very different effects upon response rates per se. Response rations are further differentiated by examination of different schedules of reinforcement. Even within a toxicant, effects on response rates can be quite different for a given schedule of reinforcement when one considers dose of toxicant or duration of treatment, as has certainly been demonstrated in studies of lead effects on schedule-controlled behavior.

This discussion of schedule-controlled behavior also fails to note that these baselines can be used not only to measure what is termed "steady-state performance," but also can be used in a learning and memory context. With regard to learning, one can measure, for example, the number of sessions to acquisition of the characteristic pattern of behavior associated with the schedule. Moreover, one can continue to impose a change in a schedule parameter (length of the fixed-interval, size of the fixed-ratio) and measure the time or number of previously utilized parameters, one can ask questions about how the treatment affected "memory" since reacquisition at a particular parameter value should be faster than the first acquisition curve.

The discussion of schedule-controlled behavior also does not refer to the similarity across a wide range of species (including humans) of the characteristic patterns of schedule-controlled behavior. This should be pointed out, since it has particular relevance to the issue of risk assessment in which extrapolation across species is an important consideration. While this aspect does not address the probability that humans will likewise show changes in schedule-controlled behavior in response to a particular toxicant (i.e., predictive validity), it does attest to the fact that similar behavioral processes are being evaluated across species. Similar evaluations across species are not being conducted for extrapolations that involve comparison of some experimental animal paradigms which purport to measure a specific behavioral function to

a computer or pen-and-paper based test which purports to measure the same function in humans.

p. 34 and Table 5. What exactly is "discriminated conditioning?" This is not a standard term from the behavioral literature and should be replaced with the appropriate terms. Does it refer to discrimination learning?

p. 36. The definitions of learning and memory are not particularly satisfactory. For example, with the definitions used, how does performance differ from memory? What does "due to experience" mean?

pp. 46-47. What happens to the risk assessment process when the dose-effect or dose-response curves are not linear, which is not unusual in neurotoxicology? Many of the dose-effect curves obtained with lead show a U-shaped function. This is not restricted to behavioral endpoints, but has been observed function. This is not restricted to behavioral endpoints, but has been observed for other CNS effects of lead as well (Davis and Svendsgaard, 1990).

p. 48. The statement that "there also appears to be little biological justification for many of the uncertainty factors" requires further explanation.

p. 50. The bottom line is that even with documents such as the one under consideration here, a great deal of appropriate scientific judgment and expertise always will be needed to make decisions on risk assessment even with the guidance provided by documents such as this. Who will these people be; is the field of neurotoxicology generating sufficient personnel for this purpose?

p. 58. The acronym MOE is never explained, or if it was, I missed it.

References

Arcia, E. and Otto, D.A. 1992. Reliability of selected tests from the neurobehavioral evaluation system. Neurotoxicol and Teratol. 14, 103-110.

Barrrett, J.E. 1986. Behavior History: Residual influences on subsequent behavior and drug effects. In: Developmental Behavioral Pharmacology, vol. 5, Advances in Behavioral Pharmacology, N. Krasnegor, B. Gray and T. Thompson, eds., Lawrence Erlbaum Associates, New Jersey, pp. 99-114.

Davis, J.M. and Svendsgaard, D.J. 1990. U-shaped dose-response curves: Their occurrence and implications for risk assessment. J. Toxicol. Environ. Health, 30, 71-83.

Gullion, C.M. and Eckerman, D.A. 1986. Field Testing. In: Neurobehavioral Toxicology, Z. Annau, ed., The Johns Hopkins University Press, Baltimore, pp. 288-330.

O'Callaghan, J.P. Assessment of neurotoxicity using assays of neuron- and glia-localized - proteins: Chronology and critique. In: Neurotoxicology, Target Organ Toxicology Series, H.A. Tilson and C.L. Mitchell, eds., Raven Press, New York, pp. 83-100.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
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The guidelines being proposed by EPA fulfill a needed function in the area of data interpretation for neurotoxicity studies carried out under a variety of regulatory programs. A considerable amount of neurotoxicity data are being generated under TSCA, FIFRA, and other regulatory initiatives that will need to be evaluated in a consistent and scientifically sound manner. These guidelines hopefully will serve as a scientifically reasonable yardstick against which newly generated data can be interpreted and appropriate conclusions drawn regarding potential neurotoxic hazard and risk.

Below are comments on specific subject areas for consideration by EPA and the peer review workgroup.

Categorization of Evidence for Neurotoxic Hazards (pp. 50-54)

EPA has proposed a scheme whereby data on a given chemical will be categorized as "Sufficient Evidence" or "Insufficient Evidence" for characterizing neurotoxic hazards. This scheme is identical in concept to the categorization scheme recently published by EPA for use in developmental toxicity risk assessment. It is clearly desirable to have a conceptually similar scheme for most noncancer endpoints, as the Agency is proposing.

In Table 7A, the "Sufficient Evidence" category is described as that which provides enough information to judge whether or not a human neurotoxic hazard could exist. However, under the two subcategory paragraphs of "Human Evidence" and "Experimental Animal

Evidence," little attention is given to the *or not* side of the judgment. For example, under Sufficient Human Evidence, it is stated that, "This category includes agents for which there is sufficient evidence from epidemiologic studies..., to judge that some neurotoxic effect is associated with exposure." Nothing more is stated about the situation in which there is sufficient evidence to conclude that a neurotoxic hazard does *not* exist. For purposes of clarity and understanding, the Agency's Guidelines would be improved if it were made more explicitly clear that the category of Sufficient Evidence also includes those situations in which the data allow a conclusion that a neurotoxic hazard does *not* exist. As presently written, one could easily get the impression from Table 7A that this category was reserved only for those agents which had demonstrated frank neurotoxic effects.

Neurochemical Endpoints of Neurotoxicity (pp. 23-25)

The guidelines state that, "Many neuroactive agents can increase or decrease neurotransmitter levels in the brain but such changes are not necessarily indicative of neurotoxicity." This is a premise with which most individuals would agree. However, the guidelines then go on to state, "However, agent-induced decreases in specific neurotransmitters in the brain, or decreases in specific brain regions, especially when such changes are persistent, are evidence of neurotoxicity." Persistent decreases in neurotransmitter levels are clearly evidence of neurotoxicity; however, the beginning of this second sentence would seem to be at odds with the earlier statement that changes are not necessarily indicative of neurotoxicity. Are transient decreases in neurotransmitter levels in discrete regions to be considered neurotoxic? It would appear that additional clarification of this matter is needed.

In discussion of NTE inhibition, no mention is made of threshold levels of inhibition for the elicitation of clinical effects. It is generally accepted that levels of NTE inhibition in the order of 60 to 70 percent are needed following an acute exposure for clinical neurotoxicity to be manifested. For repeated dose studies, inhibition levels of 45 to 65 percent have been suggested by M. K. Johnson as a threshold zone. EPA's proposed guidelines would be more informative and valuable to the regulated community if this matter were addressed in more detail. While any inhibition of NTE represents potential neurotoxic hazard, it is not at all clear what EPA's

position is with regard to inhibition levels that pose a significant risk. Is there a level of NTE inhibition that the Agency will assume to be without significant effect?

Developmental Neurotoxicity

In discussing the interpretation of developmental neurotoxicity data (p. 39), the Agency notes the potential impact of maternal toxicity on the developing organism. The guidelines make a noteworthy distinction between "minimal" maternal toxicity and "excessive" maternal toxicity. EPA states that at doses causing excessive maternal toxicity, information on developmental effects may be difficult to interpret and of limited value. In contrast, at doses that cause "minimal" maternal toxicity, the developmental effects are still considered to represent neurotoxicity. Given the obvious importance of minimal vs. excessive maternal toxicity, some general description of the two is needed. Although it is clearly not practical or desirable to attempt to explicitly define all signs, symptoms, and findings characteristic of excessive maternal toxicity, some general guidance would seem to be in order. Given the importance of this issue, the proposed guidelines would be more informative if the terms minimal and excessive were characterized.

Direct versus Indirect Effects

The draft guidelines conclude that chemicals acting through both indirect and direct means can be considered neurotoxic. The Agency's Issues Paper lists a number of suppositions and conclusions that were used to reach this position. While these suppositions are sound in principle, this logic can lead to practical difficulties when testing is carried out in accord with the neurotoxicity test guidelines established by EPA. The source of the difficulty is related to the testing requirement that the high dose produce "significant neurotoxic effects or other clearly toxic effects." As long as EPA's definition of neurotoxicity implicitly includes behavioral changes, there is a significant probability that non-specific effects such as general sickness or malaise will be operationally interpreted as representing neurotoxic effects. Thus testing in accord with the established guidelines would seem to ensure a high likelihood of observing indirect effects that will be interpreted as evidence of neurotoxicity.

Elsewhere in the proposed Risk Assessment guidelines it is noted that agent-induced changes in the FOB or motor activity that are associated with overt signs of toxicity (weight loss or systemic toxicity) or that occur only at the high doses are not necessarily evidence of neurotoxicity. It is encouraging to see this position espoused since it allows for an element of reasoned scientific judgment to be brought to bear on the interpretation of the data. Moreover, I would recommend that this phrasing also be incorporated into the "Definition" section (pp. 4-5) of the proposed guidelines, where the issue of indirect effects is initially raised.

In the final analysis it would seem there is little to be gained by identifying as neurotoxic a variety of chemical agents whose only effect on the nervous system occurs following administration of heroic doses and whose nervous system effects are comparatively insignificant and a consequence of target organ effects produced elsewhere in the body.

Interpretation of Reversible Effects

The guidelines conclude that both reversible and irreversible effects of chemicals on the nervous system should be considered adverse. An issue which might warrant further consideration by EPA and the Peer Review Group concerns terminology and it relates to effects that have generally been considered pharmacological. No one would dispute the characterization of excessive CNS depression following acute exposure to chemical agents as an adverse effect. For many classes of compounds (aliphatic hydrocarbons for instance) this effect is reversible upon cessation of exposure and recovery of function is typically complete. Rather than refer to such pharmacologic effects as "neurotoxic" however, might it not be more accurate and informative to refer to these effects using other terminology (such as neuroactive, for example). For purposes of risk assessment and public protection, one would certainly want to distinguish compounds that produce frank irreversible CNS damage from those that produce acute, reversible effects. Referring to both types of endpoints as "neurotoxic" leads to a blurring of these distinctions in terms of hazard and risk communication. It is suggested that consideration be given to modifying the definition section on neurotoxicity in the guidelines to more accurately reflect the differences between acute reversible pharmacologic effects and irreversible nervous system damage.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
Washington, DC
June 2-3, 1992

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The proposed guidelines for neurotoxicity risk assessment are generally well written and easy to read. My initial review of this April draft is divided into two sets of comments and questions.

General Comments

1. It must be noted that the authors have done an excellent job of addressing what this reviewer believes to be valid issues of conditions effecting the relevance of findings from animal studies. The guidelines now make clear that functional changes seen only at high doses or in the presence of signs of marked acute systemic toxicity are not *a priori* indicative of neurotoxicity.

2. If the purpose of the document is to provide for neurotoxicity evaluation and risk assessment, then the authors have included an overly large amount of examples, commentary, and justification for the need for such guidelines and the measures provided. Though interesting and almost entirely accurate, the lack of reference citations and the inclusion of a few cases that are not clear weakens the presentation's standing as a background document. This is particularly of concern if the same effort has taken away from addressing some issues of study design and interpretation for the task at hand, as pointed out below.

3. No guidance is provided on two key issues of design for preclinical neurotoxicity studies. The issue of model selection (what species, age, sex, or health status of animals should be employed) is not addressed. This will be a critical point in the evaluation of new or previously not evaluated chemical entities or mixtures and in evaluating the relevance of existing findings in various animal models. Likewise, the issue of how doses are to be selected is not addressed, other than by inference (i.e., the guidance that findings at agonal doses are inappropriate).

4. The only unaddressed question previously raised as to assessing the relevance of functional findings in animals to neurotoxicity is that of "pharmacological" effects of agents. That is, are agents to be considered neurotoxic if they effect some functional components (such as motor activities) for an initial brief period of peak plasma levels following dosing? I believe that purely pharmacological agents must be differentiated from toxicological ones, and truly neurotoxic agents are those that have a persistent (more than an hour after dosing/exposure) effect.

Specific Comments

1. P. 4 (second paragraph): "(2) any alteration from baseline that diminishes the ability to survive, reproduce, or adapt to the environment." This begs the "pharmacologic" or time course question—what about ethanol or vigorous exercise? If assessed immediately after either of these, both would qualify as causing "adverse effects."

2. P. 7 (last sentence): "chronic solvent toxicity" is not currently a universally accepted case, and therefore may not be compelling.

3. P. 10 (end of first paragraph): Some good citations of recent U.S. cases would be very useful here.

4. P. 14 (end of second paragraph): "An absolute loss of brain weight in adult animals should be regarded as an indication of neurotoxicity." Is this without any finding of

histopathological alteration or indication of functional change? A marginally statistically significant finding here should be considered suspect. The strength of the approach presented in these guidelines is that of broadly integrated measures.

5. P. 31 (line 9): Press (TYPO).

6. P. 34 (under incoordination): Why isn't righting reflex included as a technique? It is simple, well established, and sensitive.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
Washington, DC
June 2-3, 1992

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The draft guidelines for neurotoxicity risk assessment are well written and reflect current understanding of neurotoxicity testing. I appreciate the opportunity to review and comment on the guidelines. My comments relate my experience with guideline neurotoxicity testing (TSCA and FIFRA) to the principles espoused in the guidelines, discuss a few concerns, and suggest ways that the guidance may be modified to improve consistency.

Interpreting Functional Observational Battery Data

Behavioral screening data are relatively imprecise compared to neurochemical, anatomical, or electrophysiological data, and alterations in one or a few endpoints rarely lead to a diagnosis of neurotoxicity. Guidance for interpreting functional observational battery data should discuss the concept of functional domains of the nervous system since alterations in functional domains form the basis for interpreting the absence or presence of neurotoxicity. Grouping the data from the FOB and motor activity measurements into functional domains is a generally accepted practice and has recently been adapted for statistical analysis by investigators in a number of laboratories. This grouping technique is useful when separating changes that occur randomly or in conjunction with systemic toxicity from those treatment-related changes that are indicative of gross alterations in nervous system function. For example, a number of statistically significant findings from the isopropanol 13-week vapor inhalation neurotoxicity study were not considered to be exposure-related or neurotoxicologically significant based, in part, on the lack of a demonstrated pattern of effects in one or more functional domains of the

nervous system (Table 1). A discussion of functional domains and the importance of establishing patterns of effects within domains should be included in the guidelines. This will strengthen the guidance for data interpretation and minimize the potential for overemphasizing nonspecific findings common to screening studies.

Interpreting Motor Activity Data

I strongly support the discussion on diagnosing neurotoxicity in the presence of systemic toxicity and would like to relate it to motor activity measurements in light of the results of the 13-week triethylene glycol monomethyl ether (TGME) neurotoxicity study. A high dose of 4 g TGME/kg/day in the study resulted in decreased mean body weight and food consumption throughout the study and decreased motor activity during the latter half of the study (Table 2). There were no FOB findings, clinical signs of toxicity, or neuropathology findings to support the conclusion that the motor activity findings represented a direct effect of TGME on the nervous system. Alternatively, the effects on motor activity may have been secondary to the systemic toxicity indicated by changes in body weight and food consumption. It would be inappropriate to consider TGME to be a neurotoxicant in light of these confounding effects and the high dosages used. These data underscore the importance of evaluating the data set for supporting evidence of neurotoxicity as well as for signs of systemic toxicity.

General Comment on Interpreting Screening Data

High dose levels are required for the current screening studies to demonstrate either toxicity or neurotoxicity. Indeed, a dose level above generally accepted limit doses was required for the 13-week TGME neurotoxicity screening study. An ideal screen would be sensitive and specific. Unfortunately, the high doses required for the neurotoxicity screens result in an increase in apparent sensitivity while forfeiting specificity. A comment should be added to the guidelines that recognizes the impact of toxic doses on test specificity.

Reversibility and Adaptation versus Neurotoxicity

The determination of whether reversible functional effects reflect neurotoxicity should consider the nature of the test substance, the effects observed, and knowledge of the potential mechanisms for these effects. Reversible effects do not reflect neurotoxicity if the effects are generally expected to be reversible at the biochemical level based on knowledge of the test agent or class of test agents under investigation. The reversible apparent sedation of the central nervous system following a single 6-hour exposure to high vapor concentrations of isopropanol is one such example. The nature of the effects was consistent with expected profiles for short chain aliphatic alcohols. In addition, the time course of the effects paralleled the time course of disappearance of isopropanol from blood following vapor inhalation.

Unfortunately, limited information will be available regarding the mechanism of action for most test agents to be screened for neurotoxicity, and it will not be possible to conclude that reversible functional effects reflect reversible biochemical events. A more conservative diagnosis of neurotoxicity will be needed in these cases when significant reversible effects are detected.

Adaptation following repeated exposure may be evidence of permanent molecular and structural changes in the nervous system unless information is available to support pharmacokinetic alterations. Molecular or structural changes may alter the organism's ability to respond to challenge and should be considered to represent potential neurotoxicity.

3.2.4.3 Schedule-Controlled Behavior. A rewording of the second sentence in the first paragraph of this section is recommended since schedule-controlled behavior tests may be used to measure learning, memory, or performance depending on the design of the test. Suggested change: add "memory, and/or performance" after "learned behavior."

3.3.1 Statistical Considerations. The first two paragraphs of this section infer that a minimum statistical power is necessary to detect a true effect of an agent and that the absence of this minimum power will jeopardize the usefulness of the study. As indicated, power

depends on the variability of the behavioral measure and the sample size. Further, it is generally recognized that behavioral measures are inherently variable, and large sample sizes may be necessary for some behavioral measures to satisfy a common power requirement of 0.8. The authors of the recent FIFRA guidelines for neurotoxicity recognized this and omitted a specific power requirement in order to limit the number of animals in these studies to an ethical and manageable level. A discussion that recognizes the variability of behavioral measures, the limits placed on the number of animals used in these studies, and the potential for decreases in statistical power should be included in the guidelines.

3.3.4 In-vitro Data in Neurotoxicology. The second sentence in the last paragraph of this section should mention differences between the intact organism and the *in-vitro* system. I suggest the following change in the text: "This validation process requires consideration in study design, including defined end points of toxicity and an understanding of how a test agent would be handled by a system in comparison to the intact organism."

5. Adequacy of The Evidence for Hazard Identification and Dose Response Assessment. Page 52, line 2. The two sentences beginning with "Neurotoxicity..." and ending with "...well conducted study," contradict the previous discussions on data interpretation and should be modified. Taken literally, this passage says that a single statistically significant change reflects a hazard and should be used to estimate the risk from the test agent. Since these sentences could be interpreted out of context, this section must be changed in order to ensure consistent and appropriate guidance on data interpretation.

Table 7A. Sufficient Human Evidence. The approach taken to categorize the amount of information is not consistent throughout Table 7. This category also should include the cases when epidemiology or experimental studies provide sufficient evidence to judge that there is no neurotoxic effect associated with exposure.

Table 7B. This table argues to exclude the "weight of evidence" approach to interpreting neurotoxicity and infers that any study that was not performed in compliance with the current

guidelines would be inadequate to judge the neurotoxic potential of a test agent. However, there are test agents for which there are well conducted subchronic and chronic toxicity studies, and/or toxicity studies modified to address neurotoxicity concerns. Categorizing all chemicals that have not been through a guideline neurotoxicity screen as having "insufficient evidence" is inappropriate given the large number of industrial chemicals in commerce that need to be tested and our limited testing capacity. Clearly there are test agents with a sufficient weight of evidence for assessing human risk without additional data from guideline neurotoxicity studies. Screening methods must be allowed to evolve so that assessment of human risk can be improved. The inflexible position presented in the section on "insufficient evidence" may hinder the evolution of screening methods by occupying available laboratory resources with guideline neurotoxicity studies for years to come.

In conclusion, the draft guidelines for neurotoxicity risk assessment are well written and reflect current neurotoxicity knowledge. Thank you for the opportunity to review the guidelines, and I hope that my comments and suggestions are useful.

Table 1.

Functional Observational Battery Findings for Male and Female Rats Exposed to Isopropanol for 13 Weeks

Group (ppm)		500	1,500	5,000
Pre-exposure				
Grip Strength (hind)	Decreased	M	M	
Study Week 2				
Rearing Events	Decreased		F	F
Study Week 9				
Tail Flick	Decreased		F	
Pupil Response	Decreased	M		

F, female; M, male; M or F notations indicate statistically significant differences from the control group.

Table 2.

Body Weight, Food Consumption, and Motor Activity for Male and Female Rats Treated with 4 g/kg/day Triethylene Glycol Monomethyl Ether for 13 Weeks

		Body Weight	Food Consumption	Motor Activity	
	Study Week	Percent of Control			
Male	1	92*	78*	93	
	4	90*	88*	89	
	9	82*	89*	71*	
	13	79*	83*	82	
Female	1	100		88*	95
	4	96		86*	83
	9	95		91	84
	13	92		85*	70*

*Mean of the absolute value of the measurement was statistically significantly different from the mean for the control group.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
Washington, DC
June 2-3, 1992

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Panel 1: Neurotoxicity as an Appropriate Endpoint for Risk Assessment

The conclusion is correct, but perhaps could use more support in the body of the draft. The inclusion of the term environmental is not fully understood.

Conclusions and Suppositions

The linking supposition, that neurotoxicity causes the failure of the normal functioning of the nervous system, is omitted. In addition, the word "proper" is not defined. Attempts within the draft to do so allude to the notion of a baseline, which then requires a notion of how far the deviation of that baseline can be before normality is exceeded. Perhaps for the purposes of the draft, methods that specify that (given the amount of information on hand) an event exceeding the likelihood of $p<0.05$ (two standard deviations, etc.) may be used to define a deviation from normal functioning.

Many agents cause neurotoxicity—fuller citation would help. The best evidence of a need for neurotoxicity risk assessment is the historical record of neurotoxic events, perhaps placed in context with other endpoints. There is a minimal effort in this regard on pp. 7-8. Human exposure to neurotoxic agents either is or can be significant.

Areas of Special Concern or Focus

The amount of *neuro*toxicological information is inadequate only in that enough is not available to determine if most agents are safe at current exposure levels and conditions. There is enough toxicological information available to suspect many agents will be neurotoxic, especially in the developmental area.

Clearly standards have not been set. While this manuscript establishes a number of reasonable endpoints, it does less to provide "information that will be useful for the evaluation of the data." (p. 1, last 4 lines).

Panel 2: Interpretation of Transient Data

The problem with the position statement is that it says that *all* effects are adverse (since effects must either be reversible or irreversible). Further exclusion is required.

Conclusions and Suppositions

The first conclusion should be completely reworded. The nervous system is not composed of wires, resilient complex patterns do not appear helpful in detecting effects, and the processes of compensation and adaptation are too poorly described to use as a reason to study transient effects.

The basis and linkage for this supposition are poorly established. It does not follow the first supposition. The scientific basis for this statement, in particular reserve capacity, will have to be provided.

Areas of Special Concern or Focus

Given the concern with secondary effects, this supposition must be withdrawn. Acute exposures can be lethal through primarily neuronal effects. These effects, therefore, must be studied in lower dose ranges.

These effects, known as historical determinants in behavioral pharmacology, are well-established and clearly reveal changes in function. Their neuronal basis is not well-established.

This question is of concern, both because more data are needed to evaluate it and that different agents may result in different life-time temporal patterns. As stated in the text, and as applies throughout, each agent must be evaluated on a case by case basis.

Panel 3: Indirect and Direct Effects Should be Considered Neurotoxic

Agents produce neurotoxic effects through indirect as well as direct means. It seems that the issue addressed here is whether the inability to adapt because of exposure-related effects is mediated by neuronal processes. Clearly other processes can be affected. The inability of baroreceptors to respond to change may be due to a non-neuronal toxic effect, leading to neuronal damage (related to issues in the second area of special focus in Panel 2).

Conclusions and Suppositions

What distinctions sometimes exist between direct and indirect and primary and secondary. It would seem that they are either the same or not.

The second supposition, while true, excludes many mechanisms employed even in the example provided, not to mention an extremely wide range of other possibilities. Does the occupation of receptors result in a toxic effect, or the subsequent actions at channels, within the

cell, etc. Glutamate is a great example. Since it may function as a neurotransmitter at lower levels, risk assessments will have to stay above these. However, it does not pass the blood brain barrier.

If cell death is the functional equivalent, this can be true. However, this supposition appears to be some sort of end run to lump all neurotoxic effects together, and will require more than a little discussion.

There appear to be two logical exceptions to this supposition: (1) neurotoxins, as defined in the text are different than neurotoxicants, and (2) dose, a compound can produce neurotoxicity (at higher doses), but may have normal functioning, therapeutic, or other desirable effects at lower doses. As we learn more about peptides and other modulators, dose-effect functions not normally observed in pharmacology and toxicology have emerged, which require further understanding rather than rule-governed simplification.

Again, this may not always be the case, although an example at the moment escapes me. Essential minerals cannot be maintained at too low of a dose, without neurotoxicity occurring either. Also, for many agents, with increases in dose, the cause of death may not be related to neurotoxicology—cardiac failure for example.

Areas of Special Concern or Focus

This is true and why many of the endpoints chosen are practical.

Given that an agent is not regulated by systemic toxicity, and produces neurotoxicity, risks clearly should be assessed. It may be inappropriate to dismiss a candidate from regulation because it produces primary toxicity on some other system. Risk managers may want to take into account that risks across several categories of endpoint (cancer, systemic, etc.) increase the likelihood of effect of any one endpoint.

Panel 4: Extrapolation from Animal to Human

For neurotoxicity, where direct examination in humans frequently will not be possible, the position reached is much too apologetic for the presumed adequacy of animal research. The necessity to actively pursue characterization of neurotoxic agents in animals is based on a lack of valid alternatives. These risk assessments are valid, period. On the other hand, the discussion of validity in the draft seems wordy.

Conclusions and Suppositions

While this supposition is true, it opens the door for further questions. The relationships between dose and effect are by and large similar, similar routes can be studied, while other disciplines over-extend to use words like model, this supposition is much too shy.

True, more could be said. Since the nature of risk assessment is precision, and precision cannot be obtained with epidemiological studies or case reports, it seems the only approach.

The same things can be measured in humans and (other) animals. One could even say everything could be measured, but this might be a bit too strong. Again, this sounds too weak, even at "many procedures." A similar point is made as supposition 3 in Panel 5. The use of the word model is offensive. Neuronal degeneration in an animal is not a model of what happens in a human, it is neuronal degeneration. Its effect may be used as a model of Parkinsonism, etc. but if differences exist it will only be a partial model. If none exist it will be the same.

I suspect that the range of uncertainty factors will not always be the same. Part of this concern is that little is actually known for the animal-to-human factors for particular agents. One area in particular may be solvents, where uptake to steady-state tends to equilibrate physical differences in size etc., and respiratory rates may actually shift things to the animal being more sensitive. This is one area (neurotoxicology) where such issues can be directly compared, if sufficient human data exist. Thus a basis for safety (fudge) factors can be established from data rather than an assumption initiated long ago.

Areas of Special Concern or Focus

While I concur with the spirit of the language (verbal behavior) comment, there are clearly studies that show that chemical exposures in rodents and primates increase rates of well-defined and communicative vocalizations. I question the relevance of the statement to risk assessment. On the other hand, if an agent were shown to produce a cerebral stroke that affected speech, and that agent had no effects on other species, a basis for the focus would be substantiated. I know of no such agents.

While this supposition is true on occasion, it should not negate the process of risk assessment with animals. The possible exceptions are chemicals for which metabolism or kinetics in humans are unique and toxic. Like the point above, specific examples must be brought forth to hold these points up, otherwise speculation retards the ability to gather what meaningful data exist.

The most sensitive species exception also detracts from the process. There always will be these exceptions. If metabolism in these species is unrepresentative (i.e., producing a unique toxicant, etc.) then they shouldn't be used. If they represent different rate constants, levels, etc., then they should because then they are merely a biological extreme.

Panel 5: Interpretation of Behavioral Data

Behavioral change, or perhaps more importantly the lack of behavioral change, under challenge conditions, as a result of chemical exposure can be a clear indication of neurotoxicity. However while behavioral change alone can provide evidence of neurotoxicity, transient behavioral change is insufficient to conclude neurotoxic effects have occurred.

Conclusions and Suppositions

Behavior is not always the most sensitive indication of toxicity.

While evaluation of behavior should play an important role in efforts to understand brain function, it rarely is employed. There are many reasons for this. Many basic neuroscientists are often not equipped or do not understand behavior. Others simply avoid the issue of relevance. When behavior is studied, it is often at a primary screening level (i.e. locomotor activity, classical conditioning effects).

Some behavioral evaluations have animal counterparts (i.e., startle) not most. Some are verbal.

Probably false if extended to neurochemical effects. The issue rests with the ability to measure physiology together with behavior, and the definition of "behavioral effect." The state of the art is not advanced now, but may be by the time these guidelines are implemented. For morphology, behavioral change may be more sensitive than gross "holes in the brain," but less than growth of dendritic processes.

That the primary developmental effects of some chemicals may be behavioral seems argumentative. Very little behavior is studied in utero. This may mean that correlates or neurochemical determinants of in utero effects have not been found or explored. However, there is no question that there are developmental neurobehavioral effects of some chemicals, and theses effects alone are a basis for regulation.

Areas of Special Concern or Focus

While true, "indirect" may be preferred over "non-specific." Examples may include conditioned sickness, which can impair normal behavioral functioning without neurotoxic effects (i.e., conditioned gastrointestinal effects).

A maximum tolerated dose for behavioral function rests on the ability to assess the adaptability of the system as a function of dose.

Although behavior can change due to physiological effects (see panel 3), this is not necessarily a basis for excluding behavioral changes associated with more toxic effects (i.e., a false positive).

Premeeting Comments for
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Washington, DC
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General Comments

The *Proposed Guidelines for Neurotoxicity Risk Assessment* contained an extensive review of the actions of various classes of neurotoxicants in humans and in experimental animals. The studies presented were organized according to the guidelines established by the National Research Council. Data relevant to hazard identification, dose-response assessment and exposure assessment were discussed and brought together in a concluding two page summary of some of the issues to be addressed for the risk characterization of neurotoxicants.

Within an overall exposure-dose-response paradigm, there are key gaps in the current knowledge of the pharmacokinetics and mechanisms of action of neurotoxicants that require additional research with prototype agents in both experimental animals and appropriate *in vitro* cell models. *In vitro* models should be used to support studies on the mechanisms of action and not necessarily developed as short-term test systems for neurotoxicity. The strength of *in vitro* approaches is the elucidation of specific molecular and biochemical events evoked in a surrogate target cell by a potential neurotoxicant. Recent advances in molecular neurobiology have resulted in the cloning of ion channel proteins, receptors for neurotransmitters and regulatory proteins involved in signal transduction; the elucidation of the role of immediate early genes in memory and responsiveness to environmental stimuli; and the implementation of cloning strategies for genes involved in certain neurodegenerative diseases.

Using physiologically based pharmacokinetic approaches, models that incorporate knowledge of the biological determinants of tissue dose for volatile agents, and certain

chlorinated aromatic compounds have been developed and used to predict accurately tissue concentrations at low exposure doses. Similar approaches are needed for ongoing pharmacokinetic studies of neurotoxicants. Existing analytical techniques should permit experimental validation of models that incorporate biological determinants relevant to the distribution and/or metabolism of compounds in potential target cells within the nervous system.

Application of contemporary cell and molecular biology approaches to the study of nervous system function provide the opportunity to gain new insights into the molecular mechanisms of action of known neurotoxicants. Comparative analysis of the actions of these compounds in experimental animals and in human and animal cell culture models should be focused on elucidation of the biological determinants of target cell- and species-specificity. Integration of molecular, cellular and tissue dosimetry models for specific nervous system endpoints with physiologically-based pharmocokinetic descriptions of tissue dose at environmentally relevant exposure levels are essential for the development of biologically-based risk characterizations for neurotoxicants.

Specific Comments

Endpoints of Neurotoxicity. Table 1 lists several endpoints of neurotoxicity within five categories. The challenge is to develop a short list of quantifiable endpoints that represent potential adverse changes in the structure or function of the nervous system likely to occur as a result of chronic low level exposure to environmental agents of significant concern. It is necessary to distinguish between reversible changes with no demonstrable adverse clinical outcome versus those changes that are measurable, but based on solid experimental evidence, not linked casually to a nervous system lesion. Molecular probes currently available and the development of with well-characterized animal and cell culture models for the study of nervous system function offer the potential for detailed analysis of the mechanisms of toxicity of certain classes of neurotoxicants that act on specific protein targets within the nervous system (e.g., neurotransmitter receptors, and signal transduction proteins). The sensitivity of detection methods using DNA and/or antibody probes allow identification and quantitation of specific changes in the level or function of these proteins in specific cell populations at low

neurotoxicant exposure concentrations. Integration of studies in *in vitro* cell systems and animal models are needed to determine the linkage of early events to nervous system dysfunction. These systems also could be used to study the modulating influence of confounding factors on neurotoxicants. However, like the nervous system itself, the interaction of these factors with a given neurotoxicant is complex. For example, confounding factors such as alcohol consumption can influence the metabolism and distribution of the neurotoxicant, as well as alter its action on a target cell within the nervous system.

Pharmacokinetics. The pharmacokinetics of neurotoxicants, particularly at low exposure doses, should be an area of increased emphasis. Advancements in physiologically based pharmacokinetics provide a foundation for detailed study of the uptake, distribution and metabolism of potential neurotoxicants. Models can be developed based on increased knowledge of the xenobiotic metabolizing potential of various cell populations within the nervous system and the various components that control circulating concentrations and tissue localization of neurotoxicants; e.g., hepatic metabolism and elimination, blood flow to target tissues, the blood-brain barrier, brain lipids, and high affinity binding to target proteins. Comparative analysis of these parameters should be carried out across species, using both *in vivo* and *in vitro* models. For studies focused on low level exposures, available data on the metabolism and elimination of a given neurotoxicant in humans need to be considered in deciding on relevant exposure levels in experimental animals. It is important that known differences in the metabolism and elimination of xenobiotics in humans versus rodents be incorporated into pharmacokinetic models for predicting the concentration of a given neurotoxicant in a nervous system target tissue.

Risk Characterization—Concluding Comments. The goal of risk characterization is to develop a biological mechanisms-based risk assessment that incorporates knowledge of the biology of the target organ(s) of interest. Application of contemporary molecular biology approaches to the study of neurotoxicants should be focused in large part on elucidation of the biological determinants of tissue- and species-specific responsiveness of nervous system targets. Quantitative descriptions of low dose behavior of neurotoxicants will require the development of physiologically based pharmacokinetic models. Linkage of physiologically-based tissue dosimetry models with quantitative descriptions of relevant molecular and biochemical events elicited by

the interaction of a neurotoxicant with a nervous system target tissue are key elements in a biologically based risk assessment strategy. Given the complexity of the nervous system and the large number of neurotoxicity endpoints, it is essential to prioritize efforts and focus on endpoints that are quantifiable and linked to a clinical outcome relevant to environmental exposure scenarios of concern.

Premeeting Comments for
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Comments

In reference to structural endpoints of neurotoxicity, it needs to be made clear that such perturbations may not be evident at early exposure times or at low doses with the general type of neuropathological screening procedures employed. A more detailed evaluation may be needed for detection. Cellular alterations such as cell death and cellular organelle restructuring may be a late event in the toxicity response. This does not rule out the possibility that lower exposure levels are producing neurotoxicity. The sensitivity of each detection method used must be taken into consideration when evaluating site of structural perturbation.

Developmental exposure to a compound and alterations in structural endpoints must be evaluated in the presence of normal cellular restructuring during the process of development. The ability to detect a perturbation may be limited; however, the perturbation may indeed be more detrimental. Such is the concern for evaluating GFAP reactivity in the nervous system during development since an increase in GFAP is seen during the normal process of development indicating a differential role for the protein in the developing versus mature animal. Similar concerns exist with evaluations of neurochemical and electrophysiological endpoints during the developing process. Evaluation of the mature system following developmental exposure would not be limited by such concerns but would examine the long-term consequences of such exposure. For these reasons, it is felt that the problems of evaluating alterations in the developing organism must be fully understood when the data are to be used to evaluate risk.

One model of exposure that should be considered is the situation where the person is removed from the exposure environment to allow for reported symptoms to subside and then placed in a similar exposure environment. Evaluation of increased sensitivity following re-exposure could be used to determine the long-term compromise of the nervous system and may offer information to be used in evaluating risk.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
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The document uses the Nuclear Regulatory Commission (NRC) four-stage approach to risk assessment: hazard identification, dose-response assessment, exposure assessment, and risk characterization. The emphasis of the document is on hazard identification of neurotoxicity with some reference to dose-response assessment, but very little discussion about exposure assessment and risk characterization. Perhaps the major weakness of the document is that it lacks specificity in its recommendations. Although there is general discussion of how different kinds of data would be interpreted by the U.S. Environmental Protection Agency (EPA), the information is not presented in any detail. Although many measures of central nervous system (CNS) function and integrity are discussed, it is never clear exactly what tests or methods would actually be recommended or required by EPA when a company wished to market a new chemical. Nevertheless, the document is a good starting point for more detailed discussion of neurotoxicity testing.

An immediate problem arises from the decision to pool all functional and anatomical changes produced by chemicals into a single class, which they define as neurotoxins. This decision forces EPA to classify chemicals that produce frank irreversible CNS lesions in the same category as short-acting chemicals, whose effects may be serious in some situations, but whose effects are readily reversible upon discontinuation of exposure. By defining all chemicals that affect either the morphology or the function of the CNS as neurotoxins, it makes it likely that a chemical with modest short-term behavioral effects will be inappropriately branded as a neurotoxin. In my opinion, EPA should consider at least two categories of definitions. For example, definitions might be made on the basis of chronicity of effects (e.g., chronic

neurotoxins versus acute neurotoxins, or reversible versus irreversible neurotoxins), or anatomical versus functional effects (e.g., neurotoxins versus behavioral toxins). This would prevent a short-term behavioral change based on the odor of a chemical from being considered with the same level of concern as a chemical that produces widespread lesions in the brain.

Table 1 presents an impressive list of potential endpoints for the measurement of neurotoxicity. Any chemical making it through the battery of tests listed in this table would certainly have a high probability of receiving a clean bill of health, provided that the studies performed were of high quality. Is the purpose of the table to suggest a model test battery for neurobehavioral toxicity testing or is it merely listing a range of tests that could provide useful data about the potential neurotoxicity of a chemical? Perhaps I don't understand the purpose of the document, but someone in charge of setting up a neurotoxicity test battery for a company would not get much guidance about what kinds of testing should be conducted based on this document.

A related problem is that the document does not define what constitutes an acceptable study and whether or not all kinds of information will be treated identically. Will animal studies require placebo controls? Should active placebos be used? Should FOB be done by "blind" scorers? Are behavioral endpoints to be weighted the same as neurochemical endpoints? These and similar questions are not addressed.

A major strength of the document is the attention that it gives to functional testing and to the transient effects of chemicals. It is very important to recognize that transient effects of chemicals on behavior are important indices of toxicity, even though they may be reversible on discontinuation of exposure. Although it is difficult to argue against giving our most serious concern to those chemicals that cause permanent lesions in the CNS and produce profound functional consequences, the document recognizes, perhaps better than any similar document, the great importance that chemicals can have in producing transient functional effects. A chemical that slows reaction time, affects intellectual functions, or has other behavioral effects during and perhaps for a short period after exposure, can have devastating consequences for someone driving an automobile or operating dangerous machinery. It is very important that EPA has recognized this and is attempting to screen for these effects.

EPA also should be congratulated for choosing a broad range of functional endpoints on which to base reference doses. This is particularly true with respect to behavioral endpoints. Unfortunately, there are certain issues concerning behavioral endpoints that have been raised repeatedly by those who resist this type of testing. Although I have discussed some of these issues previously (McMillan, 1986; 1990), a few of them bear mentioning here. An issue certain to be raised is the interpretation of behavioral changes following exposure to a chemical. For example, does a change in motor activity during or following chemical exposure represent toxicity? Does a decrease in reaction time, or an improvement in memory represent toxicity? My opinion is that any change in a behavioral baseline from "normal values" represents a behavioral toxicity. This is especially true when it is recognized that the exposed population has not chosen to have their behavior altered by chemicals, but rather the population has involuntarily been exposed to a chemical that changes behavior.

A variation on this theme is that given enough of a chemical, all chemicals affect behavior. Therefore, all chemicals become "neurotoxins" at some dose and for this reason testing for behavioral toxicity is not very useful. Although the axiom that all chemicals can produce behavioral effects at some dose is probably true, this hardly constitutes a reason for challenging the importance and validity of behavioral testing. The same issues can be raised about many types of toxicological data, such as chemical-induced changes in the immune response or induction of P 450. Since all of these effects can represent responses to the stress from a chemical, perhaps with the consequence of a limited capacity for responses to further stressors, they all denote toxicity. The dosage issue can be handled adequately by other components of the NRC approach, such as dose-response assessment, exposure assessment, and risk characterization. If a chemical produces behavioral effects at doses far above those that affect other endpoints, the behavioral effects will be of little importance in establishing reference doses.

Yet another variation on this theme concerns the specificity of neurobehavioral effects. For example, a chemical may produce liver damage, leading to illness, which can be manifested in behavioral changes. It is argued that such effects should not be labeled as neurotoxic effects. I agree; however, it seems likely that indirect behavioral changes produced by chemicals can serve as markers for the general well being of the animal. It would seem that the onus of

proving that these indirect changes in behavior do not represent primary behavioral toxicity, or primary neurotoxicity, is upon the company wishing to market the chemical. It is interesting to note that the writers of the current document appear to be reaching this same conclusion (p. 37) for behavioral measurements, yet they deny that it applies for neurochemical measurements (p. 23). Why should neurochemical and behavioral changes be treated differentially?

There are several minor points in this section that need to be reconsidered. At the end of paragraph 2 on p. 31, the final statement reads that although schedule-controlled behavior has been used to study drugs in humans its use in "toxicology *is* limited." The verb should be changed to *has been*. Granted there are ethical problems with exposing humans to most neurotoxins to study their effects on behavior prospectively. However, the techniques may be quite useful in the study of occupationally or environmentally exposed populations. This remains to be explored.

On p. 32, paragraph 1, it is stated that behavioral changes may indicate neurotoxicology if they are *not producing* concurrent alterations in motivation, or overt signs of toxicity. I see no reason for these exclusions. Clearly motivation is an important aspect of behavior (e.g., the purported amotivational syndrome produced by marijuana). Most psychologists would consider motivation an important determinant of behavior and a function of the CNS. The exclusion of neurotoxicity when there are "overt signs of systemic toxicity" also seems unreasonable. Is it not possible to observe important neurobehavioral toxicity concurrent with toxicity in other systems? Again I believe that the task of determining whether behavioral toxicity is a primary effect, or is a secondary effect from other toxic effects should fall on whomever is responsible for providing the toxicity test data. Similarly, on p. 32, paragraph 3, the statement appears to rule out any interpretation of the data as showing neurobehavioral toxicity when body weight changes, or other signs of systemic toxicity occur. This is inappropriate. For example, hypothalamic effects of a chemical may reduce appetite and food intake, resulting in a weight loss. This is neurobehavioral toxicity. When behavioral effects occur concurrently with other kinds of toxicity, the task becomes one of determining the relationship among these effects. It does not mean that behavioral forms of toxicity should be ignored, just because other forms of toxicity can be documented at the same dose or exposure level.

The final paragraph on p. 31 makes a confusing assumption about the relationship among variability, sensitivity, and specificity (the latter term is not discussed). The implication of the paragraph seems to be that the lower the variability of a behavioral test, the lower the sensitivity of the test to disruption by extraneous variables (e.g., chemicals). This is not necessarily true, especially when chemicals produce specific effects on a behavior. For example, one might train animals to respond under a multiple schedule with components A and B. Behavior in component A might show much lower variability than behavior in component B, but the behavior in component A might be much more sensitive to the effects of a given chemical, especially when the chemical *specifically* affects the behavior maintained in component A. I would suggest elimination of this paragraph.

The section on developmental neurotoxicity is a welcome addition to a document of this type. Generally, this section is well developed, but some discussion about control groups would be a useful addition. It is now state of the art in developmental toxicology to use pair-fed controls and to use the technique of cross fostering to control for possible post-partum maternal effects. Are such controls needed in developmental toxicity studies? It could be argued that these techniques are not needed to establish reference doses, but are needed for more mechanism-oriented studies.. This is a debatable point. The issue of appropriate ages for testing in developmental studies also might be worth discussing.

The document appropriately emphasizes the importance of dose-effect data in neurobehavioral toxicity testing; however, it is not clear when dose-effect data should be collected in relationship to the collection of other toxicity data. Should neurobehavioral toxicity testing be independent of and proceed in parallel to other types of toxicity testing? Should neurobehavioral data be used to establish dose levels for other types of toxicity testing or vice versa? Does one proceed through a neurobehavioral screen in a hierarchical order, or should an entire screen be conducted at one time? The issue of when testing does occur, what gets tested in what order is an important issue.

The sections on exposure assessment and risk characterization are perfunctory and might be expanded. Are there any special issues that need to be considered with regard to neurobehavioral toxicity testing as regards exposure assessment and risk characterization? The

goal of replacing the current unscientific "safety factor" approach in establishing a reference dose with a more scientific approach based on pharmacokinetic and pharmacodynamic observations is strongly encouraged. The present method for calculation of reference doses based on the use of arbitrary safety factors is not based on good science and should be replaced as soon as possible. The assumptions in using a "factor of 10" as a margin of safety have not been adequately documented, although the data base for doing so is almost certainly available in the literature for some chemicals.

With respect to issues related to the extrapolation of animal data to humans, there is a growing literature on species scaling that should be consulted. It might be argued that EPA should make some specific recommendations about the use of different species in toxicity testing. For example, are data on neurotoxic effects in fish suitable for establishing reference doses? Should all neurobehavioral toxicity testing require that some tests be performed in at least two mammalian species to increase the generality of the findings?

The perspective of the document on *in vitro* testing is quite appropriate. Although such tests can provide clues as to the possible toxicity of chemicals, such methods cannot model the metabolic activation of toxins and probably never will be able to model the complex interactions of the nervous system that result in behavior. This is especially true when one considers how behavior develops from "experiences" of an organism interacting with its environment. Similarly, computer models are not useful at this time in predicting neurobehavioral effects of chemicals. Even if such models develop sufficiently to make some useful predictions, these predictions will have to be validated by animal testing. Neurobehavioral toxicity testing (and in fact all of toxicity testing) will have to rely on whole animal experiments for the foreseeable future.

REFERENCES

1. McMillan, D.E. (1986). Risk assessment for neurobehavioral toxicity. Envir. Health Perspect. 76: 155-161.

2. McMillan, D.E. (1990). The pigeon as a model for comparative behavioral pharmacology and toxicology. Neurotoxicol. Teratol. 12: 523-529.

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Neurotoxicity as an Appropriate Endpoint for Environmental Risk Assessment

The neurotoxicity risk assessment process should include the concept of developing an efficient method for reasonably assuring the public that a chemical does not present a human health concern at environmentally relevant exposure levels. The public should not be led to believe, however, that any evaluation method can absolutely ensure that a chemical can or cannot be neurotoxic under all circumstances of exposure. As the draft guidelines indicate, most of the data that will be available for analysis will be from test animals, not humans. Specific neurotoxicity data for the species of concern (humans) will, therefore, most often not be available. The public also should not be led to believe that large numbers of neurotoxicants are present in the environment. The available data do not support such a conclusion. Such a position leads to unnecessary and potentially harmful public anxiety.

Reports about the number of neurotoxic chemicals frequently overestimate the number of chemicals that need specific testing. For example, such reports frequently cite the number of chemicals on the TSCA Inventory or the number of Pre-Manufacture Notices as evidence that there are vast numbers of chemicals that could be neurotoxic. But inspection of such lists shows that the number of chemicals available that could be neurotoxic or result in environmental exposure are much smaller. Some reports cite the number of chemicals that do not have specific neurotoxicity tests as evidence that there are many undiscovered neurotoxicants. These presentations usually ignore the likelihood for potential contact with chemicals at significant exposure levels and the role that routine screening tests play in identifying a variety of chemical toxicities, including neurotoxicity. Often these reports suggest that most or many neurotoxicants

were first discovered because of human poisonings; such assertions are not supported by the data and become even weaker if the neurotoxicants discovered before the advent of modern scientific practices are eliminated from consideration.

Current estimates of the number of potential chemicals that might be neurotoxic range from approximately 5 to 30 percent. If the upper limit of these estimates is accepted as accurate, then the testing of all chemicals in a large series of specific neurotoxicity tests would be inefficient because at least 70 percent of the chemicals would not be found to be neurotoxicants. Thus the use of screening tests to indicate which chemicals need specific neurotoxicity testing and formal risk assessments becomes very important and could save significant resources that could be used to control other more significant environmental risks.

Interpretation of Neurotoxicity Data When Effects Are Transient

In considering whether or not reversible or irreversible effects should be considered adverse it is important first to consider whether or not the endpoint itself should be considered evidence of neurotoxicity. When considering whether or not behavioral changes are adverse, it is important to acknowledge that behavior is the end result of an organism's interaction with its environment. Observation of a behavioral change is not necessarily a signal that something adverse has occurred, rather it is an indication that the organism has reacted to a change in its environment. The response of the organism may be considered positive, neutral, or negative depending on other external factors that need to be considered when evaluating the observed response. Therefore, interpretation of behavioral changes, many of which will be reversible, needs to be considered very critically. Effects that are considered trivial should not be given the same weight in assessing risk as those effects that are considered serious.

While neurons that are damaged severely are considered to have limited capacity for regeneration, there is no reason to believe that neurons that are involved in readily reversible functional changes suffer a similar fate. Likewise, recovery from readily reversible functional changes would not be expected to (1) represent activation of reserve neural capacity, (2)

decrease the potential of the nervous system to adapt to future challenges, or (3) result in changes that would later be revealed through an environmental or pharmacological challenge.

Agents Acting through Indirect and Direct Means Can Be Considered Neurotoxic

The significance of direct versus indirect effects is a complicated issue that is closely associated with identifying what is neurotoxic and how the neurotoxicity testing guidelines actually are implemented. When chemicals have a direct effect on the nervous system, there is usually little disagreement about identifying them as neurotoxic. Some chemicals, like carbon monoxide, can have both direct and indirect effects on the nervous system for example by interfering with delivery of oxygen to the nervous system and interfering with respiratory enzymes in the nervous system. Such materials are routinely considered neurotoxic and are not confused with simple asphyxiants, such as nitrogen at normal atmospheric pressures, which are usually not considered neurotoxic even though exposure to them can cause behavioral changes.

Of much greater concern is how to interpret data derived from studies conducted at exposure levels that are systemically toxic and many orders of magnitude higher than anticipated environmental exposure levels. It is quite clear that chemicals given in large doses can cause behavioral changes by disrupting non-neural target tissues. Without invoking concerns about hepatic encephalopathy, it is an ordinary experience of most toxicologists to note that animals change their behavior when a chemical "makes them sick" by damaging the liver or other parenchymal organs. Chemicals that cause behavioral changes, even adverse ones, at dose levels that result in systemic toxicity should be controlled as systemic toxicants and not as neurotoxicants. To do otherwise would waste precious resources devoted to establishing chemical control procedures. In a clinical setting, we would chastise a practitioner who would warn a patient to be on the lookout for signs of convulsions and not jaundice when he/she knows that the patient will be nearly dead from liver failure before the onset of convulsions. Why in an environmental setting would we warn people about neurotoxicity when we know that liver toxicity is the critical endpoint and that if we prevent hepatotoxicity, we will prevent behavioral changes from occurring?

Much of the controversy about direct and indirect effects could be eliminated by recognizing that neurotoxicity tests should not be conducted at excessively high dose levels or dose levels that result in systemic toxicity. The practice of deliberately confounding neurotoxicity studies by using massive dose levels is one which should be discouraged. It only prevents the risk assessor from having a clear picture of the risks associated with exposure to a particular chemical.

Extrapolation of Neurotoxicity Data from Laboratory Animals to Humans

Uncertainty factors are used to express the degree of confidence that the no-effect levels that are determined in test animal studies are sound for determining safe human exposure. Generally, the less confidence one has in the data, the greater the uncertainty factor. The converse also should be considered when determining uncertainty factors: the uncertainty factors should be decreased as one becomes more confident in the data. As more and more sensitive endpoints are used to improve the reliability of the no-effect level determination, smaller uncertainty factors should be considered. In this way, there is an incentive to collect more and better data and thus provide better identification of materials that present potential human health problems.

There should be a significant concern about extrapolation of nonspecific effects in animal studies to humans. For example, in Table 1 of the draft, hemorrhage in nerve tissue, GFAP increases, increases or decreases in motor activity, and changes in brain weight are listed as examples of potential endpoints for neurotoxicity. Minor hemorrhage in nerve tissue is quite common as an agonal change in animals dying for a variety of reasons and ordinarily should not be regarded as evidence of neurotoxicity. Quantitative changes in GFAP should not be shown in Table 1 as an interpretable endpoint because there are insufficient data and experience with GFAP measurements to allow an understanding of what changes in GFAP mean. Motor activity also is an endpoint that can change because of nonspecific illness in an animal and therefore should not be considered as indicative of neurotoxicity.

The issue of brain weight is discussed in more detail on p. 14 of the draft guidelines. The draft states without support that statistically significant changes in absolute brain weight should not be discounted because relative brain weights appear normal. The draft also indicates that "the brain is usually protected in weight loss," although it doesn't indicate under what conditions it is not protected. Since most neurotoxicity studies are conducted during the growth phase of the test animals, concern about brain weight differences will be due to growth retardation, which can affect the ultimate size of the entire animal including the brain. There seems to be no basis for the draft to state without equivocation that a change in brain weight should be considered as an adverse neurotoxic effect.

Page 17 of the draft states: "An alteration in the structure of the nervous system is regarded as evidence of neurotoxicity." Alterations in structure, like other endpoints, should be interpreted carefully. While most alterations in structure may be considered adverse, all changes are not adverse. For example, antioxidants have been given to laboratory animals to reduce the deposition of lipofuscin in the brain. Such changes are usually interpreted as positive or useful changes and not neurotoxicity.

Page 45 discusses the interpretation of *in vitro* data in neurotoxicity risk assessment. The draft indicates that demonstrated neurotoxicity *in vitro* in the absence of *in vivo* data should be regarded as suggestive evidence of neurotoxicity. This conclusion is not consistent with the preceding discussion in the draft about the difficulties of interpreting *in vitro* studies. The draft also indicates that *in vitro* data confirmed by *in vivo* data are convincing evidence of neurotoxicity. The draft should indicate that for *in vivo* data to provide confirmation of *in vitro* data there should be a plausible biological association between the *in vitro* and the *in vivo* endpoints. In the absence of biological plausibility, the data sets should not be regarded as complementary.

On page 52, second paragraph, the draft seems to establish a new standard for determining that a chemical is neurotoxic in spite of finding that multiple individual studies are negative. The draft states: "In some cases, while no individual study may be judged sufficient to establish a hazard, the total available data may support such a conclusion." The rationale for this new standard appears to be given in the example on p. 52, which suggests that greater

concern should be felt for chemicals we know something about rather than those we know nothing about. The use of marginal data in this way does not appear to be appropriate.

On page 53, the draft appears to discount all studies that have not been conducted according to the EPA neurotoxicity guidelines. There is no basis for such a determination. Neurotoxicity studies have been conducted for many years in the absence of EPA guidelines, and there should be no scientific basis for the Agency to summarily dismiss the entire scientific literature developed either before or after the development of EPA guidelines. A better approach might be to describe the attributes of adequately conducted and reported studies and use these criteria as a test for the acceptability of data to be used in the risk assessment process.

Interpretation of Behavioral Data

Behavioral changes in toxicology studies often are seen as nonspecific endpoints, which generally require correlation with other endpoints before they can be considered evidence of neurotoxicity. Behavior is the end result of the many interactions the nervous system has with the environment. Increases or decreases in behavioral signs frequently indicate a response to a stimulus which allows an animal to adapt to its environment. In common with other endpoints that are considered sensitive, there should be a concern that these endpoints may be nonspecific and error prone, and their use in isolation might lead to incorrect regulatory classification of chemicals for neurotoxicity.

While behavior has been regarded by some as the most sensitive indicator of neurotoxicity, there is little evidence to support this premise, and there are substantial reasons to consider the issue irrelevant, because it is difficult to imagine how behavior could be the most sensitive endpoint for all types of neurotoxicity.

Sufficient Evidence to Categorize Neurotoxic Hazards

The criteria for classification in the "Sufficient Human Evidence" category are too broad and will force many nonneurotoxic chemicals to be improperly categorized. Before an epidemiologic study is used to determine that there is sufficient human evidence of neurotoxicity, there should be a rigorous review of all available data (including negative data) that should reveal (1) that the study meets the criteria being developed for Good Epidemiologic Practice and (2) there is biologic plausibility that explains why the observed effects should be considered causally related to a chemical exposure. The use of simple association with exposure as a criterion for the acceptability of epidemiologic data is inappropriate. Use of a case study to determine sufficient evidence is even more likely to produce errors in classification, particularly if the adequacy of "supporting data" is not rigorously defined. The "supporting data" for case studies should provide evidence of a biological plausibility between the effects observed and the observations made in the case studies. *In vitro* data and nonspecific findings in animal studies do not provide the types of data needed to support the validity of case study reports. The main support for the use of these types of data is based on the unsupported statement that "most neurotoxicants have been 'discovered' in humans," with the implication that chemicals, particularly man-made chemicals, are creating widespread neurotoxic disease.

Sufficient Experimental Animal Evidence

The discussion about what constitutes sufficient experimental animal evidence for classification includes the criteria for determining that a chemical is not a neurotoxicant. The risk assessment document should provide a clear category for such materials that are found to be not neurotoxic.

Insufficient Evidence Category

This category would contain materials for which data would not provide sufficient evidence for classification, but the text of the document also should discuss the lack of need for

extensive neurotoxicity studies for all chemicals. Chemicals that should be considered for formal risk assessment should be those that potentially present concerns to human health. Many chemicals are tested in routine toxicology tests that provide a first screen for neurotoxicity. Chemicals that are negative for neurotoxicity on routine screening tests should not be considered as insufficiently tested for neurotoxicity but rather they should be placed into a category indicating a low degree of concern.

Categorization in General

In order to address the needs of the public, the Agency should present factual information and minimize the potential for such information to be misused. The proposed classification schemes facilitate such misuse. Although the Agency has attempted to address this issue in the text, describing the "Sufficient" and "Insufficient" categories, the Agency's efforts fall short of what is needed. Many, particularly at the state level, and those pursuing legislation through the initiative process, will interpret the "Sufficient" category as meaning a hazard exists, rather than that "sufficient information exists to judge whether or not a human neurotoxic hazard could exist." I strongly suggest the title of the categories be changed to reflect the Agency's interest. A potential change might be "Sufficient/Insufficient Data to Proceed with Hazard Characterization." Ideally, categorization schemes should be eliminated. In the meantime, the Agency has an obligation to minimize the potential for misuse of the scheme.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
Washington, DC
June 2-3, 1992

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These comments are presented according to sections of the guidelines document.

2.1 Neurotoxicity

The definition of adverse effect is usefully broad. The listing of behavioral changes, however, should include: (1) motivational changes (2) degradation of skilled performance, and (3) degradation of decision quality as examples of adverse effects.

2.2 Neurotoxicant

There is a discrepancy between the number of types of validity and the number listed. Nonetheless, the comments on the different types of validity deserve a separate heading and consideration as a device to summarize the status of Sections 3.1 and 3.2. One could visualize the types of validity as a series of stages to the conclusion that a material is a neurotoxicant:

Predictive Validity

Concurrent Validity

Construct Validity

Content Validity

3.2.4.3 Schedule Controlled Behavior

I tend to think of simple schedules as the fundamental units from which the complex instrumental behavioral systems such as those used for tests of sensory systems, memory, and learning ability are synthesized.

Sections 3.2.4.3 and 3.2.4.4 could well be subcategories of a category called performance.

3.2.5 Developmental Neurotoxicity

Several of the desirable features listed on pp. 37 and 38 such as replicate study design and pharmacologic challenge also should apply to other neurotoxicity studies.

3.3.1 Pharmacokinetics

Eventually issues will need to be addressed in neurotoxicology that are similar to those addressed in the area of carcinogenesis risk assessment. Factors such as fraction of life span and relevant scaling parameters will need to be studied.

3.3.3 Statistical Considerations

The discussion of power appears to have something missing. To obtain an alpha of 0.05 and power of 0.8, you need to specify an effect size!

The issues related to repeated-measures statistics and the use of corrections for multiple comparisons deserve technical attention.

3.3.4 In-vitro Data in Neurotoxicology

This is a good discussion, however, the analog of the power issue is not addressed. Is lack of neurotoxicity *in vitro* to be considered evidence that a compound is not neurotoxic *in vivo*?

5. Adequacy. . . .

This section could be strengthened by working through a couple of examples for which some data are available.

Premeeting Comments for
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Section 3 Hazard Identification

The document does not discuss the application of tiered testing or the differences in informational value obtained at each level of testing in the assessment of neurotoxicity. There is no distinction between stage 1 screening and stage 2 or 3 in-depth neurotoxicity testing. Is it the intent to use or allow the use of screening (FOB) information as the basis for determining NOAEL or LOEL and/or for making risk assessment determinations?

Section 3.2.1 Structural Endpoints of Neurotoxicity

With regards to reductions in brain size, the absolute association of decreased brain size (particularly with reference to whole brain size) with neurotoxicity, regardless of the body size, is not warranted. While it may generally be true that "most of the body weight reduction reflects a loss of body fat" and that "dieters do not lose brain tissue," it is equally true to say that the brains of small people are not abnormally small. Body size must be taken into consideration. While an absolute reduction in (whole) brain weight in adult animals may certainly be regarded as a possible indication of neurotoxicity, a decrease in brain weight in-and-of-itself should not be taken as definitive evidence of neurotoxicity. However, if one considers the size of discrete brain regions (weight, width, or length), there may be more of a reason to argue that absolute reductions (or increases) in the size of specific brain regions may be associated with neurotoxicity regardless of body size.

Section 3.2.1 Structural Endpoints of Neurotoxicity

This section also should describe the nonneuronal components of the nervous system that also may be involved in neuropathological effects of chemical substances, i.e., the glial elements.

Section 3.2.1 Structural Endpoints of Neurotoxicity

Page 17, lines 21-25: "Since increases in GFAP may be an early indicator of neuronal injury in adults, treatment-dependent increases in GFAP are considered neurotoxic. Changes in GFAP levels have been observed in immature animals, but have not been conclusively linked to neuronal injury."

The meaning of the latter sentence is unclear. Does this mean that treatment-related changes in GFAP have been observed in immature animals but not conclusively associated with neurotoxicity? Furthermore, if changes in GFAP are considered neurotoxic in adults but not in immature animals, it seems that the document should make some statement as to when this age-related transition in significance of GFAP occurs from the immature to adult animal.

Section 3.2.2 Neurophysiological Endpoints of Neurotoxicity

As an endpoint of neurotoxicity, neurophysiology encompasses two broad areas: (1) electrophysiology, which deals primarily with the electrical activity associated with the nervous system, and (2) general physiology, which involves the functioning of peripheral organs that are controlled or modulated by the nervous system. In general, the use of electrophysiological techniques (e.g., EEG, sensory-evoked responses, nerve-conduction velocity) provides a means of directly assessing neuronal function, whereas general physiological status (e.g., blood pressure, lacrimation, salivation, body temperature) provides an indirect means of assessing neuronal function. One of the key roles played by the nervous system is to orchestrate the general physiological functions of the body to help maintain homeostasis. To this end, the nervous

system and many of the peripheral organ systems are integrated and functionally interdependent. Since many peripheral organ functions involve neuronal components, changes in such physiological endpoints as blood pressure, heart rate, EKG, body temperature, respiration, lacrimination, or salivation may indirectly reflect possible treatment-related effects on the functional integrity of the nervous system. However, since physiological endpoints also depend on the integrity of the related peripheral organ itself, changes in physiological function also may reflect systemic toxicity involving that organ. Consequently, the neurotoxicological significance of a physiological change must be interpreted within the context of other signs of toxicity. When performed properly, neurophysiological techniques provide information on the integrity of defined portions and/or functional operations of the nervous system.

Section 3.2.2.3 Convulsions

The following two statements are made in this section: (1) "Behavioral convulsions that occur only at legal or near lethal dose levels do not necessarily constitute evidence of neurotoxicity," and (2) "Convulsions that occur in the presence of systemic toxicity are not necessarily evidence of neurotoxicity." Both of these statements seem to be in contradiction with the statement on p. 5/1.2-3 that "Chemicals may produce neurotoxicity effects by either direct or indirect means." In the instance of convulsion, it seems that the endpoint of neurotoxicity is the convulsion. The mechanism whereby the convulsion is produced is not necessarily the determining issue. The presence of convulsion, by whatever mechanism, indicates that neurotoxicity is a component of the toxicological profile for that chemical treatment. It may be of little significance in terms of the overall toxicity of that chemical, particularly if convulsions only occur at lethal or near lethal doses, but none the less the convulsion indicates a treatment-related neurotoxic effect. On the other hand, the convulsion may be the most significant part of the toxicological profile, including the systemic toxicity, for that particular chemical treatment.

Section 3.2.2.3 Convulsions

The statement is made that "Convulsions that occur in the presence of systemic toxicity are not necessarily evidence of neurotoxicity." But then the next sentence states that "In such cases, neurophysiological recordings of electrical activity in the brain that is indicative of seizures provides evidence of neurotoxicity." Does this mean that only electrical recordings are to be accepted as reliable measures of seizures and as evidence of neurotoxicity? Why isn't observation of convulsion accepted as evidence of neurotoxicity? Are electrical recordings accepted only when there is systemic toxicity present? This needs some clarification.

Section 3.2.3 Neurochemical Endpoints of Neurotoxicity

This section makes some apparent contradictory statements. On p. 23/lines 26-30, the statement is made that "By themselves [neurochemical effects], demonstrated does-related effects on these endpoints are not evidence of neurotoxicity. Many neuroactive agents can increase or decrease neurotransmitter levels in the brain but such changes are not necessarily indicative of neurotoxicity." . Yet, on p. 24/lines 1-3, it states rather definitively that "...agent-induced increases in specific neurotransmitters in the brain, or decreases in specific brain regions, especially when such changes are persistent, are evidence of neurotoxicity." This section needs some clarification.

Section 3.2.4 Behavioral Endpoints of Neurotoxicity

The point of the examples in the second paragraph is unclear. The statement is made that "...toxicant-induced changes in behavior can result from a variety of physiological changes in addition to effects on the nervous system." But it is not very clear how this statement relates to the examples given, i.e., "changes in relative and absolute organ weights may be signs of systemic toxicity" and "Food and water consumption data are necessary in determining the relative contribution of general toxicity..." It might be worth reiterating that indirect toxicant-related effects on behavior (e.g., behavioral changes elicited via toxicant-induced effects on physiological

systems) are regarded as neurotoxic, but then stating that it is still important to discern, whenever possible, whether systemic toxicity may be involved in the resulting behavioral or neurotoxic effects. Several common endpoints used as signs of systemic toxicity include relative and absolute organ weight changes, altered food and water consumption, etc.

Section 3.2.4.1 Functional Observational Batteries

Page 27, lines 7-10. The test results from the FOB should certainly be judged, as the document states, according to the number of signs affected, the dose at which neurotoxic signs are observed, and the persistence of the effects. But, consideration should also be given to the *nature of the effects* observed as well as their *severity*.

Section 3.2.4.1 Functional Observational Batteries

Section 3.2.4.2 Motor Activity

Section 3.2.4.3 Schedule-Controlled Behavior

Section 3.2.4.4 Specialized Tests for Neurotoxicity

Page 27, lines 13-18; p. 31, lines 1-3; p. 32, lines 9-14; and p. 32, 3rd paragraph. Some discussion at the workshop should be centered around the issue of whether neurological signs occurring at the high dose in conjunction with other overt signs of toxicity should or should not be considered neurotoxicity. Why is this not considered a possible indirect neurotoxic effect associated with treatment? It should be clear that dose is a very significant factor in the neurotoxic potential of any chemical substance. For some chemicals, neurotoxicity may only occur at high doses which produce general systemic toxicity. It is important to consider also, as is alluded to on p. 39 with reference to maternal/pup toxicity, that the systemic toxic effects may be reversible but the neurotoxic effects may not be. Should exceptions be made? If such effects are not considered neurotoxic, then it seems that the position that "Chemicals may produce

neurotoxicity effects by either direct or indirect means" (p. 5) should be reconsidered or, at least, restated.

Section 3.2.4.1 Functional Observational Batteries

Page 27, line 22. The developing organism is referred to in this section. Does this means that the FOB is to be applied in developmental studies, as well as adult studies?

Section 3.2.4.3 Schedule-Controlled Behavior

Page 32, line 5. Define quarter life.

Page 34, Table 5. The word "pyrethroids" is in the wrong column.

Section 3.2.4.4 Specialized Test for Neurotoxicity: Cognitive Function

Page 37, lines 5-8. The sentence "...it is not necessarily the case that a decrease in responding on a learning memory task is adverse...," as written, may be misinterpreted to mean that decreased learning/memory responding may not be considered adverse.

Section 3.2.5 Developmental Neurotoxicity

Pages 38-39. There are a number of indented sentences. What are these supposed to be? Are they factors to consider in evaluating developmental neurotoxicity data as part of the risk assessment process? Some introductory statement should be inserted to explain what these indented sentences represent.

Page 40, lines 7-16. The issues presented in this paragraph are generally applicable for the interpretation of all types of neurotoxicity data. It would be appropriate to expand the discussion of these ideas and to include this in one of the introductory sections of this document or in the guidelines for interpretation in Section 5.

Section 3.3.3 Statistical Considerations

If power is defined as one minus the probability of a Type II error (1.13), then how can power increase with sample size *if the probabilities of Type I and Type II errors are held constant* (1.21-22)?

Section 5 Adequacy of Evidence...

Page 51, lines 11-12. The statement that "most...neurobehavioral changes are regarded as adverse" is too unqualified and too general and does not address the critical issue of how adverse should be defined. Obviously, there are a number of factors that must be considered in determining whether any particular behavioral change should be considered adverse, including severity and nature of the effect. In making this determination of adversity, consideration should be given to Section 2: Definitions (p. 4 of this document) which states that adverse effects include: (1) unwanted effects and/or (2) any alteration from baseline that diminishes the ability to survive, reproduce, or adapt to the environment.

Section 5. Adequacy of Evidence

Page 52, bottom paragraph. This paragraph brings out an excellent point and expresses it very well—the potential for neurotoxic hazard may be very dependent on exposure route and exposure level.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
Washington, DC
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The following comments are presented according to section of the proposed guidelines document.

2.1 Neurotoxicity

Expand definition to: "Any acute and chronic adverse change in the structure or function of the developing, mature, or aged central and/or peripheral neuro-muscular system (including sensory and special sensory organs) attributable to a chemical, physical, or biological agent." This represents the true scope of the guidelines and introduces the specific notion of cause and effect. It explicitly includes : acute and chronic effects; changes at all stages of life; actions on brain, spinal cord, nerve, muscle, sense organs, and special sensory organs. Still unclear, however, is *whether the definition is intended to include adverse effects on the neuroendocrine system as implied by the statement: an "alteration from baseline that diminishes the ability to reproduce."* Failure to "adapt to the environment" could result from many perturbations unassociated with an action on the developing or mature nervous system.

If such an all-encompassing definition is selected, it will be important to discuss the long-term consequences of agent-induced changes. On the basis of current understanding (always limited), most agent-induced disorders of the adult nervous system improve after exposure has ceased. In some case, improvement may not occur until the disorder has played out for some weeks after the exposure terminated (a phenomenon known as coasting). The degree of recovery (reduction of the signs of the disorder) varies with the agent and the exposure condition. All of the following are possible: complete recovery with no functional or anatomical residua or susceptibility to re-exposure to the agent; apparent complete recovery with subclinical

anatomical or functional residua and increased susceptibility upon re-exposure to the agent; partial recovery with overt but stable anatomical and functional abnormalities (which may slowly increase in severity with the advance of age). Some conditions may not recover (e.g., OP-induced spasticity), and others may even be progressive (e.g., tardive malignant neurological degeneration following acute exposure to carbon monoxide). The problem of long-latency effects is treated in Section 2.2.

Paragraph 2: Change "chemical" to "biochemical" or "molecular and cellular." Add to the end of the paragraph: "neurological syndromes spanning a wide variety of usually mixed behavioral abnormalities."

2.2 Neurotoxicant

The tautologous distinction of "neurotoxicant" and "neurotoxin" has been dropped in most professional circles. The distinction almost always causes confusion: "neurotoxicant is a. . . biological, ..." and Neurotoxins are naturally occurring. . ." More important is to state that "*many if not all chemicals have neurotoxic potential in certain dose and exposure conditions, including chemicals which are required for normal physiological function.*" Use of glutamate as an example of a neurotoxicant/neurotoxin (?) then makes sense.

The issue of long-latency neurotoxicity is not addressed in this section, although it is alluded to on p. 55, lines 14-16. While available data are too meager to impose regulatory standards, it is important to mention this concept. There are two ides, neither of which is inconsistent with the other: (1) chemical exposure may elicit a subclinical lesion which, as a result of age-related attrition of the same cell population, may surface years or decades later as a progressive disorder (e.g., MPTP and subclinical Parkinsonism); and (2) certain agents may act as "slow toxins" which trigger clinically silent events that come to clinical significance weeks (organophosphates), months (carbon monoxide), years/decades (cycad toxins and western Pacific amyotrophic lateral sclerosis/Parkinsonism-dementia complex) later. Concern for the existence of long-latency effects justifies inclusion of subclinical anatomical and functional changes in the guidelines.

3 Hazard Identification

An explicit hierarchy of studies by which to judge relevance and attribute weight is proposed. For example, if the goal is to evaluate hazard to humans from specific agent(s), then the following sources of data should be given descending weight in the evaluation process:

1. Controlled human studies. Studies of humans exposed to an identified agent/mixture for a defined period at a known dose. (Most of these are to be found in the literature dealing with the side effects of therapeutic drugs, a literature often ignored by neurotoxicologists.)
2. Controlled animal studies. Experimental studies of a species exposed to an identified agent/mixture for a defined period at a known dose.
3. Uncontrolled human studies. Non-epidemiological reports of humans exposed to an identified agent/mixture.
4. Uncontrolled epidemiological studies. Studies that offer unproven associations between agents and health effects in humans and animals.
5. Uncontrolled animal studies. Reports of animals exposed to an identified agent/mixture. (Mostly from veterinary studies, another source of literature commonly ignored by neurotoxicologists.)

3,1.1 Epidemiological Studies

The classification in #3 gives less weight to reports of epidemiological associations between agents and effect than a series of well performed case reports or controlled animal studies. Indeed, epidemiological studies are usually *unable to demonstrate causation* and must rely on other studies (i.e., controlled studies with experimental animals) for this purpose. Regulating agents on the basis of epidemiological conjecture is inappropriate.

3.1.1.3 Outcome Measurement

Neurotoxicants that cause central-peripheral distal axonopathies (a neologism I am guilty of introducing!) are hardly "the most prominent categories. . . ." It seems to be a largely stereotyped response of the nervous systems to a wide variety of chemicals of disparate structure, but so are many other types of chemically induced neurological abnormalities of equally great or greater clinical significance.

Far too little weight is given to the neurological examination. This has been the cornerstone of diagnosis of nervous system disease and deserves to be represented as such. Interpretation of the results of the sensory-motor examination *vis-a-vis* overall neurological status is far superior than anything presently offered by behavioral techniques. However, the neurological examination is weak on the precise assessment of mental state.

The discussion of the electrophysiological methods is poor: e.g., peripheral neuropathy requires assessment of nerve conduction properties (nor velocity alone). There is no mention of contemporary human brain examination/assessment techniques, such as computerized tomograhy (CT), nuclear magnetic resonance spectroscopy (NMR), single photon emission tomography (SPET), or positron emission tomography (PET).

3.1.1.4 Confounding Variables

The document seems to treat age as a confounding variable. Presumably, the guidelines are being developed for the protection of individuals of all ages.

Table 1. Spell out acronyms. Change "Neurophysiological" to "*Neurophysiological and Functional*" and add endpoints relating reproducible changes identified by contemporary techniques, such as "*change in the neurotransmitter marker by PET.*"

Add a new table listing the rich variety of neurological endpoints following exposure to chemicals. Include chemical/physical agents with direct/indirect effects on the brain, spinal cord, special sensory organs, sensory motor and autonomic nerve fibers/end organs, muscle and (?) neuroendocrine system.

The number of examples listed under "Behavior Endpoints" is disproportionately high.

3.2 Animal Studies

This section should begin with a description of gross physical/behavioral changes, including convulsions (move from Section 3.2.2.3).

3.2.1 Structural Endpoints of Neurotoxicity

Brain edema may be seen grossly as a loss of the normal pattern of convolutions. Brain weight: Reduce discussion to 1-2 lines; it is rarely used or useful. It is unclear that breakdown of cells may be preceded by accumulation, proliferation, or rearrangement of structural elements. Part of the classification used was introduced by P.S. Spencer and H.H. Schaumburg (1980), Experimental and Clinical Neurotoxicology, Williams and Wilkins, Baltimore, MD, and not as cited.

"Neurodegeneration" is not used to refer to changes in nerve terminals; most commonly, the word refers to neuronal loss seen in progressive neurodegenerative diseases.

Developing nervous system: The key vulnerability of the nervous system to *exposures at particular sensitive developmental times* needs to be emphasized (an agent that is refractory at some points during development may cause devastative abnormalities if exposure occurs at certain states of organogenesis).

The aged appear to be uncommonly vulnerable to chemicals with neurotoxic potential because: liver and kidney metabolism may be compromised; body weight may be lower; and certain groups of nerve cells and their processes may have undergone 'normally occurring' age-related compromise.

The section dealing with neuropathology needs to address the issue of artefactual changes (induced by preparative trauma, fixatives, dehydration steps, etc.) which all too often are mistakenly taken as evidence of neurotoxicity.

Table 2. This is very poor and reflects much misunderstanding. Buckthorn toxin probably causes an axonopathy, not as listed under myelinopathy. MPTP induces degeneration of nerve terminals and substantia nigra neurons. An organophosphate anticholinesterase agent is an example of a chemical that acts at (certain) nerve terminals. Lead and peripheral neuropathy are adequate examples, although diphtheria toxin (a biological agent) might be a useful addition. Acrylamide, hexacarbons and carbon disulfide are all associated with

"Peripheral Neuropathy," not "Vitamin Deficiency." Proximal axonopathy and motor neuron disease are not equivalent.

3.2.2.1 Nerve Conduction Studies

Refractory period, chronaxy, and rheobase are rarely used and difficult to interpret in relation to neurotoxicity.

3.2.3 Neurochemical Endpoints of Neurotoxicity

All endpoints, but most especially neurochemical endpoints, acquire validity and standing when the magnitude of the change is shown to vary as a function of the dose of the agent administered and, if possible, the duration of chemical exposure.

Agents that perturb axonal transport function (fast or slow anterograde, retrograde) should be included as neurochemical endpoints of neurotoxicity.

3.2.4 Behavioral Endpoints

This section has received much more detailed treatment than sections dealing with morphology, neurophysiology, neurochemistry. Does it reflect a bias that neurobehavioral methods are more important than other methods for the detection of neurotoxicity? Little guidance is offered as to the difficulty, reliability, and reproducibility of the techniques, or their relevance to human neurological dysfunction. Even more troubling is the recognition on p. 51 that "there are adverse behavioral effects that may not reflect a direct action of the nervous system."

Paragraph 2. Define "general toxicity."

3.2.4.1 Functional Observational Battery

The statement on p. 27 regarding the distinction between evidence of neurotoxicity and lack thereof is crucially important. The discussion as written is unclear. A concrete example of what EPA has in mind would be more helpful.

3.2.4.2 Motor Activity

It is doubtful that "neurotoxic agents generally decrease motor activity." Many compounds increase the central nervous system (CNS) excitation and, at certain dosages, increase motor activity (see also p. 31, last two lines). EPA is advised to avoid these types of generalizations, especially if they are untrue.

3.2.4.4 Specialized Tests for Neurotoxicity

Ataxia may result from a motor (cerebellar) or sensory (vestibular, proprioceptive) defect.

3.2.5 Developmental Neurotoxicity

Emphasize critical role of the timing of agent exposure (see Section 3.2.1). This thought is currently buried in the text. Table 6 reinforces the notion that the agent, rather than the agent and the timing of exposure, are factors dictating developmental neurotoxicity.

Replicate (or triplicate) study design adds confidence to any study.

3.3 Other Considerations

Parts of the CNS (circumventricular organs) and peripheral nervous system (dorsal root ganglia and sympathetic ganglia) normally lack a blood-brain regulatory interface. Access of blood-borne chemicals to these regions is immediate and total.

3.3.3 Statistical Considerations

This discussion should be placed after Section 3.3.4.

3.3.4 *In vitro* Data in Neurotoxicity

The second paragraph is very negative. The use of organotypic explants has been extensively explored. They reproduce the structural and functional features of nervous tissue *in vivo*, and they respond to chemical, physical and biological agents in a manner that reproduces changes *in vivo*. They have been useful in demonstrating the site of chemical action and the resulting effect, and they are also predictive of neurotoxicological effects in animals.

4 Dose Response Assessment

It is important to recognize that a single agent may act at multiple points in the nervous system at a single dose/time level, and may attack different sites at other dose/time levels. The discussion ignores the problem of extrapolation between acute and chronic effects on the nervous system and behavior. A NOAEL may be critically important to avoid a chronic neurological effect (e.g., *n*-hexane neuropathy) but of little relevance to short-term exposures where much greater levels may be tolerated without harm.

5 Adequacy of the Evidence for the Determination of Hazard

(And in Table 7) An explicit hierarchy of studies by which to judge relevance and attribute weight is proposed. For example, if the goal is to evaluate hazard to humans from specific agent(s), then the following sources of data should be given descending weight in the evaluation process:

1. Controlled human studies. Studies of humans exposed to an identified agent/mixture for a defined period at a known dose. (Most of these are to be found in the literature dealing with the side effects of therapeutic drugs.)
2. Controlled animal studies. Experimental studies of a species exposed to an identified agent/mixture for a defined period at a known dose.
3. Uncontrolled human studies. Non-epidemiological reports of humans exposed to an identified agent/mixture.
4. Uncontrolled epidemiological studies. Studies that offer unproven associations between agents and health effects in humans and animals.
5. Uncontrolled animal studies. Reports of animals exposed to an identified agent/mixture. (Mostly from veterinary studies.)

Use of structure-activity considerations should be limited to those classes of chemicals for which an understanding exists.

The notion that "correlations support a coherent and logical link between behavioral effects and biochemical mechanisms" is very valuable. Another important concept is the issue of replicability. Another omitted concept is that of dose-dependent changes which allow the

researcher to distinguish spurious effects from those related specifically to the agent under study.

7 Risk Characterization

There is an implicit understanding that guidelines will be developed for the protection of normal healthy individuals (of differing ages or ethnicity), rather than those with some inherent susceptibility (e.g., diabetics with subclinical neuropathy who work with acrylamide or *n*-hexane). Should this issue be addressed?

Some ethnic groups are more susceptible to certain chemical agents because of specific genetic phenotypes that dictate metabolic capacity. The best known example is the regulation of acetylation for drugs like isoniazid: genetically slow acetylators metabolize (acetylate) the agent slowly and are more susceptible than fast acetylators to isoniazid neuropathy and encephalopathy.

Premeeting Comments for
Peer Review of Neurotoxicity Risk Assessment Guidelines Workshop
Washington, DC
June 2-3, 1992

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My comments on the proposed guidelines deal first with the panel outlines and then with the draft guidelines themselves.

Panel 1: Neurotoxicity as an Appropriate Endpoint for Environmental Risk Assessment

I agree with the general statement that neurotoxicity is an appropriate endpoint for assessing risk. I also concur that the opinion is properly founded on the importance of the nervous system to human health, the large number of agents known to cause neurotoxicity, and the possibility that there may be significant exposure of humans to neurotoxic agents. I also agree with the caveats that inadequate information is available on many potentially harmful chemicals and that better standards for evaluating neurotoxic potential are needed, especially with regard to low-level chronic exposures and possible synergistic effects.

One issue that touches upon many parts of the report is deciding what constitutes a no-observed adverse-effect level (NOAEL). Phrased another way, when do behavioral, physiological and/or biochemical effects that are acceptable as markers that exposure to a chemical has occurred become signals of an adverse effect? The definition of an adverse effect on p. 4, "(1) unwanted effects, and/or (2) any alteration from baseline that diminishes the ability to survive, reproduce, or adapt to the environment" specifically in the "structure and function of the central and/or peripheral nervous system...," is generally a reasonable one. But converting it into practice requires detailed knowledge of exposure levels and effects for probably both humans and experimental animals.

Panel 2: Interpretation of Neurotoxicity Data When Effects Are Transient

The statements that "nerve cells have limited capacity for regeneration" and that "apparent recovery ...represents activation of reserve capacity..." need clarification and perhaps some delimiting. For example, there is rapid regrowth of axons and reinnervation of denervated motor neurons, especially in young animals, presumably without loss of reserve capacity. Is there evidence that recovery from exposure to compounds like acrylamide and n-hexane that require repeated exposures to produce major neural damage uses up some "capacity" of the cells? Once a cell body dies, the axon and other cell processes will die too, but the synthetic capacity of nerve cells often would permit eventual full recovery if the cell body was not destroyed. Perhaps different kinds of "recoveries" could be considered. For example, some recoveries may be due to the destruction of the chemical by metabolism or its loss by excretion; in other cases recovery may require synthesis of new proteins and other tissue constituents.

Whether or not pharmacological effects are traditionally considered short-acting and toxicants long-acting, many naturally occurring toxicants such as curare and eserine are acute in their actions, and some nerve gases that can hardly be considered "pharmacological," act acutely and have been shown to have little long-term effects after repeated exposures (Wilson et al., 1992b).

The proposal that some effects of exposure to a chemical may only appear following environmental or pharmacological stresses is an interesting one, since it points towards different paradigms than are currently used in testing for neurotoxicity. This proposal should be buttressed by examples.

Panel 3: Agents Acting through Indirect and Direct Means Can Be Considered Neurotoxic

I am not sure that I agree that "effects...produced through direct or indirect means are functionally equivalent... ." Few chemicals are so specific in their action as to have only one major effect, and events that occur downstream from an initial chemical-receptor interaction may be multiple. Indeed, most chemicals affect the body at more than a single site of action.

Tetrodotoxin, and other similar natural channel blocking toxins, are some of the few examples compared to the many "dirty" neuroactive drugs.

Perhaps the report could make more of the phenomenon of bioactivation and less of whether an effect is primary or secondary with respect to risk assessment. The report itself makes the point that "knowledge of exact mechanisms of action is not...necessary to reach the conclusion that a chemical produces neurotoxicity." Setting reliable NOAELS may require more knowledge and study. Risk assessment should proceed from clearly established endpoints that occur at specific levels of exposure regardless of the mechanisms of the toxicity. One issue to discuss is how one establishes whether direct and indirect actions are the most sensitive indicators of an effect. It may be important to determine whether or not specific damage occurs to the nervous system at levels below that which causes damage to other parts of the body. This determination may be important in defining a chemical as a neurotoxicant and thereby assessing its risk from its ability to injure the nervous system rather than do damage to other parts of the body.

Panel 4: Extrapolation of Neurotoxicity Data from Laboratory Animals to Humans

The idea that a neurotoxicant in one species is a neurotoxicant in others, including the human, neglects examples of neurotoxicants that differ in their actions from species to species presumably due to differences in bioactivation, metabolic detoxification, excretion, induction of oxidases, etc. Given the present state of knowledge of the action mechanisms of many chemicals and their effects on humans the use of experimental animals in establishing the scientific bases for risk assessment seems unavoidable. The more is learned about the mechanisms of action of an agent in humans and experimental animals the more likely arbitrary uncertainty factors can be replaced by factors based on solid data. The fact that other animals do not have the full repertoire of behavior of the human has a flip side if one considers that many animals "see" into the ultraviolet, "hear" above the human range, and some even sense magnetic frequencies. One issue is whether speech, or some other feature of human behavior (or physiology or biochemistry), constitutes the most sensitive endpoint for detecting a specific chemical's effect.

In vitro systems played little role in the report, even though they have been much studied as possible primary screens for neurotoxicants in the hopes that cell level effects could be revealed in cultured cell systems and followed up in experimental animals and humans.

Panel 5: Interpretation of Behavioral Data

What is the evidence that behavior is "one of the most sensitive indicators of toxicity?" Is it not also often the most readily altered by experimental conditions? Can the statement that behavioral changes appear prior to physiological or morphological endpoints be documented? The concerns expressed for maximum tolerated doses and chemicals that produce neurotoxic and/or behavioral changes at high doses may not be as important as worrying about establishing the lowest doses that yield detectable effects and the systems and species that are the most sensitive.

If the primary effect of a toxicant is defined as the first biochemical action of the activated chemical, usually at the receptor level, then it follows that no primary event can be a behavioral one. Behavior is a consequence of biochemical, molecular, usually multiple events in the nervous system. One advantage of emphasizing behavioral endpoints is that alterations in them may reflect more than one molecular event; one disadvantage is that important biochemical level events could be masked from expression elsewhere in the nervous system.

Another question is whether a behavioral change, by definition, is always an "adverse effect," in contrast to biochemical or physiological events that may not be harmful, in and of themselves?

How many behavioral endpoints have been validated as screens for neurotoxicants?

Acetylcholinesterase Determinations

The report recommends that "inhibition of "...AChE..." is evidence of neurotoxicity." It is safe to say that decreases in specific acetylcholinesterases (AChE, E.C. 3.1.1.7) and nonspecific cholinesterases (BChE, E.C. 3.1.1.8) are generally accepted as biomarkers of exposure to organophosphate (OP) and organocarbamate anti-ChE esters, providing that disorders leading to decreased levels of cholinesterases (ChEs) are not present, regardless of the tissues concerned. One issue is whether such decreases constitute an adverse effect in and of themselves. An SAB/SAP Joint Panel on Cholinesterases did not recommend using statistically significant decreases in plasma and red blood cell ChEs as indicators of adverse effects (Weiss, 1990). The panel, like the present report, supported using decreases in brain AChE levels as an adverse effect. However, a recent EPA workshop on cholinesterase methodologies (Wilson et al., 1992a) found there were large differences between laboratories in the determinations of tissue ChEs, raising doubts on the use of such data for regulatory purposes. (Variances on the order of 20 percent were commonplace.) Problems in assay methods were especially important with carbamates, since many inhibited carbamylated-ChE complexes are readily reactivatable. Steps are under way to standardize the procedures used by the laboratories, reducing both inter- and intra- laboratory variances. Until standards are set, one approach would be to recommend that AChE measurements be considered on a case by case basis, and referred to EPA's Office of Pesticide Programs and the panel of scientists they convened.

Another issue is what level of enzyme decrease is acceptable as "adverse." There have been proposals to make "a statistically significant" decrease in enzyme activity the baseline for an adverse effect. At first glance, such a criterion seems clear-cut and readily applicable by trained personnel. But there are several major problems:

1. So long as there are no standard operating procedures that specify reasonable confidence limits and variances, the company with the sloppier methods, and thus the higher variances, will be given higher permissible levels of residues. In other words, the criterion of a statistically significant difference only works when there is an even playing field based on standard procedures and internal controls.

2. Whether or not the criterion of a statistically significant difference is accepted and adhered to by all laboratories, scientists do not agree about what level of decrease of brain AChE produces an adverse effect.

3. There are large differences in AChE levels between different parts of the brain, and dissection and sampling procedures must be strictly adhered to for brain AChE assays to be meaningful.

Organophosphate Induced Delayed Neurotoxicity

The statement in the report that "inhibition of neurotoxic esterase...has been associated with agents that produce OPIDN...and is considered evidence of neurotoxicity," needs clarification to accurately reflect current thinking and data. Presently, Organophosphate-induced Delayed Neuropathy (OPIDN) is screened by specific animal tests, and determination of neuropathy target esterase (neurotoxic esterase [NTE]) activity is a part of the testing. Inhibition of NTE is not evidence *per se* that a compound is neuropathic. For example, phenylmethanesulfonyl fluoride (PMSF), carbamates (e.g., physostigmine), and phosphinates inhibit NTE activity but do not cause OPIDN. There is a body of evidence (Meredith and Johnson, 1989; Johnson, 1990) to indicate that most OPs that cause OPIDN not only inhibit NTE, but also "age" (shift an alkyl group from the phosphorylated active site to another part of the NTE molecule). The precise role of "aging" is still unclear; indeed recent experiments have led Johnson and others to question the role of aging in the onset of the neuropathy (Johnson et al., 1991; Lotti, 1991). Regardless, overt symptoms of OPIDN will not appear with acute exposures unless inhibition of brain NTE exceeds 70 to 80 percent (Johnson, 1990). (Levels of lymphocyte NTE activity and brain NTE activity after repeated exposures have not been as reliable predictors). It is safe to say that it is generally agreed that, even though inhibition of NTE alone is not sufficient to brand a chemical as neuropathic, or to use the NTE assay, by itself, as a screen for the disorder, OPs that inhibit NTE activity are possible neuropathic compounds and deserve further investigation.

References

Johnson M.K., Vilanova E., and Read D.J. 1991. Anomalous biochemical responses in tests of the delayed neuropathic potential of methamidophos (O,S-dimethyl phosphorothioamidate), its resolved isomers and of some higher O-alkyl homologues. Archives of Toxicology, 65(8):618-24.

Johnson M.K. 1990. Organophosphates and delayed neuropathy--is NTE alive and well? Toxicology and Applied Pharmacology, 102(3):385-99.

Meredith C., Johnson M.K. 1989. Species distribution of paraoxon-resistant brain polypeptides radiolabelled with diisopropyl phosphorofluoridate ([3H]DiPF): electrophoretic assay for the aged polypeptide of [3H]DiPF-labelled neuropathy target esterase. Journal of Neurochemistry, 52(4):1248-52.

Lotti M. 1991. The pathogenesis of organophosphate polyneuropathy. Critical Reviews in Toxicology, 21(6):465-87.

Weiss, B. (Chair). 1990. Review of cholinesterase inhibition and its effects. Report of the SAB/SAP Joint Study Group on Cholinesterase, U.S. Environmental Protection Agency.

Wilson, B.W., Jaeger, B. and Baetke, K. (Editors). 1992a. Proceedings of the U.S. EPA Workshop on Cholinesterase Methodologies, Dec. 4-5, 1991, Office of Pesticide Programs.

Wilson, B.W., Hooper, M.J., Hansen, M.E. and Nieberg, P.S. 1992b. Reactivation of organophosphate inhibited AChE with oximes. In "Organophosphates, Chemistry, Fate and Effects," Chambers, J.E. and Levi, P.E. (Eds.) Academic Press. pp. 107-137.

APPENDIX D

POSTMEETING COMMENTS

APPENDIX D

Postmeeting Comments

Dr. Barry Wilson, Ph.D., Workgroup Chair of Direct and Indirect Effects Panel

One group that has considered the definition of an "adverse health effect" is the "Scientific Assembly for Environmental and Occupational Health of the American Thoracic Society" with reference to air pollution and the Clean Air Act (Am. Rev. Respir. Dis. 131:666-668, 1985). The concerns discussed in their report are similar to those discussed by the neurotoxicology panels. Health effects were classed in an ascending triangle with mortality, morbidity and pathophysiologic changes being classed as adverse health effects at the top, and physiologic changes of uncertain significance and pollutant burdens at the bottom. The approach was epidemiological to the extent that the proportion of the population affected decreased as one progressed from the bottom (physiologic changes) to the top (mortality) of the pyramid. Factors singled out as most important in considering when a physiologic change should be considered an adverse effect included: (a) differences between "statistical significance and medical or biological significance," and (b) the idea that "not all changes (e.g. physiologic) are necessarily adverse." The reaction of carbon monoxide with hemoglobin was used as an example; (c) the problem of reversible effects, and (d) when an effect occurs in the lifetime of an individual were also considered.

The report defines "adverse respiratory health effects" as medically significant physiologic or pathologic changes generally evidenced by one or more of the following: (1) interference with the normal activity of the affected person or persons; (2) episodic respiratory illness; (3) incapacitating illness; (4) permanent respiratory injury; and/or (5) progressive respiratory dysfunction. The report stressed human epidemiological, clinical and human exposure studies more than animal studies. (Parenthetically, the area of neurotoxicology has much better molecular, cellular and organismal models for human toxicities than does that of respiratory pollution.) Nevertheless, it is striking that both panels independently generated hierarchal schemes for establishing adverse health effects and physiologic events of "uncertain significance."

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