May 2, 1997

EPA-SAB-EHC-97-004

Honorable Carol M. Browner
Administrator
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Subject: Science Advisory Board's review of the revised Guidelines for Neurotoxicity Risk Assessment

Dear Ms. Browner:

The proposed Neurotoxicity Guidelines for Risk Assessment were developed as part of an interoffice guidelines development program under the auspices of the Risk Assessment Forum within EPA's Office of Research and Development. The Draft Guidelines were developed initially by an Agency work group composed of scientists from throughout the Agency, and selected drafts were peer reviewed internally and by experts from universities, environmental groups, industry and other government agencies. The proposed Guidelines are based on recommendations derived from these reviews and on those made at various scientific meetings and workshops on neurotoxicology. The proposed Guidelines were published for public comment in the October 4, 1995, issue of the *Federal Register*.

The Science Advisory Board's (SAB) Environmental Health Committee (EHC) met on July 18, 1996 in Washington DC to review the proposed Guidelines.

In general, the Committee considers the revised Guidelines document to be quite successful, and, all things considered, well suited to its intended task. It addresses a wide range of subjects of considerable complexity and (often) of considerable subtlety, and constitutes a clear step forward in the state of the art. Naturally, as with any such ambitious undertaking, the Committee's review has identified areas where improvements could be made. Specifics on such improvements, which generally lie outside the area of the specific Charge for the review, and reflect the thoughts of individual Committee Members rather than a Committee consensus, have been

provided separately to Agency staff.

The various issues comprising the Charge are addressed in detail in the body of the enclosed report, and are summarized (with the specific Charge question italicized) below:

a) *The combining of hazard identification and dose-response evaluation to reflect more accurately the process used for noncancer health effects.* It was noted that this issue had been addressed in depth during the EHC review of the reproductive toxicity guidelines and in following discussions of the SAB Executive Committee. There was a clear consensus that EPA should follow the SAB position articulated in the recent "Commentary on Hazard identification" from the Executive Committee (EPA-SAB-EC-COM-96-001, December 8, 1995) which called for keeping hazard identification as an identifiable qualitative step in the risk assessment process. The Committee recommended that revisions be made to decouple the qualitative step of hazard identification from the more quantitatively rigorous steps of exposure evaluation and dose response assessment. The Committee encourages the Agency to adopt an approach similar to that used in the proposed Cancer Risk Assessment Guidelines.

b) *The issue of compensation and recovery of function in neurotoxicological studies and how to account for compensation in neurotoxicology risk assessment*. The Committee believes that reversibility can not be ignored in risk assessment, but that an assessor had to be very careful and search out particularly good evidence if recovery or an apparent transient effect is cited to support evidence for relatively benign effects. In general, assessors should be advised to look carefully at instances where reversibility is involved and be alert to the possibility of re-occurrence of effects. The Committee recommended that EPA address specifically differences related to type of damage, nature of the insulting agent, and age of the organism, as well providing case examples relating to possible reversibility.

c) *The use of blood and/or brain acetylcholinesterase activity as an indication of neurotoxicity for risk assessment* and *Considering the available data and the state of the science, does the SAB agree with the recommendation that inhibition of RBC and/or plasma cholinesterase can serve <u>only</u> as a biomarker of exposure?* The Committee addressed these two issues

together because of their close relationship. The EHC concurred with the findings of previous SAB reviews regarding the consideration of data on the inhibition of RBC and/or plasma cholinesterase. In the absence of clinical signs in humans or animals or the absence of morphological data in animals, the quantitative nature of the inhibition of red blood cell (RBC) and/or plasma cholinesterase is considered unreliable for assessing significant biological adverse changes, but can be used as a biomarker of exposure. The Committee also recommended that a noted decline in brain ChE should be evaluated by risk assessors in terms of possible effects that are biologically significant, and that the term "statistically significant" needed to be better explicated -- perhaps in terms of the benchmark dose or by some measure which reflected information about the distribution of the effect under study. The Committee also suggested that further details concerning reversibility and possible tolerance effects (which could enhance sensitivity to other agents) be provided.

d) *Are there endpoints indicative of neurotoxicity that may not be covered by these proposed Guidelines, e.g., endocrine disruption or neuroendocrine-mediated neurotoxicity?* The Committee considered that the description of endpoints indicative of neurotoxicity was extensive and it does not suggest additional endpoints based on current scientific knowledge. The Committee also recognizes that some chemical agents may cause both neurotoxic and other effects such as endocrine disruption; however endocrine disruption without evidence of a neurochemical or neuro-physiological causation would not be a basis to label that chemical agent as a neurotoxicant. This notwithstanding, the Guidelines should recognize that a finding of neuroendocrine dysfunction might have to be amplified by neurobehavioral assessment techniques not discussed or described in the current draft. Observation of a widening or narrowing of male-female differences on certain neurotoxic endpoints could be a reflection of interference with neuroendocrine function. Specific recommendations should be included in the Guidelines for dealing with such multi-system actions because risk assessors will typically not be sensitive to their ramifications.

e) *Are the descriptions of the endpoints used in human and animal neurotoxicological assessments complete?* In the broadest sense, the Committee found the treatment of this issue by the Guidelines document to be "..not complete, but complete enough for its purposes," meaning

that, although the Committee has many refinements and improvements to suggest, the Guidelines provided sufficient information on various end-points to enable a risk assessor to do his/her job. More specifically, the Committee found that coverage of human studies was quite good, but that the treatment of animal studies (particularly with regard to warning caveats) was, by comparison, weaker. It was also suggested that better coverage be given to various possible tests (including references for various test methodologies).

g) *Treatment of the possibility of no threshold for some neurotoxic agents.* There was considerable debate/discussion as to what the concept of a threshold, or of no threshold, *actually* meant in terms of a risk assessment, and whether the concept itself was meaningful or useful in this context. It was proposed that the term "non-linear (at low doses) dose-response curve for most neurotoxicants" be substituted for the term "threshold." The EPA was urged to harmonize its treatment of this (as well as several other issues) with the presentation and positions taken in the draft Carcinogen Risk Assessment Guidelines currently out for public comment.

g) *Adequacy of the treatment of susceptible populations and individuals by the proposed Guidelines.* The Committee noted that the aged were the only specific sub-population noted, and that the Guidelines do not discuss means of modifying NOAELS or dose-response curves in this context. It was suggested that the Guidelines be modified to identify other susceptible groups (e.g., those with poor nutritional status) and provide suggestions as to dealing with them.

h) *The use of the Benchmark Dose in Neurotoxicity Risk Assessment*. There was considerable discussion within the Committee and between the Committee, EPA staff, and members of the audience as to the nature of the results yielded by the application of the benchmark dose methodology and how it compared with the more common NOAEL measure. No consensus on this aspect of the issue was reached. The Committee did note that definition of the benchmark dose in the Guidelines was not clear and should be improved; it further suggested that the basic position of the Guidelines be retained, i.e., that the use of the benchmark dose should be explored in specific situations, but that the NOAEL measure was the default approach.

Finally, during the SAB Executive Committee (EC) review of this report, several Members raised one additional concern which we believe should be brought to EPA's attention. The Guidelines note that there are a large number of indicators of neurotoxicity and neurobehavioral effects. They recognize that the complexity of these multiple indicators requires a great deal of scientific judgement, including expertise both within and without the field of neurotoxicology. We recommend that the Guidelines describe a mechanism for risk assessors and other users to obtain the resources for expert advice.

We appreciate the opportunity to review this document, and look forward to your response to the issues we have raised.

Genevieve M. Matanoski

Dr. Genevieve Matanoski
Chair, Science Advisory Board

/signed/
Dr. Emil Pfitzer
Chair, Environmental Health Committee

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Science Advisory Board
Washington DC

EPA-SAB-EHC-97-004
May 1997

AN SAB REPORT: GUIDELINES FOR NEUROTOXICITY RISK ASSESSMENT

REVIEW OF THE OFFICE OF RESEARCH AND DEVELOPMENT'S GUIDELINES FOR NEUROTOXICITY RISK ASSESSMENT BY THE ENVIRONMENTAL HEALTH COMMITTEE

NOTICE

This report has been written as a part of the activities of the Science Advisory Board, a public advisory group providing extramural scientific information and advice to the Administrator and other officials of the Environmental Protection Agency. The Board is structured to provide balanced, expert assessment of scientific matters relating to problems facing the Agency. This report has not been reviewed for approval by the Agency and, therefore, the contents of this report do not necessarily represent the views and policies of the Environmental Protection Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

ABSTRACT

The proposed Neurotoxicity Guidelines for Risk Assessment were developed by the Risk Assessment Forum within EPA's Office of Research and Development. The Science Advisory Board's (SAB) Environmental Health Committee met on July 18, 1996 in Washington DC to review the proposed Guidelines.

In general, the Committee considers the revised Guidelines document to be quite successful, and, all things considered, well suited to its intended task. On specific issues, the Committee noted that:

a) EPA should keep hazard identification as an identifiable qualitative step in the risk assessment process.

b) Reversibility can not be ignored in risk assessment, but that an assessor had to be very careful and search out particularly good evidence if recovery or an apparent transient effect is cited to support evidence for relatively benign effects.

c) In the absence of clinical signs in humans or animals or the absence of morphological data in animals, the quantitative nature of the inhibition of red blood cell (RBC) and/or plasma cholinesterase is considered unreliable for assessing significant biological adverse changes, but can be used as a biomarker of exposure.

d) The description of endpoints indicative of neurotoxicity was extensive and it does not suggest additional endpoints based on current scientific knowledge.

e) The Guidelines provided sufficient information on various endpoints to enable a risk assessor to do his/her job.

g) The term "non-linear (at low doses) dose-response curve for most neurotoxicants" be substituted for the term "threshold." The EPA was urged to harmonize its treatment of this (as well as several other issues) with the presentation and positions taken in the draft Carcinogen Risk Assessment Guidelines currently out for public comment.

g) The Guidelines be modified to identify other susceptible groups (e.g., those with poor nutritional status) and provide suggestions as to dealing with them.

h) The definition of the benchmark dose in the Guidelines was not clear and

should be improved, and the basic position of the Guidelines be retained, i.e., that the use of the benchmark dose should be explored in specific situations, but that the NOAEL measure was the default approach.

KEYWORDS: neurotoxicology; neurobehavioral; risk assessment; benchmark dose; threshold.

U.S. ENVIRONMENTAL PROTECTION AGENCY
SCIENCE ADVISORY BOARD
ENVIRONMENTAL HEALTH COMMITTEE
MEETING

July 18, 1996

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*Dr. Donald R. Mattison served as Chair of the public meeting, but resigned from the Board prior to development of this report; Dr. Pfitzer suceeded him as Chair.

**Chair of the Scientific Advisory Panel

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1. EXECUTIVE SUMMARY

In general, the Committee finds the revisions to the Guidelines document to be quite successful, constituting a clear step forward in the state of the art. As with any such ambitious undertaking, the Committee has identified some areas where improvements could be made. The areas were, in general, outside the area of the specific Charge for the review, and, in some instances, reflect the thoughts of individual Committee Members rather than a Committee consensus. Specific technical comments of this nature were provided to Agency staff.

The various items of the Charge are addressed in detail in the body of this report (Section 3), and are summarized (with the specific question italicized)below:

a) *The combining of hazard identification and dose-response evaluation to reflect more accurately the process used for noncancer health effects.*

There was a clear consensus that EPA should follow the SAB position articulated in the recent "Commentary on Hazard identification" from the Executive Committee (EPA-SAB-EC-COM-96-001, December 8, 1995) which called for keeping hazard identification as an identifiable qualitative step in the risk assessment process. The Committee recommended that the qualitative hazard identification be decoupled from the more quantitatively rigorous steps of exposure evaluation and dose response assessment, and that the Agency adopt an approach similar to that used in the proposed Cancer Risk Assessment Guidelines.

b) *The issue of compensation and recovery of function in neurotoxicological studies and how to account for compensation in neurotoxicology risk assessment*.

The Committee believes that reversibility can not be ignored in risk assessment, but that assessors should be advised to look carefully at instances where reversibility is involved and be alert to the possibility of re-occurrence of effects. EPA should address specifically differences related to type of damage, nature of the insulting agent, and age of the organism, as provide case examples relating to possible reversibility.

c) *The use of blood and/or brain acetylcholinesterase activity as an indication of neurotoxicity for risk assessment* and *Considering the available data*

and the state of the science, does the SAB agree with the recommendation that inhibition of RBC and/or plasma cholinesterase can serve only as a biomarker of exposure?

The Committee concurred with the findings of previous SAB reviews regarding the consideration of data on the inhibition of RBC and/or plasma cholinesterase. The quantitative nature of the inhibition of red blood cell (RBC) and/or plasma cholinesterase is considered unreliable for assessing significant biological adverse changes, but can be used as a biomarker of exposure. A noted decline in brain ChE should be evaluated by risk assessors in terms biologically significant effects. The Committee also suggested that further details concerning reversibility and possible tolerance effects (which could enhance sensitivity to other agents) be provided.

d) *Are there endpoints indicative of neurotoxicity that may not be covered by these proposed Guidelines, e.g., endocrine disruption or neuroendocrine-mediated neurotoxicity?*

The Committee does not suggest additional endpoints based on current scientific knowledge. The Committee also recognizes that some chemical agents may cause both neurotoxic and other effects such as endocrine disruption; however endocrine disruption without evidence of a neurochemical or neurophysiological causation would not be a basis to label that chemical agent as a neurotoxicant. A finding of neuroendocrine dysfunction might have to be amplified by neurobehavioral assessment techniques not discussed or described in the current draft. Specific recommendations should be included in the Guidelines for dealing with such multi-system actions because risk assessors will typically not be sensitive to their ramifications.

e) *Are the descriptions of the endpoints used in human and animal neurotoxicological assessments complete?*

Although the Committee has many refinements and improvements to suggest, the Guidelines provided sufficient information on various endpoints to enable a risk assessor to do his/her job. More specifically, the Committee found that coverage of human studies was quite good, but that the treatment of animal studies (particularly with regard to warning

caveats) was. by comparison, weaker.

g) *Treatment of the possibility of no threshold for some neurotoxic agents.*

The EHC suggests that the term “non-linear (at low doses) dose-response curve for most neurotoxicants” be substituted for the term “threshold.” Again, EPA is urged to harmonize its treatment of this (as well as several other issues) with the presentation and positions taken in the draft Carcinogen Risk Assessment Guidelines.

g) *Adequacy of the treatment of susceptible populations and individuals by the proposed Guidelines*.

The Committee noted that the aged were the only specific sub-population identified. The Guidelines should be modified to identify other susceptible groups (e.g., those with poor nutritional status) and provide suggestions as to dealing with them.

h) *The use of the Benchmark Dose in Neurotoxicity Risk Assessment*.

No consensus on the issue of comparing the results yielded by applications of the benchmark dose methodology and the more common NOAEL measure. The Committee noted that definition of the benchmark dose in the Guidelines was not clear and should be improved and suggested that the basic position of the Guidelines be retained, i.e., that the use of the benchmark dose should be explored in specific situations, but that the NOAEL (No-Observed-Adverse-Effect-Level) measure remained the default approach.

2. BACKGROUND AND CHARGE

2.1 Background

The proposed Guidelines were developed as part of an interoffice guidelines development program under the auspices of the Risk Assessment Forum within EPA's Office of Research and Development. Draft Guidelines were developed by an Agency work group composed of scientists from throughout the Agency, and selected drafts were peer reviewed internally and by experts from universities, environmental groups, industry and other government agencies. A preliminary draft underwent peer review in a workshop held on June 2-3, 1992, and has received internal review by the Concordance and Oversight Subcommittees of the Risk Assessment Forum. These proposed Guidelines also were reviewed on August 15, 1995, by the Committee on the Environment and Natural Resources of the Office of Science and Technology Policy. The proposed Guidelines are based on recommendations derived from these reviews and on those made at various scientific meetings and workshops on neurotoxicology. The proposed Guidelines were published for public comment in the October 4, 1995, issue of the *Federal Register*.

After completion of the SAB review, the proposed Guidelines will be revised according to the public comments and responses to the SAB. The revised Guidelines will then undergo Agency review before being published in final form in the *Federal Register*.

2.2 Charge

a) The combining of hazard identification and dose-response evaluation to reflect more accurately the process used for noncancer health effects (Section I).

The draft Guidelines recognize that, in practice, hazard identification for noncancer health effects is usually done in conjunction with an evaluation of dose-response relationships in studies to identify hazard. This process of hazard characterization provides an evaluation of a hazard within the context of the dose, route, duration and timing of exposure. With one exception, public comment supported the combining of hazard identification and dose-response evaluation.

In 1994, EPA requested SAB comment on this issue during the EHC's review of the draft Guidelines for Reproductive Toxicity Risk Assessment (July 19, 1994 Meeting;

EPA-SAB-EHC-95-014, and December 8, 1995 Memorandum; EPA-SAB-EC-COM-96-001). EPA's draft proposed Neurotoxicity Guidelines reflect the SAB's earlier opinions by incorporating the EHC/SAB comments into these proposed Guidelines.

b) The issue of compensation and recovery of function in neurotoxicological studies and how to account for compensation in neurotoxicology risk assessment (Section II)

Once damaged, neurons, particularly in the central nervous system, have a limited capacity for regeneration. Reversibility of effects resulting from cell death or from destruction of cell processes may represent an activation of repair capacity, decreasing future potential adaptability. The nervous system has a reserve capacity that, once exceeded, results in permanent, irreversible loss of nervous system function and structure. Reversible neurotoxic effects should be of concern to the risk assessor.

c-1) The use of blood and/or brain acetylcholinesterase activity as an indication of neurotoxicity for risk assessment (Section IIIA).

As written, the proposed Guidelines state that statistically significant decreases in brain cholinesterase can be considered to be a biologically significant event. However, the proposed Guidelines indicate that there is a lack of consensus as to whether RBC and/or plasma cholinesterase activity represents biologically significant events. Instead, inhibition of RBC and/or plasma cholinesterase can serve as a biomarker of exposure.

Many comments were received from the public on this issue, including many that supported the position that a 20% inhibition in brain acetylcholinesterase should be considered as evidence of neurotoxicity and that inhibition of erythrocyte, whole-blood, or plasma acetylcholinesterase could provide only indirect evidence of neurotoxicity. Measures of blood acetylcholinesterase could be used to support clinical observations of cholinesterase inhibition.

c-2) Considering the available data and the state of the science, does the SAB agree with the above recommendations?

d) Endpoints indicative of neurotoxicity that may not be covered by these proposed Guidelines, e.g., endocrine disruption or neuroendocrine-mediated neurotoxicity

The proposed Guidelines are inclusive of the major neurotoxicity endpoints of concern and the Agency does not plan to develop additional topics. Few public comments were received on this issue although several that were received supported this position. No additional neurochemical, neurophysiological, or structural endpoints were suggested. Comments indicated no need to consider endocrine disruptors differently then other potential neurotoxic agents.

e) The completeness of the description of the endpoints used in human and animal neurotoxicological assessments (Section III).

The proposed Guidelines are relatively complete in describing endpoints used in human and animal neurotoxicological assessments. Some modification will be made to the human studies section to include discussion on the evaluation of more subjective effects, such as changes in mood, irritability, and well-being as well as additional discussion on neurobehavioral and neurochemical measures.

f) The possibility of no threshold for some neurotoxic agents.

In general, a threshold is assumed for the dose-response curve for most neurotoxicants. This is based on the known capacity of the nervous system to compensate for or to repair a certain amount of damage at the cellular, tissue, or organ level. In addition, because of the multiplicity of cells in the nervous system, multiple insults at the molecular or cellular level may be required to produce an affect on the whole organism.

Recent data suggest that there may be no threshold for some suspected neurotoxic agents such as developmental exposure to lead. During the SAB's July 1994 review of the Guidelines for Reproductive Toxicity Risk Assessment, the EHC recommended that use of the threshold assumption should occur only after an evaluation of the likely biological mechanisms and available data provide evidence that linear responses would not be expected. The Agency agrees that all available mechanistic information should be reviewed carefully and analyzed to determine the most suitable approach when information is available to make such a decision. However, in the absence of data to make a judgment based on biological mechanisms, the Agency will assume a threshold for neurotoxic effects as a default. This approach is consistent with the Agency's long-standing practice used in other EPA guidelines for health effects other than cancer.

g) Adequate treatment of susceptible populations and individuals by the

proposed Guidelines (Section IV).

Several public comments indicated that additional categories of susceptible populations, e.g., the elderly, should be addressed. EPA agrees, but considers parallel questions such as modification of uncertainty factors for susceptible populations to be outside the scope of these proposed Guidelines.

h) The use of the Benchmark Dose in Neurotoxicity Risk Assessment (Section IVB).

There was consensus among public commentors in support of the Agency exploring the use of other models for quantitative risk assessment. However, a majority of respondents indicated that the Benchmark Dose (BMD) was not yet ready for actual use in neurotoxicity risk assessment. Several responders provided a number of qualifiers that should be included in the Guidelines' discussion on the BMD, including the conservative nature at the lower end of the dose-response curve, the need for computational formulas, guidance as to how the BMD would be used in risk assessment, and the need for studies that compare the performance of the BMD relative to the RfD approach.

3. SPECIFIC ISSUES

3.1 Combining Hazard Identification and Dose-response Evaluation

The presence or absence of a dose response relationship is an important consideration in the hazard identification and characterization step of a risk assessment. Further, a discussion of the hazard in terms of dose, route, duration and timing of exposure issues can be important feature of the hazard characterization in some cases. However, this can be achieved without combining the hazard identification and dose response steps, as has been done in the draft neurotoxicity guidelines. Hazard characterization and dose response should remain distinct, separate steps in risk assessment. **The EPA, in its proposed cancer guidelines has identified an approach which accomplishes this while providing for considerations of dose response issues at the hazard identification/characterization stage, and the EHC encourages the Agency to adopt this approach in guidance documents for non-cancer endpoints as well**.

The importance of maintaining hazard characterization and dose response evaluation as separate steps in risk assessment has been previously discussed, in the SAB Environmental Health Committee findings on the Guidelines for Reproductive Risk Assessment SAB, 1995), and in the subsequent December 1995 letter by the SAB chair, Dr. Matanoski to Administrator Browner, which further clarifies the SAB position on this issue (SAB, 1996). The Agency released the draft neurotoxicity guidelines in October 1995, before the letter from the SAB chair was sent to Administrator Browner.

In revising the draft neurotoxicity guidelines, the Agency should consider the SAB position on this matter, as outlined in the above noted letter from the SAB chair:

> "The National Academy of Sciences (NAS) risk assessment framework, described in their 1983 report *Decision Making in the Federal Government: Managing the Process* has proven a useful and durable tool for assessing risk. It identifies easily understandable and recognizable steps for assessing risk and using the results for decision-making. In 1994, the National Academy of Sciences report *Science and Judgment in Risk Assessment*, recommended that EPA and others should broaden the types of information considered in the hazard identification phase of risk assessment. The SAB supports EPA's intent to expand the hazard identification and evaluation phases to include additional data. However we believe that hazard identification/characterization is too

multidimensional to be merged into dose-response assessment in a fixed

manner, and the Committee recommend that the phases of the hazard identification process remain clearly discernable.

The hazard identification should continue to be essentially qualitative in nature on the following where data are available: overall consistency of data, nature of effects observed, relevance of the effect(s) to human health, mechanisms of action if known, pattern of dose response relationships in the studies reviewed, and phamacokinetic data where appropriate (especially in terms of qualitative differences in metabolic pathways between species). In contrast, the dose-response analysis step evaluates in quantitative terms the relationship between dose or exposure and severity or probability of effect in humans."

Finally we note in passing that it can be useful to characterize an agent as a "neurotoxicant," and the Agency has characterized agents as such throughout the current draft guidelines. Yet, the current Guidelines would appear to call for such characterizations only with the indication of the conditions of exposure under which such characterizations would apply.

3.2 Compensation and Recovery of Function in Neurotoxicological Studies

Dealing with reversible neurotoxic effects is a matter of considerable concern to the risk assessor. In some organ systems, such as the liver, functional recovery and tissue regeneration can follow even extensive injury. Tissue regeneration does not occur in nerve cells, however. When functional recovery appears, as after a stroke, it is due basically to the remaining neurons compensating for those lost to injury. This is an extreme example of how apparent reversibility may prove deceptive. A more subtle illustration may be found by examining the post-polio syndrome. Individuals who suffered an episode of polio in their early years, then evidently recovered, experience a reappearance of symptoms as they age. The most popular hypothesis to account for this phenomenon posits compensatory mechanisms as the culprit. Remaining nerve cells in the spinal cord are believed to develop additional connections to overcome the loss of the failed cells. The added metabolic burden, combined with the natural loss of nerve cells with aging, erodes the compensatory margin and the previously masked deficit then reemerges.

Another setting in which apparent reversibility could prove deceptive is the workplace. Recurrent episodes of clinically detectable neurotoxicity due to solvent

exposure, although each is followed by evident recovery, may eventually lead to enough cumulative impairment to be detectable. Perhaps such a history accounts for some

proportion of the deficits observed in workers exposed to solvents during past eras in which monitoring of exposure levels was absent, haphazard, or intermittent.

Laboratory data add another dimension--the aftermath of exposure during early brain development. In rats, brain injury suffered via lesions or ionizing radiation early in life may produce behavioral deficits visible late in life. During adulthood, the deficits often become submerged. During senescence, they emerge again. Prenatal drug treatment can produce similar fluctuations in function over the lifespan.

Stage of the lifespan, in fact, is another variable influencing reversibility. Older animals recover from treatment with MPTP (methylphenyltetrahydraopyridine), AChE antagonists, and other chemical agents less completely and rapidly than younger animals. Apparent reversibility in young organisms cannot be extrapolated to older organisms.

Given such data, the SAB supports the presumption that what appear to be reversible neurotoxic effects, especially those arising from gestational or neonatal exposure and observed before adulthood, should not be dismissed as of little practical consequence. They may be indices of silent toxicity that emerge later in life or may suggest more robust and enduring responses in aged individuals.

3.3 Blood And/or Brain Acetylcholinesterase Activity as an Indication of Neurotoxicity for Risk Assessment

The proposed Guidelines state that statistically significant decreases in brain cholinesterase can be considered to be a biologically significant event, and the Charge for this review asked for the Committee's position on this issue. There is a historic lack of consensus on this issue, however, as to whether RBC and/or plasma cholinesterase activity represents biologically significant events or is primarily a biomarker of exposure.

Many comments were received from the public on this issue, including many that supported the position that a 20% inhibition in brain acetylcholinesterase should be considered as evidence of neurotoxicity and that inhibition of erythrocyte, whole-blood, or plasma acetylcholinesterase could provide only indirect evidence of neurotoxicity.

Measures of blood acetylcholinesterase could be used to support clinical observations of cholinesterase inhibition.

The subject of neurotoxic criteria for acetylcholinesterase (AChE) inhibitors was addressed by the SAB in 1990 (SAB, 1990). This review addressed many of the issues posed to the Committee by the Charge for the review of the current document. The 1990 review concluded that RBC and plasma cholinesterase levels serve as indices of exposure rather than as direct measures of neurotoxicity or other biologically significant effects. This conclusion contradicts the position that any “statistically significant” (a term which is discussed below) reduction in these peripheral ChE levels is evidence of toxicity. The Committee believes that the position taken by the SAB in 1990 is still valid. The 1990 report also noted that reduced brain ChE is not necessarily inherently adverse because of the large functional reserves of AChE in the brain, and compensatory mechanisms such as up- and down-regulation of receptor populations. Inhibitory muscarinic AChE receptors have been located presynaptically, for example, and act as autoreceptors regulating acetylcholine release. Prolonged exposure to cholinergic agonists or AChE inhibitors such as DFP reduces the density of these receptors, even to the point of inducing behavioral deficits despite other indications of tolerance. Defining neurotoxicity on the basis of a statistically significant change in brain ChE activity, however, evades the question of biological significance. As is true for peripheral ChE measures, the brain contains large reserves of AChE. A fall in brain ChE, however, does merit a thorough appraisal of the criteria noted above so that a more decisive relationship between the degree of ChE inhibition, its time course, and its consequences can be established; it is not the same for all organophophates. Such data will indicate the biological significance of a specified fall in ChE. It would help to establish a margin of exposure for the selected agent.

The Guidelines, in discussing changes in ChE levels, use the term "statistically significant." Although seemingly precise, this term is actually vague and undefined. One way to overcome this problem would be to adopt a more tangible and quantitative index, such as the Benchmark Dose and its variations, as discussed in the Guidelines. Various methods are now available to derive such indexes. A promising approach for AChE inhibitors is that outlined by Gaylor and Slikker (1990). It examines the distribution of some measure in a control sample, then determines, in an exposed sample, the proportion of responses beyond a specified measure (e.g., three standard deviations of the control mean). It would lend itself to risk quantification of criteria ranging, say, from 10% to 90% reductions in AChE.

Two additional facets of AChE neurotoxicity should also be discussed in the

Guidelines because they may pertain to other classes of chemicals as well and to the reversibility question (discussed in general terms above). The first is the development of tolerance, possibly arising from down-regulation of receptors, which may mask enhanced sensitivity to other agents or be followed by withdrawal compensation. Or, it may reflect behavioral adaptation, a phenomenon seen with other kinds of agents such as opiates. Again, this is a problem in experimental design and methodology that cannot be dissociated from the discussion in the Guidelines. The second facet, reversibility, is confounded with the enduring consequences of an acute exposure great enough to have induced neurotoxic signs. Long after apparent recovery, neuro-behavioral deficits may still be detectable with sensitive test methods. These lingering effects have been observed in both human and animal studies.

In summary, the Committee does not accept the current position of the Guidelines *vis-a-vis* the significance of ChE decreases. In the absence of clinical signs in humans or animals or the absence of morphological data in animals, the quantitative nature of the inhibition of red blood cell (RBC) and/or plasma cholinesterase is considered unreliable for assessing significant biological adverse changes, but can be used as a biomarker of exposure.

3.4 Other Endpoints Indicative of Neurotoxicity

The proposed Guidelines are inclusive of the major neurotoxicity endpoints of concern and the Agency does not plan to develop additional topics. Few public comments were received on this issue although several that were received supported this position. No additional neurochemical, neurophysiological, or structural endpoints were suggested. Comments indicated no need to consider endocrine disruptors differently then other potential neurotoxic agents.

The Committee recognizes that the description of endpoints indicative of neurotoxicity is extensive, and, in most circumstances, reflects the actions of conventionally neurotoxic agents. The Committee also recognizes the mutual dependence and influences among the nervous, immune, and endocrine systems that have been revealed during the past two decades. In addition to such indirect effects, many agents identified as neurotoxic also exert potent effects on immune and endocrine function. Such broad systemic, interactive effects might not necessarily imply that an agent provoking impaired endocrine function, for example, would need concurrently to be assayed for neurotoxicity. That need would depend upon the nature of the effect. The Guidelines should recognize, however, that a finding of neuroendocrine dysfunction might have to be amplified by neurobehavioral

assessment techniques not discussed or described in the current draft. Observation of a widening or narrowing of male-female differences on certain neurotoxic endpoints could be a reflection of interference with neuroendocrine function. Specific recommendations should be included in the Guidelines for dealing with such multi-system actions because risk assessors will typically not be sensitive to their ramifications.

3.5 Description of Endpoints for Neurotoxicological Assessments

In general the descriptions of endpoints used in human and animal neurotoxicological assessments are thorough, easy to read, adequately documented and, in fact, appear to be exhaustive. There are a few areas for improvement, however. The observations in the functional observational battery (FOB) could be "sharpened;" FOBs should be conducted according to the need for inter-observer reliability and thoroughly defined responses.

As a caution to the inexperienced reader on the use of single animal observations of a behavioral parameter to reach a conclusion on the NOAEL, the Guidelines state (correctly) that "It is reasonable to assume that a NOAEL or LOAEL could be based on one or more of these endpoints." It would be useful to move this sentence from its current location (the third column, middle of page 52044) to be closer to the discussion of the relevance of statistically significant test results at the bottom of the page.

Finally, adding caveats about the need to be sure the data are reliable, particularly for case reports and neurologic examinations, to the discussion of tests used in clinical studies would improve the document, and changing the word "Summary" in the title of Table 5 to "Examples" would make it consistent with the other tables.

3.6 The Possibility of No Threshold for Some Neurotoxic Agents

In general, a threshold is assumed for the dose-response curve for most neurotoxicants. This is based on the known capacity of the nervous system to compensate for or to repair a certain amount of damage at the cellular, tissue, or organ level. In addition, because of the multiplicity of cells in the nervous system, multiple insults at the molecular or cellular level may be required to produce an affect on the

whole organism.

Recent data suggest that there may be no threshold for some suspected neurotoxic agents such as developmental exposure to lead. During the SAB's July 1994 review of the Guidelines for Reproductive Toxicity Risk Assessment, the EHC recommended that use of the threshold assumption should occur only after an evaluation of the likely biological mechanisms and available data provide evidence that linear responses would not be expected. The Agency agrees that all available mechanistic information should be reviewed carefully and analyzed to determine the most suitable approach when information is available to make such a decision. However, in the absence of data to make a judgment based on biological mechanisms, the Agency assumes a threshold for neurotoxic effects as a default. This approach is consistent with the Agency's long-standing practice used in other EPA guidelines for health effects other than cancer.

A strict threshold is not always clear in the human population because of the wide variation in background levels of neurobehavioral function. Cumulative neuro-damage may alter the response of some individuals within a special population. Therefore, EPA risk assessors should have the option of establishing one level for the general healthy population and a second level to protect a separate population of compromised individuals. It must be remembered that the concept of "threshold" is physiological and structural in the context of general neurological function, and the wide range of "normal" has to be surpassed before damage is considered clinically significant. This is not necessarily true in laboratory animals where function was apparently normal in the face of data to the contrary at autopsy.

Although the Committee does not disagree with the guidelines assumption of a threshold as a default for neurotoxic effects, it urges EPA to harmonize its treatment of this issue with the 'weighing of evidence' positions taken in the draft Carcinogen Risk Assessment Guidelines (which, at the time of the public meeting were out for public comment).

3.7 Treatment of Susceptible Populations and Individuals

Several public comments indicated that additional categories of susceptible populations, e.g., the elderly, should be addressed. EPA agreed, but considered parallel questions such as modification of uncertainty factors for susceptible populations to be outside the scope of the proposed Guidelines.

There was, however, a consensus among the Committee members that the elderly should be considered a susceptible population. The elderly might be at increased risk of toxic effects for several age-related reasons, including:

a) loss of neurons with age which may result in a decline in the reserve capacity for the development of compensatory neural pathways required with various types of injury;

b) decrease in the ability to detoxify or excrete xenobiotics with age which may result in increased body levels of the biologically active agent for the same exposure; and

c) increase with age in the use of medications which may increase the potential for synergistic interactions between toxic chemicals and medications.

There may be other special populations that also deserve consideration as potentially susceptible to the effects of neurotoxicants. These populations include:

a) individuals with other chronic and debilitating conditions (e.g., stroke, cancer, renal failure);

b) certain groups of workers with potential exposure to other chemicals that may be neurotoxic such as solvents or pesticides;

b) some ethnic subgroups because genetic polymorphism for susceptibility to toxicity in humans may vary with ethnicity;

c) some disadvantaged communities because of their interactions with the environment (e.g., may cluster around exposures to mixtures of other chemicals), or diet (e.g., subclinical nutritional deficiencies or a diet that may be rich in an item prone to certain contamination).

In the absence of data indicating whether or how the uncertainty factor should be modified, the risk assessor should be advised to consider the extent to which the exposed population includes susceptible subgroups and, if possible, conduct risk assessment separately for such subgroups.

3.8 The use of the Benchmark Dose

This issue was the subject of prolonged discussion at the Committee's public meeting. There were differences within the EHC on many aspects of the use and interpretation of the "Benchmark Dose" (BMD), and **there was not a consensus that the BMD was ready for immediate incorporation into adjustment-factor-based safety assessment and could serve as a substitute or replacement for the more familiar No-Observed-Adverse-Effect-Level (NOAEL)**. Research and development on the BMD should be aggressively encouraged and actively supported by both the public and private sectors. In the meantime, trials to gain experience with the BMD and its use in safety assessment should also be encouraged. Attention should be directed at developing a broad consensus among informed scientists on:

a) guidance on the characteristics and qualities of specific data sets that make them appropriate (or inappropriate) for use in BMD estimation

b) a definition for the term, "observable range" and guidance on the limits for that range

c) bases for selecting from among the various methods and models to calculate the BMD

d) guidance on how to incorporate information on the dose-response slope into safety assessment judgments

e) means to prevent inappropriate extrapolation of BMD statistics

f) mathematically robust and biologically meaningful means to deal with continuous, as well as quantal, data

4. SUMMARY

In general, the Committee finds the revised Guidelines document to be quite successful, and, all things considered, well suited to its intended task. It addresses a wide range of subjects of considerable complexity and (often) of considerable subtlety, and constitutes a clear step forward in the state of the art. Naturally, as with any such ambitious undertaking, the Committee's review has identified areas where improvements could be made. Specifics on such improvements, which generally lie outside the area of the specific Charge for the review, and reflect the thoughts of individual Committee Members rather than a Committee consensus, have been provided to Agency staff.

The various items of the Charge are addressed in detail in the body of this report (Section 3), and are summarized (with the specific question italicized)below:

a) *The combining of hazard identification and dose-response evaluation to reflect more accurately the process used for noncancer health effects.* There was a clear consensus that EPA should follow the SAB position articulated in the recent "Commentary on Hazard identification" from the Executive Committee (EPA-SAB-EC-COM-96-001, December 8, 1995) which called for keeping hazard identification as an identifiable qualitative step in the risk assessment process. The Committee recommended that revisions be made to decouple the qualitative of hazard identification from more quantitatively rigorous steps of exposure evaluation and dose response assessment. The Committee encourages the Agency to adopt an approach similar to that used in the proposed Cancer Risk Assessment Guidelines.

b) *The issue of compensation and recovery of function in neurotoxicological studies and how to account for compensation in neurotoxicology risk assessment*. The Committee took the position that reversibility can not be ignored in risk assessment, but that an assessor had to be very careful and search out particularly good evidence if recovery or an apparent transient effect is cited to support evidence for relatively benign effects. In general, assessors should be advised to look carefully at instances where reversibility is involved and be alert to the possibility of re-occurrence of effects. The Committee recommended that EPA address specifically differences related to type of damage, nature of the insulting agent,

and age of the organism, as well providing case examples relating to possible reversibility.

c) *The use of blood and/or brain acetylcholinesterase activity as an indication of neurotoxicity for risk assessment* and *Considering the available data and the state of the science, does the SAB agree with the recommendation that inhibition of RBC and/or plasma cholinesterase can serve only as a biomarker of exposure?* The Committee addressed these two issues together because of their close relationship. The EHC concurred with the findings of previous SAB reviews regarding the consideration of data on the inhibition of RBC and/or plasma cholinesterase. In the absence of clinical signs in humans or animals or the absence of morphological data in animals, the quantitative nature of the inhibition of red blood cell (RBC) and/or plasma cholinesterase is considered unreliable for assessing significant biological adverse changes, but can be used as a biomarker of exposure. The Committee also recommended that a noted decline in brain ChE should be evaluated by risk assessors in terms of possible effects that are biologically significant, and that the term "statistically significant" needed to be better explicated -- perhaps in terms of the benchmark dose or by some measure which reflected information about the distribution of the effect under study. The Committee also suggested that further details concerning reversibility and possible tolerance effects (which could enhance sensitivity to other agents) be provided.

d) *Are there endpoints indicative of neurotoxicity that may not be covered by these proposed Guidelines, e.g., endocrine disruption or neuroendocrine-mediated neurotoxicity?* The Committee considered that the description of endpoints indicative of neurotoxicity was extensive and it does not suggest additional endpoints based on current scientific knowledge. The Committee also recognizes that some chemical agents may cause both neurotoxic and other effects such as endocrine disruption; however endocrine disruption without evidence of a neurochemical or neurophysiological causation would not be a basis to label that chemical agent as a neurotoxicant. This notwithstanding, the Guidelines should recognize that a finding of neuroendocrine dysfunction might have to be amplified by neurobehavioral assessment techniques not discussed or described in the current draft. Observation of a widening or narrowing of male-female differences on certain neurotoxic endpoints could be a

reflection of interference with neuroendocrine function. Specific recommendations should
be included in the Guidelines for dealing with such multi-system actions because risk assessors will typically not be sensitive to their ramifications.

e) *Are the descriptions of the endpoints used in human and animal neurotoxicological assessments complete?* In the broadest sense, the Committee found the treatment of this issue by the Guidelines document to be "..not complete, but complete enough for its purposes," meaning that, although the Committee has many refinements and improvements to suggest, the Guidelines provided sufficient information on various endpoints to enable a risk assessor to do his/her job. More specifically, the Committee found that coverage of human studies was quite good, but that the treatment of animal studies (particularly with regard to warning caveats) was. by comparison, weaker. It was also suggested that better coverage be given to various possible tests (including references for various test methodologies); the Committee also agreed with the comment by EPA staff that the Guidelines were not a "Testing Guideline," and that details of specific tests were not called for. Re tests, the EHC also suggested that Table V list the cited tests in alphabetical order to avoid the implication of priority noted in the current arrangement. It was also noted that addition treatment of maternal toxicity and possible effects on offspring, particularly re interpreting dose-response curves, would be useful.

g) *Treatment of the possibility of no threshold for some neurotoxic agents.* There was considerable debate/discussion as to what the concept of a threshold, or of no threshold, *actually* meant in terms of a risk assessment, and whether the concept itself was meaningful or useful in this context. It was proposed that the term "non-linear (at low doses) dose-response curve for most neurotoxicants" be substituted for the term "threshold." The EPA was urged to harmonize its treatment of this (as well as several other issues) with the presentation and positions taken in the draft Carcinogen Risk Assessment Guidelines currently out for public comment.

g) Adequacy of the treatment of susceptible populations and individuals by the proposed Guidelines. The Committee noted that the aged were the

only specific sub-population noted, and that the Guidelines do not discuss means of modifying NOAELS or dose-response curves in this context. It was suggested that the Guidelines be modified to identify other susceptible groups (e.g., those with poor nutritional status) and provide suggestions as to dealing with them.

h) *The use of the Benchmark Dose in Neurotoxicity Risk Assessment*. There was considerable discussion within the Committee and between the Committee, EPA staff, and members of the audience as to the nature of the results yielded by the application of the benchmark dose methodology and how it compared with the more common NOAEL measure. No consensus on this aspect of the issue was reached. The Committee did note that definition of the benchmark dose in the Guidelines was not clear and shout be improved; it further suggested that the basic position of the Guidelines be retained, i.e., that the use of the benchmark dose should be explored in specific situations, but that the NOAEL measure was the default approach.

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