ENVIRONMENTAL PROTECTION AGENCY

[FRL–5306–2]

Proposed Guidelines for Neurotoxicity Risk Assessment

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed guidelines for Neurotoxicity Risk Assessment and request for comments.

SUMMARY: The U.S. Environmental Protection Agency (EPA; Agency) is today issuing proposed guidelines for assessing the risks for neurotoxicity from exposure to environmental agents. As background information for this guidance, this notice describes the scientific basis for concern about exposure to agents that cause neurotoxicity and outlines the general process for assessing potential risk to humans because of environmental contaminants.

These proposed Guidelines for Neurotoxicity Risk Assessment (hereafter ''Guidelines'') are intended to guide Agency evaluation of agents that are suspected to cause neurotoxicity in line with the policies and procedures established in the statutes administered by the EPA. The 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 governmental agencies. A subsequent draft has undergone 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. Most recently, the Committee on the Environment and Natural Resources of the Office of Science and Technology Policy reviewed the guidelines at a meeting held on August 15, 1995. The proposed Guidelines are based, in part, on recommendations derived from these reviews and on those made at various scientific meetings and workshops on neurotoxicology.

The public is invited to comment, and public comments will be considered in EPA decisions in formulating the final Guidelines. Commenters are asked to focus on several special issues, particularly, (1) the issue of compensation and recovery of function in neurotoxicological studies and how to account for compensation in neurotoxicology risk assessment; (2) the use of blood and/or brain acetylcholinesterase activity as an indication of neurotoxicity for risk assessment; (3) endpoints indicative of neurotoxicity that may not be covered by these guidelines, i.e., endocrine disruption or neuroendocrine-mediated neurotoxicity; and (4) the possibility of no threshold for some neurotoxic agents.

The EPA Science Advisory Board (SAB) also will review these proposed Guidelines at a meeting to be announced in a future Federal Register. Agency staff will prepare summaries of the public and SAB comments, analyses of major issues presented by commenters, and Agency responses to those comments. Appropriate comments will be incorporated, and the revised Guidelines will be submitted to the Risk Assessment Forum for review. The Agency will consider comments from the public, the SAB, and the Risk Assessment Forum in its recommendations to the EPA Administrator.

DATES: The Proposed Guidelines are being made available for a 120-day public review and comment period. Comments must be in writing and must be postmarked by February 1, 1996. Please submit one unbound original with pages consecutively numbered, and three copies. If there are attachments, include an index numbered consecutively with comments, and three copies.

FOR FURTHER INFORMATION CONTACT: Dr. Hugh A. Tilson, Tel: 919–541–2671; Fax: 919–541–4849.

ADDRESSES: Comments on the proposed Guidelines may be mailed or delivered to: Dr. Hugh A. Tilson, Neurotoxicology Division (MD–74B), National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711. Please note that all comments received in response to this notice will be placed in a public record. Commenters should not send any item of personal information, such as medical information or home address, if they do not wish it to be part of the public record.

SUPPLEMENTARY INFORMATION: In its 1983 book, Risk Assessment in the Federal Government: Managing the Process, the National Academy of Sciences recommended that Federal regulatory agencies establish ''inference guidelines'' (1) to promote consistency and technical quality in risk assessment, and (2) to ensure that the risk assessment process is maintained as a scientific effort separate from risk management. A task force within EPA accepted that recommendation and requested that Agency scientists begin to develop such guidelines.

In 1984, EPA scientists began work on risk assessment guidelines for carcinogenicity, mutagenicity, suspect developmental toxicants, chemical mixtures, and exposure assessment. Following extensive scientific and public review, these first five guidelines were issued on September 24, 1986 (51 FR 33992–34054). Since 1986, additional risk assessment guidelines have been proposed for male and female reproductive risk (53 FR 24834–847; 53 FR 24850–869), and two of the 1986 guidelines, suspect developmental toxicants (56 FR 63798–826) and exposure assessment (57 FR 22888–938), have been revised, reproposed, and finalized.

The Guidelines proposed today continue the guidelines development process initiated in 1984. These Guidelines set forth principles and procedures to guide EPA scientists in the conduct of Agency risk assessments and to inform Agency decision makers and the public about these procedures. In particular, the Guidelines emphasize that risk assessments will be conducted on a case-by-case basis, giving full consideration to all relevant scientific information. This case-by-case approach means that Agency experts study scientific information on each chemical under review and use the most scientifically appropriate interpretation to assess risk. The Guidelines also stress that this information will be fully presented in Agency risk assessment documents, and that Agency scientists will identify the strengths and weaknesses of each assessment by describing uncertainties, assumptions, and limitations, as well as the scientific basis and rationale for each assessment.

The Guidelines are formulated in part to bridge gaps in risk assessment methodology and data. By identifying these gaps and the importance of the missing information to the risk assessment process, EPA wishes to encourage research and analysis that will lead to new risk assessment methods and data.

Dated: September 25, 1995.

Carol M. Browner,

Administrator.

Proposed Guidelines for Neurotoxicity Risk Assessment Contents

I. Introduction

These proposed Guidelines describe the principles, concepts, and procedures that the U.S. Environmental Protection Agency (EPA; Agency) would follow in evaluating data on potential neurotoxicity associated with exposure to environmental toxicants. The Agency's authority to regulate substances that have the potential to interfere with human health is derived from a number of statutes that are implemented through multiple offices within the EPA. The procedures outlined here are intended to help develop a sound scientific basis for neurotoxicity risk assessment, promote consistency in the Agency's assessment of toxic effects on the nervous system, and inform others of the approaches used by the Agency in those assessments.

A. Organization of These Guidelines

This Introduction (section I) summarizes the purpose of these proposed Guidelines within the overall framework of risk assessment at the EPA. It also outlines the organization of the guidance and describes several default assumptions to be used in the risk assessment process as discussed in the recent National Research Council report ''Science and Judgment in Risk Assessment (NRC, 1994).''

Section II sets forth definitions of particular terms widely used in the field of neurotoxicology. These include ''neurotoxicity'' and ''behavioral alterations.'' Also included in this section are discussions concerning reversible and irreversible effects and direct versus indirect effects.

Risk assessment is the process by which scientific judgments are made concerning the potential for toxicity to occur in humans. The National Research Council (NRC, 1983) has defined risk assessment as including some or all of the following components (paradigm): hazard identification, dose-response assessment, exposure assessment, and risk characterization. In its 1994 report ''Science and Judgment in Risk Assessment'' the NRC extended its view of the paradigm to include characterization of each component (NRC, 1994). In addition, it noted the importance of an approach that is less fragmented and more holistic, less linear and more interactive, and one that deals with recurring conceptual issues that cut across all stages of risk assessment. These Guidelines propose a more interactive approach by organizing the process around components that focus on evaluation of the toxicity data (hazard characterization), the quantitative dose-response analysis, the exposure assessment, and the risk characterization. This is done because, in practice, hazard identification for neurotoxicity and other noncancer health effects is usually done in conjunction with an evaluation of dose-response relationships in the studies used to identify the hazard. Determining a hazard often depends on whether a dose-response relationship is present (Kimmel et al., 1990). Thus, the hazard characterization provides an evaluation of a hazard within the context of the dose, route, duration, and timing of exposure. This approach combines the information important in comparing the toxicity of a chemical to potential human exposure scenarios (Section V). Secondly, it avoids the potential for labeling chemicals as ''neurotoxicants'' on a purely qualitative basis. This organization of the risk assessment process is similar to that discussed in the Guidelines for Developmental Toxicity Risk Assessment (56 FR 63798), the main difference being that the quantitative dose-response analysis is discussed under a separate section in these guidelines.

Hazard characterization involves examining all available experimental animal and human data and the associated doses, routes, timing, and durations of exposure to determine if an agent causes neurotoxicity in that species and under what conditions. From the hazard characterization and criteria provided in these Guidelines, the health-related data base can be characterized as sufficient or insufficient for use in risk assessment (section III.C). Combining hazard identification and some aspects of dose-response evaluation into hazard characterization does not preclude the evaluation and use of data when quantitative information for setting reference doses (RfDs) and reference concentrations (RfCs) are not available.

The next step, the dose-response analysis (section IV) is the quantitative analysis, and includes determining the no-observed-adverse-effect-level (NOAEL) and/or the lowest-observed-adverse-effect-level (LOAEL) for each study and type of effect. Because of the limitations associated with the use of the NOAEL, the Agency is beginning to use an additional approach, i.e., the

benchmark dose approach (Crump, 1984; U.S. EPA, 1995a), for more quantitative dose-response evaluation when sufficient data are available. The benchmark dose approach takes into account the variability in the data and the slope of the dose-response curve, and provides a more consistent basis for calculation of the RfD or RfC. If data are considered sufficient for risk assessment, and if neurotoxicity is the effect occurring at the lowest dose level (i.e., the critical effect), an oral or dermal RfD or an inhalation RfC, based on neurotoxic effects, is then derived. This RfD or RfC is derived using the NOAEL or benchmark dose divided by uncertainty factors to account for interspecies differences in response, intraspecies variability and other factors of study design or the data base. A statement of the potential for human risk and the consequences of exposure can come only from integrating the hazard characterization and dose-response analysis with the human exposure estimates in the final risk characterization.

The section on exposure assessment (section V) identifies human populations exposed or potentially exposed to an agent, describes their composition and size, and presents the types, magnitudes, frequencies, and durations of exposure to the agent. The exposure assessment provides an estimate of human exposure levels for particular populations from all potential sources.

In risk characterization (section VI), the hazard characterization, dose-response analysis, and the exposure assessment for given populations are combined to estimate some measure of the risk for neurotoxicity. As part of risk characterization, a summary of the strengths and weaknesses of each component of the risk assessment is given along with major assumptions, scientific judgments, and, to the extent possible, qualitative and quantitative estimates of the uncertainties. This characterization of the health-related data base is always presented in conjunction with information on the dose, route, duration and timing of exposure as well as the dose-response analysis including the RfD or RfC. If human exposure estimates are available, the exposure basis used for the risk assessment is clearly described, e.g., highly exposed individuals or highly sensitive or susceptible individuals. The NOAEL may be compared to the various estimates of human exposure to calculate the margin(s) of exposure (MOE). The considerations for judging the acceptability of the MOE are similar to those for determining the appropriate size of the uncertainty factor for calculating the RfD or RfC.

The Agency recently issued a policy statement and associated guidance for risk characterization (U.S. EPA, 1995b, 1995c), which is currently being implemented throughout EPA. This policy statement is designed to ensure that critical information from each stage of a risk assessment is used in forming conclusions about risk and that this information is communicated from risk assessors to risk managers (policy makers), from middle to upper management, and from the Agency to the public. Additionally, the policy provides a basis for greater clarity, transparency, reasonableness, and consistency in risk assessments across Agency programs. Final neurotoxicity risk assessment guidelines may reflect additional changes in risk characterization practices resulting from implementation activities.

Risk assessment is just one component of the regulatory process and defines the potential adverse health consequences of exposure to a toxic agent. The other component, risk management, combines risk assessment with statutory directives regarding socioeconomic, technical, political, and other considerations, to decide whether to control future exposure to the suspected toxic agent and, if so, the nature and level of control. One major objective of these risk assessment Guidelines is to help the risk assessor determine whether the experimental animal or human data indicate the potential for a neurotoxic effect. Such information can then be used subsequently to categorize evidence to identify and characterize neurotoxic hazards as described in section III.3.C, Characterization of the Health-Related Data Base, and Table 8 of these Guidelines. Risk management is not dealt with directly in these Guidelines because the basis for decision making goes beyond scientific considerations alone, but the use of scientific information in this process is discussed. For example, the acceptability of the MOE is a risk management decision, but the scientific bases for establishing this value are discussed here.

B. The Role of Environmental Agents in Neurotoxicity

Chemicals are an integral part of life, with the capacity to improve as well as endanger health. The general population is exposed to chemicals with neurotoxic properties in air, water, foods, cosmetics, household products, and drugs used therapeutically or illicitly. Naturally occurring neurotoxins, such as animal and plant toxins, present additional hazards. During daily life, a person experiences a multitude of exposures, both voluntary and unintentional, to neuroactive substances, singly and in combination. Levels of exposure vary and may or may not pose a hazard depending on dose, route, and duration of exposure.

A link between human exposure to some chemical substances and neurotoxicity has been firmly established (Anger, 1986; OTA, 1990). Because many natural and synthetic chemicals are present in today's environment, there is growing scientific and regulatory interest in the potential for risks to humans from exposure to neurotoxic agents. If sufficient exposure occurs, the effects resulting from such exposures can have a significant adverse impact on human health. It is not known how many chemicals may be neurotoxic in humans (Reiter, 1987). The EPA's inventory of toxic chemicals is greater than 65,000 and increasing yearly. An overwhelming majority of the materials in commercial use have not been tested for their neurotoxic potential (NRC, 1984). Estimates of the number of chemicals with neurotoxic properties have been made for subsets of substances. For instance, a large percentage of the more than 500 registered active pesticide ingredients are neurotoxic to varying degrees. Of 588 chemicals listed by the American Conference of Governmental Industrial Hygienists, 167 affected the nervous system or behavior at some exposure level (Anger, 1984). Anger (1990) estimated that of the approximately 200 chemicals to which one million or more American workers are exposed, more than one-third may have adverse effects on the nervous system, if sufficient exposure occurs. Anger (1984) also recognized neurotoxic effects as one of the 10 leading workplace disorders. A number of therapeutic substances, including some anticancer and antiviral agents and abused drugs, can cause adverse or neurotoxicological side effects at therapeutic levels (OTA, 1990). Thus, estimating the risks of exposure to chemicals with neurotoxic potential is of concern with regard to the overall impact of these exposures on human health.

C. Neurotoxicity Risk Assessment

In addition to its primary role in cognitive functions, the nervous system controls most, if not all, other bodily processes. It is sensitive to perturbation from various sources and has limited ability to regenerate. There is evidence that even small anatomical, biochemical, or physiological insults to the nervous system may result in

adverse effects on human health. Therefore, there is a need for consistent guidance on how to evaluate data on neurotoxic substances and assess to what degree, if any, they have the potential to cause transient or persistent, direct or indirect effects on human health.

To help address these needs, these Guidelines develop principles and concepts in several areas. First, these Guidelines outline the scientific basis for evaluating effects due to exposure to neurotoxicants and discuss principles and methods for evaluating data from human and animal studies on behavior, neurochemistry, neurophysiology, and neuropathology. This guidance document also discusses adverse effects on neurological development and function in infants and children following prenatal and perinatal exposure to chemical agents. Other sections of these Guidelines outline the method for calculating reference doses or reference concentrations when neurotoxicity is the critical effect, discuss the availability of alternative mathematical approaches to dose-response analyses, characterize the health-related data base for neurotoxicity risk assessment, and discuss integration of exposure information with the results of the dose-response assessment to characterize risks of exposures of concern. These Guidelines do not advocate developing reference doses specific for neurotoxicity, but rather the use of neurotoxicity as one possible end point to develop reference doses.

EPA offices have published guidelines for neurotoxicity testing in animals (U.S. EPA, 1986, 1987, 1988a, 1991a). The testing guidelines address the development of new data for use in risk assessment. These proposed neurotoxicity risk assessment Guidelines provide the Agency's first comprehensive guidance on the use and interpretation of neurotoxicity data. These proposed Guidelines are part of the Agency's risk assessment guidelines development process, which was initiated in 1984. As part of its neurotoxicity guidelines development program, the EPA has sponsored or participated in several conferences on relevant issues (Tilson, 1990); these and other sources (see references) provide the scientific basis for these proposed risk assessment Guidelines. This guidance is intended for use by Agency risk assessors and is separate and distinct from the recently published document on principles of neurotoxicity risk assessment (U.S. EPA, 1993). The document on principles was prepared under the auspices of the Subcommittee on Risk Assessment of the Federal Coordinating Council for Science, Engineering, and Technology and was not intended to provide specific directives for how neurotoxicity risk assessment should be performed.

It is expected that, like other EPA risk assessment guidelines (U.S. EPA, 1991b), this document will encourage research and analysis leading to new risk assessment methods and data, which in turn would be used to revise and improve the Guidelines and better guide Agency risk assessors.

D. Assumptions

There are a number of unknowns in the extrapolation of data from animal studies to humans. Therefore, a number of default assumptions are made that are generally applied in the absence of data on the relevance of effects to potential human risk. Default assumptions should not be applied indiscriminantly. First, all available mechanistic and pharmacokinetic data should be considered. If these data indicate that an alternative assumption is appropriate or obviate the need for applying an assumption, such information should be used in the risk assessment of that agent. The following default assumptions form the basis of the approaches taken in these Guidelines.

It is assumed that an agent that produces detectable adverse neurotoxic effects in experimental animal studies will pose a potential hazard to humans. This assumption is based on the comparisons of data for known human neurotoxicants (Anger, 1990; Kimmel et al., 1990; Spencer and Schaumburg, 1980), which indicate that experimental animal data are frequently predictive of a neurotoxic effect in humans.

It is assumed that behavioral, neurophysiological, neurochemical, and neuroanatomical manifestations are of concern. In the past, the tendency has been to consider only neuropathological changes as end points of concern. Based on the data on agents that are known human neurotoxicants (Anger, 1990; Kimmel et al., 1990; Spencer and Schaumberg, 1980), there is usually at least one experimental species that mimics the types of effects seen in humans, but in other species tested, the type of neurotoxic effect may be different or absent. Thus, a biologically significant increase in any of the manifestations is considered indicative of an agent's potential for disrupting the structure or function of the human nervous system.

It is assumed that the types of neurotoxic effects seen in animal studies may not always be the same as those produced in humans. Therefore, it may be difficult to determine which will be the most appropriate species in terms of predicting the specific types of effects seen in humans. The fact that every species may not react in the same way is probably due to species-specific differences in maturation of the nervous system, differences in timing of exposure, metabolism, or mechanisms of action.

It is assumed that the most appropriate species will be used when data are available to estimate human risk. In the absence of such data, the most sensitive species is used, based on the fact that for the majority of known human neurotoxicants, humans are as sensitive or more so than the most sensitive animal species tested.

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 effect on the whole organism.

These assumptions are ''plausibly conservative'' (NRC, 1994) in that they are protective of public health and are also well-founded in scientific knowledge about the effects of concern.

II. Definitions and Critical Concepts

This section defines the key terms and concepts that the EPA will use in the identification and evaluation of neurotoxicity. The various health effects that fall within the broad classification of neurotoxicity are described and examples are provided.

Adverse effects include alterations from baseline that diminish an organism's ability to survive, reproduce, or adapt to the environment. Neurotoxicity is an adverse change in the structure or function of the central and/or peripheral nervous system following exposure to a chemical, physical, or biological agent (Tilson, 1990). Neurotoxic effects include changes in somatic/autonomic, sensory, motor, and/or cognitive function. Structural effects are defined as neuroanatomical changes occurring at any level of nervous system organization; functional changes are defined as neurochemical, neurophysiological, or behavioral alterations. Changes in function can also result from toxicity to other specific organ systems, and these indirect changes may be considered adverse but not necessarily neurotoxic.

The risk assessor also should know that there are different levels of concern based on the magnitude of effect and reversibility of some neurotoxic effects. Neurotoxic effects may be irreversible, i.e., cannot return to the state prior to exposure, resulting in a permanent change in the organism, or reversible, i.e., can return to the pre-exposure condition, allowing the organism to return to its state prior to exposure. Clear or demonstrable irreversible change in either the structure or function of the nervous system causes greater concern than do reversible changes. If neurotoxic effects are observed at some time during the life span of the organism but are slowly reversible, the concern is also high. There is lesser concern for effects that are rapidly reversible or transient, i.e., measured in minutes, hours, or days, and appear to be associated with the pharmacokinetics of the causal agent and its presence in the body. Reversible changes that occur in the occupational setting or environment, however, may be of high concern if, for example, exposure to a short-acting solvent interferes with operation of heavy equipment in an industrial plant. The context of the exposure should be considered in evaluating reversible effects. The risk assessor should note that once damaged, neurons, particularly in the central nervous system, have a limited capacity for regeneration. 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. Therefore, even reversible neurotoxic changes should be of concern. Evidence of progressive effects, i.e., those that continue to worsen even after the causal agent has been removed; or delayed effects, i.e., those that occur at a time distant from the last contact with the causal agent; or residual effects, i.e., those that persist beyond a recovery period; or latent effects, i.e., those that become evident only after an environmental challenge or aging, have a high level of concern. Environmental challenges can include stress, increased physical or cognitive workload, pharmacological manipulations, and nutritional deficiency or excess.

Neurotoxic effects can be observed at various levels of organization of the nervous system, including neurochemical, anatomical, physiological, or behavioral. At the neurochemical level, for example, an agent that causes neurotoxicity might inhibit macromolecule or transmitter synthesis, alter the flow of ions across cellular membranes, or prevent release of neurotransmitter from the nerve terminals. Anatomical changes may include alterations of the cell body, the axon, or the myelin sheath. At the physiological level, a chemical might change the thresholds for neural activation or reduce the speed of neurotransmission. Behavioral alterations can include significant changes in sensations of sight, hearing, or touch; alterations in simple or complex reflexes and motor functions; alterations in cognitive functions such as learning, memory or attention; and changes in mood, such as fear or rage, disorientation as to person, time, or place, or distortions of thinking and feeling, such as delusions and hallucinations. At present, relatively few neurotoxic syndromes have been thoroughly characterized in terms of the initial neurochemical change, structural alterations, physiological consequence, and behavioral effects. Knowledge of exact mechanisms of action is not, however, necessary to conclude that a chemically induced change is a neurotoxic effect.

Neurotoxic effects can be produced by chemicals that do not require metabolism prior to interacting with their target sites in the nervous system, i.e., primary neurotoxic agents, or those that require metabolism prior to interacting with their target sites in the nervous system, i.e., secondary neurotoxic agents. Chemically induced neurotoxic effects can be direct, i.e., due to an agent or its metabolites acting directly on target sites in the nervous system, or indirect, i.e., due to agents or metabolites that produce their effects primarily by interacting with target sites outside the nervous system, which subsequently affect target sites in the nervous system. Excitatory amino acids such as domoic acid damage specific neurons directly by activating excitatory amino acid receptors in the nervous system, while carbon monoxide decreases oxygen availability, which indirectly kills neurons. Other examples of indirect effects of chemicals that could lead to altered structure and/or function of the nervous system include cadmium-induced spasms in blood vessels supplying the nervous system, dichloroacetate-induced perturbation of metabolic pathways, and chemically induced alterations in skeletomuscular function or structure and effects on the endocrine system. Professional judgment may be required in making determinations about direct versus indirect effects.

The interpretation of data as indicative of a potential neurotoxic effect involves the evaluation of the validity of the data base. This approach and these terms have been adapted from the literature on human psychological testing (Sette, 1987; Sette and MacPhail, 1992) where they have long been used to evaluate the level of confidence in different measures of intelligence or other abilities, aptitudes, or feelings. There are four principal questions that should be addressed: whether the effects result from exposure (content validity); whether the effects are adverse or toxicologically significant (construct validity); whether there are correlative measures among behavioral, physiological, neurochemical, and morphological end points (concurrent validity); and whether the effects are predictive of what will happen under various conditions (predictive validity). Addressing these issues can provide a useful framework for evaluating either human or animal studies or the weight of evidence for a chemical (Sette, 1987; Sette and MacPhail, 1992). The next sections indicate the extent to which chemically induced changes can be interpreted as providing evidence of neurotoxicity.

III. Hazard Characterization

A. Neurotoxicological Studies: End Points and Their Interpretation

Identification and characterization of neurotoxic hazard can be based on either human or animal data (Anger, 1984; Reiter, 1987; U.S. EPA, 1993). Such data can result from accidental, inappropriate, or controlled experimental exposures. This section describes many of the general and some of the specific characteristics of human studies and reports of neurotoxicity. It then describes some features of animal studies of neuroanatomical, neurochemical, neurophysiological, and behavioral effects relevant to risk assessment. The process of characterizing the sufficiency or insufficiency of neurotoxic effects for risk assessment is described in section III.C. Additional sources of information relevant to hazard characterization, such as comparisons of molecular structure among compounds and in vitro screening methods, are also discussed.

The hazard characterization should:

a. Identify strengths and limitations of the database:

—Epidemiological studies (case reports, cross-sectional, case-control, cohort, or human laboratory exposure studies);

—Animal studies including (structural or neuropathological, neurochemical, neurophysiological, behavioral or neurological, or developmental end points).

b. Evaluate the validity of the database:

—Content validity (effects result from exposure);
—Construct validity (effects are adverse or toxicologically significant);
—Concurrent validity (correlative measures among behavioral, physiological, neurochemical, or morphological end points);
—Predictive validity (effects are predictive of what will happen under various conditions).

c. Identify and describe key toxicological studies.

d. Describe the type of effects:

—Structural (neuroanatomical alternations);
—Functional (neurochemical, neurophysiological, behavioral alterations).

e. Describe the nature of the effects (irreversible, reversible, transient, progressive, delayed, residual, or latent effects).

f. Describe how much is known about how (through what biological mechanism) the chemical produces adverse effects.

g. Discuss other health end points of concern.

h. Comment on any non-positive data in humans or animals.

i. Discuss the dose-response data (epidemiological or animal) available for further dose-response analysis.

j. Discuss the route, level, timing, and duration of exposure in studies demonstrating neurotoxicity as compared to expected human exposures.

k. Summarize the hazard characterization:

—Confidence in conclusions;
—Alternative conclusions also supported by the data;
—Significant data gaps; and
—Highlight of major assumptions.

1. Human Studies

It is well established that information from the evaluation of human exposure can identify neurotoxic hazards (Anger and Johnson, 1985; Anger, 1990). Prominent among historical episodes of neurotoxicity in human populations are the outbreaks of methylmercury poisoning in Japan and Iraq and the neurotoxicity seen in miners of metals, including mercury, manganese, and lead (Carson et al., 1987; Silbergeld and Percival, 1987; OTA, 1990). In the last decade, lead poisoning in children has been a prominent issue of concern (Silbergeld and Percival, 1987). Neurotoxicity in humans has been studied and reviewed for many pesticides (Hayes, 1982; NRDC, 1989; Ecobichon and Joy, 1982; Ecobichon et al., 1990). Organochlorines, organophosphates, carbamates, pyrethroids, certain fungicides, and some fumigants are all known neurotoxicants. They may pose occupational risks to manufacturing and formulation workers, pesticide applicators and farm workers, and consumers through home application or consumption of residues in foods. Families of workers may also be exposed by transport into the home from workers' clothing. Data on humans can come from a number of sources, including clinical evaluations, case reports, and epidemiologic studies. A more extensive description of issues concerning human neurotoxicology and risk assessment has been published elsewhere (U.S. EPA, 1993).

a. Clinical Evaluations. Clinical methods are used extensively in neurology and neuropsychology to evaluate patients suspected of having neurotoxicity. An extensive array of examiner-administered and paper-and-pencil tasks are used to assess sensory, motor, cognitive, and affective functions and personality states/traits. Neurobehavioral data are synthesized with information from neurophysiologic studies and medical history to derive a working diagnosis. Brain imaging techniques based on magnetic resonance imaging or emission tomography may also be useful in helping diagnose neurodegenerative disorders following chemical exposures in humans (Omerod et al., 1994; Callender et al., 1994). Clinical diagnostic approaches have provided a rich conceptual framework for understanding the functions (and malfunctions) of the central and peripheral nervous systems and have formed the basis for the development of methods for measuring the behavioral expression of nervous system disorders. Human neurobehavioral toxicology has borrowed heavily from neurology and neuropsychology for concepts of nervous system impairment and functional assessment methods. Neurobehavioral toxicology has adopted the neurologic/neuropsychologic model, using adverse changes in behavioral function to assist in identifying chemically or drug-induced changes in nervous system processes.

Neurologic and neuropsychologic methods have long been employed to identify the adverse health effects of environmental workplace exposures (Sterman and Schaumburg, 1980). Peripheral neuropathies (with sensory and motor disturbances), encephalopathies, organic brain syndromes, extrapyramidal syndromes, demyelination, autonomic changes, and dementia are well-characterized consequences of acute and chronic exposure to chemical agents. The range of exposure conditions that produce clinical signs of neurotoxicity also has been defined by these clinical methods. It is very important to make external/internal dose measurements in humans to determine the actual dose(s) that can cause unwanted effects.

Aspects of the neurologic examination approach limit its usefulness for neurotoxicologic risk assessment. Information obtained from the neurologic exam is mostly qualitative and descriptive rather than quantitative. Estimates of the severity of functional impairment can be reliably placed into only three or four categories (for example, mild, moderate, severe). Much of the assessment depends on the subjective judgment of the examiner. For example, the magnitude and symmetry of muscle strength are often judged by having the patient push against the resistance of the examiner's hands. The end points are therefore the absolute and relative amount of muscle load sensed by the examiner in his or her arms.

Compared with other methods, the neurologic exam may be less sensitive in detecting early neurotoxicity in peripheral sensory and motor nerves. While clinicians' judgments are equal in sensitivity to quantitative methods in assessing the amplitude of tremor, tremor frequency is poorly quantified by clinicians. Thus, important aspects of the clinical neurologic exam may be insufficiently quantified and lack sufficient sensitivity for detecting early neurobehavioral toxicity produced by environmental or workplace exposure conditions. However, a neurologic evaluation of persons with documented neurobehavioral impairment would be helpful for identifying nonchemical causes of neurotoxicity, such as diabetes and cardiovascular insufficiency.

Administration of a neuropsychological battery also requires a trained technician, and interpretation requires a trained and experienced neuropsychologist. Depending on the capabilities of the patient, 2 to 4 hours may be needed to administer a full battery; 1 hour may be needed for the shorter screening versions. These practical considerations may limit the usefulness of neuropsychological assessment in large field studies of suspected neurotoxicity.

In addition to logistical problems in administration and interpretation, neuropsychological batteries and neurologic exams share two disadvantages with respect to neurotoxicity risk assessment. First,

neurologic exams and neuropsychological test batteries are designed to confirm and classify functional problems in individuals selected on the basis of signs and symptoms identified by the patient, family, or other health professionals. Their usefulness in detecting low base-rate impairment in workers or the general population is generally thought to be limited, decreasing the usefulness of clinical assessment approaches for epidemiologic risk assessment.

Second, neurologic exams and neuropsychologic test batteries were developed to assess the functional correlates of the most common forms of nervous system dysfunction: brain trauma, focal lesions, and degenerative conditions. The clinical tests were validated against these neurologic disease states. With a few notable exceptions, chemicals are not believed to produce impairment similar to that from trauma or lesions; neurotoxic effects are more similar to the effects of degenerative disease. There has been insufficient research to demonstrate which tests designed to assess functional expression of neurologic disease are useful in characterizing the modes of central nervous system impairment produced by chemical agents and drugs.

b. Case reports. The first type of human data available is often the case report or case series, which can identify cases of a disease and are reported by clinicians or discerned through active or passive surveillance, usually in the workplace. However, case reports where exposure involved a single neurotoxic agent, although informative, are rare in the literature; for example, farmers are likely to be exposed to a wide variety of potentially neurotoxic pesticides. Careful case histories assist in identifying common risk factors, especially when the association between the exposure and disease is strong, the mode of action of the agent is biologically plausible, and clusters occur in a limited period of time.

Case reports are inexpensive compared with epidemiologic studies and can be obtained more quickly than more complex studies. However, they provide little information about disease frequency or population at risk, but their importance has been clearly demonstrated, particularly in accidental poisoning or acute exposure to high levels of toxicant. They remain an important source of index cases of new diseases and for surveillance.

c. Epidemiologic Studies. Epidemiology has been defined as ''the study of the distributions and determinants of disease and injuries in human populations'' (Mausner and Kramer, 1985). Knowing the frequency of illness in groups and the factors that influence the distribution is the tool of epidemiology that allows the evaluation of causal inference with the goal of prevention and cure of disease (Friedlander and Hearn, 1980). Epidemiologic studies are a means of evaluating the effects of neurotoxic substances on human populations, but such studies are limited because they must be performed shortly after exposure if the effect is acute. Most often these effects are suspected to be a result of occupational exposures due to the increased opportunity for exposure to industrial and other chemicals. Frequently, determining the precise dose or exposure concentration can be difficult in epidemiological studies.

(1) Cross-sectional studies. In cross-sectional studies or surveys, both the disease and suspected risk factors are ascertained at the same time, and the findings are useful in generating hypotheses. A group of people are interviewed, examined, and tested at a single point in time to ascertain a relationship between a disease and a neurotoxic exposure. This study design does not allow the investigator to determine whether the disease or the exposure came first, rendering it less useful in estimating risk. These studies are intermediate in cost and time required to complete compared with case reports and more complex analytical studies but should be augmented with additional data.

(2) Case-control (retrospective) studies. Last (1986) defines a case-control study as one that ''starts with the identification of persons with the disease (or other outcome variable) of interest, and a suitable control population (comparison, reference group) of persons without the disease.'' He states that the relationship of an ''attribute'' to the disease is measured by comparing the diseased with the nondiseased with regard to how frequently the attribute is present in each of the groups. The cases are assembled from a population of persons with and without exposure, and the comparison group is selected from the same population; the relative distribution of the potential risk factor (exposure) in both groups is evaluated by computing an odds ratio that serves as an estimate of the strength of the association between the disease and the potential risk factor. The statistical significance of the ratio is determined by calculating a p-value and is used to approximate relative risk.

The case-control approach to the study of potential neurotoxicants in the environment provides a great deal of useful information for the risk assessor. In his textbook, Valciukas (1991) notes that the case-control approach is the strategy of choice when no other environmental or biological indicator of neurotoxic exposure is available. He further states: ''Considering the fact that for the vast majority of neurotoxic chemical compounds, no objective biological indicators of exposure are available (or if they are, their half-life is too short to be of any practical value), the case-control paradigm is a widely accepted strategy for the assessment of toxic causation.'' The case-control study design, however, can be very susceptible to bias. The potential sources of bias are numerous and can be specific to a particular study. Many of these biases also can be present in cross-sectional studies. For example, recall bias or faulty recall of information by study subjects in a questionnaire-based study can distort the results of the study. Analysis of the case-comparison study design assumes that the selected cases are representative persons with the disease—either all cases with the disease or a representative sample of them have been ascertained. It further assumes that the control or comparison group is representative of the nondiseased population (or that the prevalence of the characteristic under study is the same in the control group as in the general population). Failure to satisfy these assumptions may result in selection bias, but violation of assumptions does not necessarily invalidate the study results.

An additional source of bias in case-control studies is the presence of confounding variables, i.e., factors known to be associated with the exposure and causally related to the disease under study. These must be controlled either in the design of the study by matching cases to controls on the basis of the confounding factor or in the analysis of the data by using statistical techniques such as stratification or regression. Matching requires time to identify an adequate number of potential controls to distinguish those with the proper characteristics, while statistical control of confounding factors requires a larger study.

The definition of exposure is critical in epidemiologic studies. In occupational settings, exposure assessment often is based on the job assignment of the study subjects, but can be more precise if detailed company records allow the development of exposure profiles. Positive results from a properly controlled retrospective

study should weigh heavily in the risk assessment process.

(3) Cohort (prospective, followup) studies. In a prospective study design, a healthy group of people is assembled and followed forward in time and observed for the development of disease. Such studies are invaluable for determining the time course for development of disease (e.g., followup studies performed in various cities on the effects of lead on child development). This approach allows the direct estimate of risks attributed to a particular exposure since disease incidence rates in the cohort can be determined. Prospective study designs also allow the study of chronic effects of exposure. One major strength of the cohort design is that it allows the calculation of rates to determine the excess risk associated with an exposure. Also, biases are reduced by obtaining information before the disease develops. This approach, however, can be very time-consuming and costly.

In cohort studies information bias can be introduced when individuals provide distorted information about their health because they know their exposure status and may have been told of the expected health effects of the exposure under study.

A special type of cohort study is the retrospective cohort study in which the investigator goes back in time to select the study groups and traces them over time, often to the present. The studies usually involve specially exposed groups and have provided much assistance in estimating risks due to occupational exposures. Occupational retrospective cohort studies rely on company records of past and current employees that include information on the dates of employment, age at employment, date of departure, and whether diseased (or dead in the case of mortality studies). Workers can then be classified by duration and degree of exposure. Positive results from a properly controlled prospective study should weigh heavily in the risk assessment process.

d. Human Laboratory Exposure Studies. Neurotoxicity assessment has an advantage not afforded the evaluation of other toxic end points, such as cancer or reproductive toxicity, in that the effects of some chemicals are short in duration and reversible. This makes it ethically possible to perform human laboratory exposure studies and obtain data relevant to the risk assessment process. Information from experimental human exposure studies has been used to set occupational exposure limits, mostly for organic solvents that can be inhaled. Laboratory exposure studies have contributed to risk assessment and the setting of exposure limits for several solvents and other chemicals with acute reversible effects.

Human exposure studies sometime offer advantages over epidemiologic field studies. Combined with appropriate sampling of biologic fluids (urine or blood), it is possible to calculate body concentrations, examine toxicokinetics, and identify metabolites. Bioavailability, elimination, dose-related changes in metabolic pathways, individual variability, time course of effects, interactions between chemicals, and interactions between chemical and environmental/biobehavioral processes (stressors, workload/respiratory rate) are factors that are generally easier to collect under controlled conditions.

Other goals of laboratory studies include the indepth characterization of effects, the development of new assessment methods, and the examination of the sensitivity, specificity, and reliability of neurobehavioral assessment methods across chemical classes. The laboratory is the most appropriate setting for the study of environmental and biobehavioral variables that affect the action of chemical agents. The effects of ambient temperature, task difficulty, rate of ongoing behavior, conditioning variables, tolerance/sensitization, sleep deprivation, motivation, and so forth are sometimes studied.

From a methodologic standpoint, human laboratory studies can be divided into two categories—between-subjects and within-subjects designs. In the former, the neurobehavioral performance of exposed volunteers is compared with that of nonexposed participants. In the latter, preexposure performance is compared with neurobehavioral function under the influence of the chemical or drug. Within-subjects designs have the advantage of requiring fewer participants, eliminating individual differences as a source of variability, and controlling for chronic mediating variables, such as caffeine use and educational achievement. A disadvantage of the within-subjects design is that neurobehavioral tests must be administered more than once. Practice on many neurobehavioral tests often leads to improved performance that may confound the effect of the chemical/drug. There should be a sufficient number of test sessions in the pre-exposure phase of the study to allow performance on all tests to achieve a relatively stable baseline level.

Participants in laboratory exposure studies may have been recruited from populations of persons already exposed to the chemical/drug or from naive populations. Although the use of exposed volunteers has ethical advantages, can mitigate against novelty effects, and allows evaluation of tolerance/sensitization, finding an accessible exposed population in reasonable proximity to the laboratory is difficult. Naive participants are more easily recruited but may differ significantly in important characteristics from a representative sample of exposed persons. Naive volunteers are often younger, healthier, and better educated than the populations exposed environmentally, in the workplace, or pharmacotherapeutically.

Compared with workplace and environmental exposures, laboratory exposure conditions can be controlled more precisely, but exposure periods are much shorter. Generally only one or two relatively pure chemicals are studied for several hours while the population of interest may be exposed to multiple chemicals containing impurities for months or years. Laboratory studies are therefore better at identifying and characterizing effects with acute onset and the selective effects of pure agents.

Neurobehavioral test methods may have been selected according to several strategies. A test battery that examines multiple neurobehavioral functions may be more useful for screening and the initial characterization of acute effects. Selected neurobehavioral tests that measure a more limited number of functions in multiple ways may be more useful for elucidating mechanisms or validating specific effects.

Both chemical and behavioral control procedures are valuable for examining the specificity of the effects. A concordant effect among different measures of the same neurobehavioral function (e.g., reaction time) and a lack of effect on some other measures of psychomotor function (e.g., untimed manual dexterity) would increase the confidence in a selective effect on motor speed and not on attention or nonspecific motor function. Likewise, finding concordant effects among similar chemical or drug classes along with different effects from dissimilar classes would support the specificity of chemical effect. For example, finding that the effects of a solvent were similar to those of ethanol but not caffeine would support the specificity of solvent effects on a given measure of neurotoxicity.

2. Animal Studies

This section provides an overview of the major types of end points that may be evaluated in animal neurotoxicity

studies, describes the kinds of effects that may be observed and some of the tests used to detect and quantify these effects, and provides guidance for interpreting data. Compared with human studies, animal studies are more often available for specific chemicals, provide more precise exposure information, and control environmental factors better (Anger, 1984). For these reasons, risk assessments tend to rely heavily on animal studies.

Many tests that can measure some aspect of neurotoxicity have been used in the field of neurobiology in the last 50 years. The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has published animal testing guidelines that were developed in cooperation with the Office of Research and Development (U.S. EPA, 1991a). While the test end points included serve as a convenient focus for this section, there are many other end points for which there are no current EPA guidelines. The goal of this document is to provide a framework for interpreting data collected with tests frequently used by neurotoxicologists.

Five categories of end points will be described: Structural or neuropathological, neurophysiological, neurochemical, behavioral, and developmental end points. Table 1 lists a number of end points in each of these categories.

Table 1.—Examples of Possible Indicators of a Neurotoxic Effect

I. Structural or Neuropathological End Points
1. Gross changes in morphology, including brain weight
2. Hemorrhage in nerve tissue
3. Breakdown of neurons, glial cells
4. Accumulation, proliferation, or rearrangement of structural elements
5. Glial fibrillary acidic protein increases (in adults)

II. Neurochemical End Points
1. Alterations in synthesis, release, uptake, degradation of neurotransmitters
2. Alterations in second messenger associated signal transduction
3. Alterations in membrane-bound enzymes regulating neuronal activity
4. Inhibition of neuropathy target enzyme (≥40%)

III. Neurophysiological End Points
1. Change in velocity, amplitude, or refractory period of nerve conduction
2. Change in latency or amplitude of sensory-evoked potential
3. Change in electroencephalographic pattern

IV. Behavioral and Neurological End Points
1. Increases or decreases in motor activity
2. Changes in touch, sight, sound, taste, or smell sensations
3. Changes in motor coordination, weakness, paralysis, abnormal movement or posture, tremor, ongoing performance
4. Absence or decreased occurrence, magnitude, or latency of sensorimotor reflex
5. Altered magnitude of neurological measurement, including grip strength, hindlimb splay
6. Seizures
7. Changes in rate or temporal patterning of schedule-controlled behavior
8. Changes in learning, memory, intelligence, attention

V. Developmental End Points
1. Chemically induced changes in the time of appearance of behaviors during development
2. Chemically induced changes in the growth or organization of structural or neurochemical elements.

a. *Structural End Points of Neurotoxicity.* Structural end points are typically defined as neuropathological changes measured through gross observation or with the aid of a microscope. Gross changes in morphology can include discrete or widespread lesions in nerve tissue. Changes in brain size (weight, width, or length) are considered to be indicative of neurotoxic events. This is true regardless of changes in body weight, because brain size is generally protected during undernutrition or weight loss, unlike many other organs or tissues. It is inappropriate to express brain weight changes as a ratio of body weight and thereby dismiss changes in absolute brain weight. The risk assessor should be aware that a unit of measurement that is biologically meaningful should be used for analysis. Brain length measurements, for example, expressed to 1 or 10 micron units is biologically meaningless. The same is true for brain width.

Neurons are composed of a neuronal body, axon, and dendritic processes. Various types of neuropathological lesions may be classified according to the site where they occur (WHO, 1986; Krinke, 1989; Griffin, 1990). Neurodegenerative lesions in the central or peripheral nervous system may be classified as a neuronopathy (changes in the neuronal cell body), axonopathy (changes in the axons), myelinopathy (changes in the myelin sheaths), or terminal degeneration. For axonopathies, a more precise location of the changes may also be described (i.e., proximal, central, or distal axonopathy). In the case of some developmental exposures, a neurotoxic chemical might delay or accelerate the differentiation or proliferation of cells or cell types. Alteration in the axonal termination site might also occur with exposure. In an aged population, exposure to some neurotoxicants might accelerate the normal loss of neurons associated with aging (Reuhl, 1991). In rare cases, neurotoxic agents have been reported to produce neuropathic conditions resembling neurodegenerative disorders in humans such as Parkinson's disease (WHO, 1986). Table 2 lists examples of such neurotoxic chemicals, their putative site of action, the type of neuropathology produced, and the disease or condition that each typifies.

TABLE 2.—NEUROTOXICANTS AND DISEASES WITH SPECIFIC NEURONAL TARGETS

Site of action	Neuropathology	Neurotoxicant	Corresponding neurodegenerative disease or condition
Neuron cell body	Neuronopathy	Methylmercury Quinolinic acid 3-Acetylpyridine.	Minamata disease, Huntington's disease, Cerebellar ataxia.
Nerve terminal	Terminal destruction	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (dopaminergic).	Parkinson's disease.
Schwann cell Myelin	Myelinopathy	Hexachlorophene	Congenital hypomyelinogenesis.
Central-peripheral distal axon.	Distal axonopathy	Acrylamide Carbon disulfide n-Hexane	Peripheral neuropathy.
Central axons	Central axonopathy	Clioquinol	Subacute myeloopticoneuropathy.
Proximal axon	Proximal axonopathy	B,B′-iminodipropionitrile	Motor neuron disease.

Alterations in the structure of the nervous system (i.e., neuronopathy, axonopathy, myelinopathy, terminal degeneration) are regarded as evidence of a neurotoxic effect. The risk assessor should note that pathological changes in many cases require time for the perturbation to become observable, especially with evaluation at the light

microscopic level. Neuropathological studies should control for potential differences in the area(s) and section(s) of the nervous system sampled, in the age, sex, and body weight of the subject, and in fixation artifacts (WHO, 1986). Concern for the structural integrity of nervous system tissues derives from its functional specialization and the lack of regenerative capacity in the central nervous system.

In general, chemical effects can lead to two types of structural alteration at the cellular level: the breakdown of cells, in whole or in part, or the accumulation, proliferation, or rearrangement of structural elements (e.g., intermediate filaments, microtubules) or organelles (e.g., mitochondria). Some changes may be associated with regenerative processes that reflect adaptive changes associated with exposure to a toxicant.

Chemically induced injury to the central nervous system may be associated with astrocytic hypertrophy. Such changes may be seen using immunocytochemical techniques visualized by light microscopy or quantified more precisely by radioimmunoassay (RIA) procedures. Assays of glial fibrillary acidic protein (GFAP), the major intermediate filament protein of astrocytes, have been proposed as a biomarker of this response (O'Callaghan, 1988). The interpretation of a chemical-induced change in GFAP is facilitated by corroborative data from the neuropathology or neuroanatomy evaluation. A number of chemicals known to injure the central nervous system, including trimethyltin, methylmercury, cadmium, 3-acetylpyridine, and methylphenyltetrahydropyridine (MPTP), have been shown to increase levels of GFAP. Measures of GFAP are now included in the Neurotoxicity Test Battery testing guidelines (U.S. EPA, 1991a).

Increases in GFAP above control levels may be seen at dosages below those necessary to produce damage seen by standard microscopic or histopathological techniques. Because increases in GFAP reflect an astrocyte response in adults, treatment-related increases in GFAP are considered to be evidence that a neurotoxic effect has occurred. Decreases in GFAP are not clearly interpretable as indicative of neurotoxicity. The absence of a change in GFAP following exposure does not necessarily mean that the chemical is devoid of neurotoxic potential. Known neurotoxicants such as cholinesterase-inhibiting pesticides, for example, would not be expected to increase brain levels of GFAP. Interpretation of GFAP changes prior to weaning is confounded by the possibility that chemically induced increases in GFAP may be masked by changes in the concentration of this protein associated with maturation of the central nervous system, and these data may be difficult to interpret.

b. *Neurophysiological End Points of Neurotoxicity.* Neurophysiological studies are those that measure the electrical activity of the nervous system. The term ''neurophysiology'' is often used synonymously with ''electrophysiology'' (Dyer, 1987). Neurophysiological techniques provide information on the integrity of defined portions of the nervous system. Several neurophysiological procedures are available for application to neurotoxicological studies. Examples of neurophysiological measures of neurotoxicity are listed in Table 3. They range in scale from procedures that employ microelectrodes to study the function of single nerve cells or restricted portions of them, to procedures that employ macroelectrodes to perform simultaneous recordings of the summed activity of many cells. Microelectrode procedures typically are used to study mechanisms of action and are frequently performed in vitro. Macroelectrode procedures are generally used in studies to detect or characterize the potential neurotoxic effects of agents of interest because of potential environmental exposure. The present discussion concentrates on macroelectrode neurophysiological procedures because it is more likely that they will be the focus of decisions regarding critical effects in risk assessment. All of the procedures described below for use in animals also have been used in humans to determine chemically induced alterations in neurophysiological function.

TABLE 3.—EXAMPLES OF NEUROPHYSIOLOGICAL MEASURES OF NEUROTOXICITY

System/function	Procedure	Representative agents
Retina	Electroretinography (ERG)	Developmental lead.
Visual pathway	Flash evoked potential (FEP)	Carbon disulfide.
Visual function	Pattern evoked potential (PEP) pattern size and contrast).	Carbon disulfide.
Auditory pathway	Brain stem auditory evoked potential (BAER) (clicks).	Aminoglycoside, Antibiotics, Toluene, styrene.
Auditory function	BAER (tones)	Aminoglycoside, Antibiotics, Toluene, styrene.
Somatosensory pathway	Somatosensory evoked potential (SEP) (shocks).	Acrylamide, n-Hexane.
Somatosensory function	SEP (tactile)	Acrylamide n-Hexane.
Spinocerebellar pathway	SEP recorded from cerebellum	Acrylamide n-Hexane.
Mixed nerve	Peripheral nerve compound action potential (PNAP).	Triethyltin.
Motor axons	PNAP isolate motor components	Triethyltin.
Sensory axons	PNAP isolate sensory components	Triethyltin.
Neuromuscular	Electromyography (EMG), H-reflex, M-response.	Dithiobiuret.
General central nervous system/level of arousal.	Electroencephalography (EEG)	Anesthetics.

(1) Nerve conduction studies. Nerve conduction studies, generally performed on peripheral nerves, can be useful in investigations of possible peripheral neuropathy. Most peripheral nerves contain mixtures of individual sensory and motor nerve fibers, which may or may not be differentially sensitive to neurotoxicants. It is possible to distinguish sensory from motor effects in peripheral nerve studies by measuring activity in purely sensory

nerves such as the sural nerve or by measuring the muscle response evoked by nerve stimulation to measure motor effects. While a number of end points can be recorded, the most critical variables are (1) nerve conduction velocity, (2) response amplitude, and (3) refractory period.

Nerve conduction measurements are influenced by a number of factors, the most important of which is temperature. An adequate nerve conduction study will either measure the temperature of the limb under study and mathematically adjust the results according to well-established temperature factors or control limb temperature within narrow limits. Studies that measure peripheral nerve function without regard for temperature are not adequate for risk assessment.

In well-controlled studies, statistically significant decreases in nerve conduction velocity are indicative of a neurotoxic effect. While a decrease in nerve conduction velocity is indicative of demyelination, it frequently occurs later in the course of axonal degradation because normal conduction velocity may be maintained for some time in the face of axonal degeneration. For this reason, a measurement of normal nerve conduction velocity does not rule out peripheral axonal degeneration if other signs of peripheral nerve dysfunction are present.

Decreases in response amplitude reflect a loss of active nerve fibers and may occur prior to decreases in conduction velocity in the course of peripheral neuropathy. Hence, changes in response amplitude may be more sensitive measurements of axonal degeneration than conduction velocity. Measurements of response amplitude, however, can be more variable and require careful application of experimental techniques, a larger sample size, and greater statistical power than measurements of velocity to detect changes. The refractory period refers to the time required after stimulation before a nerve can fire again and provides a measure reflecting the functional status of nerve membrane ion channels. Chemically induced changes in refractory periods in a well-controlled study indicate a neurotoxic effect.

In summary, alterations in peripheral nerve response amplitude and refractory period in studies that are well controlled for temperature are indicative of a neurotoxic effect. Alterations in peripheral nerve function are frequently associated with clinical signs such as numbness, tingling, or burning sensations or with motor impairments such as weakness. Examples of compounds that alter peripheral nerve function in humans or experimental animals include acrylamide, carbon disulfide, n-hexane, lead, and some organophosphates.

(2) Sensory, motor, and other evoked potentials. Evoked potential studies are electrophysiological procedures that measure the response elicited from a defined stimulus such as a tone, a light, or a brief electrical pulse. Evoked potentials reflect the function of the system under study, including visual, auditory, or somatosensory; motor involving motor nerves and innervated muscles; or other neural pathways in the central or peripheral nervous system (Rebert, 1983; Dyer, 1985; Mattsson and Albee, 1988; Mattsson et al., 1992; Boyes, 1992, 1993). Evoked potential studies should be interpreted with respect to the known or presumed neural generators of the responses, and their likely relationships with behavioral outcomes, when such information is available. Such correlative information strengthens the confidence in electrophysiological outcomes. In the absence of such supportive information, the extent to which evoked potential studies provide convincing evidence of neurotoxicity is a matter of professional judgment on a case-by-case basis. Judgments should consider the nature, magnitude, and duration of such effects, along with other factors discussed elsewhere in this document.

Data are in the form of a voltage record collected over time and can be quantified in several ways. Commonly, the latency (time from stimulus onset) and amplitude (voltage) of the positive and negative voltage peaks are identified and measured. Alternative measurement schemes may involve substitution of spectral phase or template shifts for peak latency and spectral power, spectral amplitude, root-mean-square, or integrated area under the curve for peak amplitude. Latency measurements are dependent on both the velocity of nerve conduction and the time of synaptic transmission. Both of these factors depend on temperature, as discussed in regard to nerve conduction, and similar caveats apply for sensory evoked potential studies. In studies that are well controlled for temperature, increases in latencies or related measures can reflect deficits in nerve conduction, including demyelination or delayed synaptic transmission, and are indicators of a neurotoxic effect.

Decreases in peak latencies, like increases in nerve conduction velocity, are unusual, but the neural systems under study in sensory evoked potentials are complex, and situations that might cause a peak measurement to occur earlier are conceivable. Two such situations are a reduced threshold for spatial or temporal summation of afferent neural transmission and a selective loss of cells responding late in the peak, thus making the measured peak occur earlier. Decreases in peak latency should not be dismissed outright as experimental or statistical error, but should be examined carefully and perhaps replicated to assess possible neurotoxicity. A decrease in latency is not conclusive evidence of a neurotoxic effect.

Changes in peak amplitudes or equivalent measures reflect changes in the magnitude of the neural population responsive to stimulation. Both increases and decreases in amplitude are possible following exposure to chemicals. Whether excitatory or inhibitory neural activity is translated into a positive or negative deflection in the sensory evoked potential is dependent on the physical orientation of the electrode with respect to the tissue generating the response, which is frequently unknown. Comparisons should be based on the absolute change in amplitude. Therefore, either increases or decreases in amplitude may be indicative of a neurotoxic effect.

Within any given sensory system, the neural circuits that generate various evoked potential peaks differ as a function of peak latency. In general, early latency peaks reflect the transmission of afferent sensory information. Changes in either the latency or amplitude of these peaks are considered convincing evidence of a neurotoxic effect that is likely to be reflected in deficits in sensory perception. The later-latency peaks, in general, reflect not only the sensory input but also the more nonspecific factors such as the behavioral state of the subject, including such factors as arousal level, habituation, or sensitization (Dyer, 1987). Thus, changes in later-latency evoked potential peaks must be interpreted in light of the behavioral status of the subject and would generally be considered evidence of a neurotoxic effect.

(3) Seizures/convulsions. Neurophysiological recordings of brain electrical activity that demonstrate seizure-like activity are indicative of a neurotoxic effect. Occasionally, behaviors resembling convulsions might follow actions outside the nervous system, such as direct effects on muscle. When convulsion-like behaviors are observed, as described in the behavioral section, neurophysiological recordings can determine if these behaviors

originate from seizure activity in the brain.

In addition to producing seizures directly, neurotoxicants also may alter the frequency, severity, duration, or threshold for eliciting seizures produced through other means. Such changes can occur after acute exposure or after repeated exposure to dose levels below the acute threshold and are considered to be neurotoxic effects. Examples of agents that produce convulsions include lindane, DDT (dichloro-diphenyl-trichloroethane), pyrethroids, and trimethyltin.

(4) Electroencephalography (EEG). EEG analysis is used widely in clinical settings for the diagnosis of neurological disorders and less often for the detection of subtle toxicant-induced dysfunction (WHO, 1986; Eccles, 1988). The basis for using EEG in either setting is the relationship between specific patterns of EEG waveforms and specific behavioral states. Because states of alertness and stages of sleep are associated with distinct patterns of electrical activity in the brain, it is generally thought that arousal level can be evaluated by monitoring the EEG.

Dissociation of EEG activity and behavior can, however, occur after exposure to certain chemicals. Normal patterns of transition between sleep stages or between sleeping and waking states are known to remain disturbed for prolonged periods of time after exposure to some chemicals. Changes in the pattern of the EEG can be elicited by stimuli producing arousal (e.g., lights, sounds) and anesthetic drugs. In studies with toxicants, changes in EEG pattern can sometimes precede alterations in other objective signs of neurotoxicity (Dyer, 1987).

EEG studies must be done under highly controlled conditions, and the data must be considered on a case-by-case basis. Chemically induced seizure activity detected in the EEG pattern is evidence of a neurotoxic effect.

c. *Neurochemical End Points of Neurotoxicity*. Many different neurochemical end points have been measured in neurotoxicological studies, and some have proven useful in advancing the understanding of mechanisms of action of neurotoxic chemicals (Bondy, 1986; Mailman, 1987; Morell and Mailman, 1987; Costa, 1988). Normal functioning of the nervous system depends on the synthesis and release of specific neurotransmitters and activation of their receptors at specific presynaptic and postsynaptic sites. Chemicals can interfere with the ionic balance of a neuron, act as a cytotoxicant after transport into a nerve terminal, block reuptake of neurotransmitters and their precursors, act as a metabolic poison, overstimulate receptors, block transmitter release, and inhibit transmitter synthetic or catabolic enzymes. Table 4 lists several chemicals that produce neurotoxic effects at the neurochemical level (Bondy, 1986; Mailman, 1987; Morell and Mailman, 1987; Costa, 1988).

TABLE 4.—EXAMPLES OF NEUROTOXICANTS WITH KNOWN NEUROCHEMICAL MECHANISMS

Site of action	Examples
1. Neurotoxicants Acting on Ionic Balance:	
A. Inhibit sodium entry	Tetrodotoxin.
B. Block closing of sodium channel	p,p′-DDT, pyrethroids.
C. Increase permeability to sodium	Batrachotoxin.
D. Increase intracellular calcium	Chlordecone.
2. Cytotoxicants—Depend on uptake into nerve terminal	MPTP.
3. Uptake blockers	Hemicholinium.
4. Metabolic poisons	Cyanide.
5. Hyperactivation of receptors	Domoic acid.
6. Blocks transmitter release (Acetylcholine [ACh])	Botulinum toxin.
7. Inhibition of transmitter degradation (ACh)	Pesticides of the organophosphate and carbamate classes.
8. Blocks axonal transport	Acrylamide.

As stated previously, any neurochemical change is potentially neurotoxic, but each determination requires professional judgment. Persistent or irreversible chemically induced neurochemical changes are indicative of neurotoxicity. Because the ultimate functional significance of some biochemical changes is not known at this time, neurochemical studies should be interpreted with reference to the presumed neurotoxic consequence(s) of the neurochemical changes. For example, many neuroactive agents can increase or decrease neurotransmitter levels, but such changes are not necessarily indicative of a neurotoxic effect. If, however, these neurochemical changes may be expected to have neurophysiological, neuropathological, or neurobehavioral correlates, then the neurochemical changes could be classified as neurotoxic effects.

Some neurotoxicants, such as the organophosphate and carbamate pesticides, are known to inhibit the activity of a specific enzyme, acetylcholinesterase (for a review see Costa, 1988), which hydrolyzes the neurotransmitter acetylcholine. Inhibition of the enzyme prolongs the action of the acetylcholine at the neuron's synaptic receptors and is responsible for the autonomic stimulation and death that these agents cause.

Within EPA and elsewhere, questions have arisen as to whether inhibition of cholinesterase activity constitutes an adverse effect for defining hazard potential and evaluating risk. There is agreement among scientists that statistically significant inhibition of cholinesterase activity in multiple organs and tissues accompanied by clinical effects constitutes a hazard. However, there is scientific uncertainty and related controversy about the risk assessment implications of data describing inhibition of cholinesterase enzyme activity in the absence of observable clinical effects. While there is agreement that such inhibition is a biomarker of exposure, there is continued disagreement over whether cholinesterase inhibition, especially in blood, constitutes an adverse effect.

At this point, it can be stated that there is general agreement among scientists that objective clinical measures of dysfunction/impairment can be overt manifestations of inhibition of cholinesterase in the nervous system. On the basis of clinical manifestations, e.g., muscle weakness, tremor, blurred vision, one should be able to evaluate dose-response and dose-effect relationships and define the presence and absence of given effects. A relationship between the effect and cholinesterase inhibition should be

confirmed by biochemical measures of reduced cholinesterase activity.

In addition, a reduction in brain cholinesterase activity may or may not be accompanied by clinical manifestations. Most experts in the field acknowledge that when significant reductions in brain cholinesterase activity alone occur, reduced cholinesterase levels either are themselves toxic or would lead to a neurotoxic effect if exposure were to persist over time or increase in magnitude. Therefore, statistically significant decreases in brain cholinesterase could be considered to be a biologically significant effect.

A reduction in RBC and/or plasma cholinesterase activity also may or may not be accompanied by clinical manifestations. At this time, there is general agreement that the observation of inhibition of RBC and/or plasma cholinesterase contributes to the overall hazard identification of cholinesterase inhibiting agents by serving as biomarkers. As such, these enzyme parameters can provide information that will help scientists evaluate whether reported clinical effects are associated with cholinesterase inhibition. There remains, however, a lack of consensus as to whether RBC and/or plasma cholinesterase represent biologically significant events. Discussions on this topic are continuing within the Agency.

A subset of organophosphate agents also produces organophosphate-induced delayed neuropathy (OPIDN) after acute or repeated exposure. Prolonged inhibition (i.e., aging) of neurotoxic esterase (or neuropathy target enzyme) has been associated with agents that produce OPIDN (Johnson, 1990), a clear neurotoxic effect.

d. *Behavioral End Points of Neurotoxicity.* EPA's testing guidelines developed for the Toxic Substances Control Act and the Federal Insecticide, Fungicide and Rodenticide Act describe the use of functional observational batteries (FOB), motor activity, and schedule-controlled behavior for assessing neurotoxic potential (U.S. EPA, 1991a). There are many other measures of behavior, including specialized tests of motor and sensory function and of learning and memory (Tilson, 1987; Anger, 1984). Examples of behavioral end points that have been used to detect neurotoxicity are included in Table 1. The risk assessor should know that the literature is clear that a number of other behaviors besides those listed in Tables 1 and 5 could be affected by chemical exposure. For example, alterations in food and water intake, reproduction, sleep, temperature regulation, and circadian rhythmicity are controlled by specific regions of the brain and chemical-induced alterations in these behaviors could be indicative of neurotoxicity. It is reasonable to assume that a NOAEL or LOAEL could be based on one or more of these end points.

TABLE 5.—SUMMARY OF MEASURES IN A REPRESENTATIVE FUNCTIONAL OBSERVATIONAL BATTERY, AND THE TYPE OF DATA PRODUCED BY EACH

Home cage and open field	Manipulative	Physiologic
Posture (D)	Ease of removal (R)	Body temperature (I).
Convulsions, tremors (D)	Handling reactivity (R)	Body weight (I).
Palpebral closure (R)	Palpebral closure (R).	
Lacrimation (R)	Approach response (R).	
Piloerection (Q)	Click response (R).	
Salivation (R)	Touch response (R).	
Vocalizations (Q)	Tail pinch response (R).	
Rearing (C)	Righting reflex (R).	
Urination (C)	Landing foot splay (I).	
Defecation (C)	Forelimb grip strength (I).	
Gait (D, R)	Hindlimb grip strength (I).	
Arousal (R)	Pupil response (Q).	
Mobility (R).		
Stereotypy (D).		
Bizarre behavior (D)		

D—descriptive data; R—rank order data; Q—quantal data; I—interval data; C—count data.

Behavior is an indication of the overall well-being of the organism. Changes in behavior can arise from a direct effect of a toxicant on the nervous system or indirectly from its effects on other physiological systems. Understanding the interrelationship between systemic toxicity and behavioral changes is extremely important (e.g., the relationship between liver damage and motor activity). The presence of systemic toxicity may complicate, but does not necessarily preclude, interpretation of behavioral changes as evidence of neurotoxicity. In addition, a number of behaviors (e.g., schedule-controlled behavior) may require a motivational component for successful completion of the task. In such cases, experimental paradigms designed to assess the motivation of an animal during behavior might be necessary to interpret the meaning of some chemical-induced changes in behavior.

The following sections describe in general behavioral tests and their uses and offer guidance on interpreting data.

(1) Functional observational battery. A functional observational battery is designed to detect and quantify major overt behavioral, physiological, and neurological signs (Gad, 1982; O'Donoghue, 1989; Moser, 1989). A number of batteries have been developed, each consisting of tests generally intended to evaluate various aspects of sensorimotor function (Tilson and Moser, 1992). Many FOB tests are essentially clinical neurological examinations that rate the presence or absence, and in many cases the severity, of specific neurological signs. Some FOBs in animals are similar to clinical neurological examinations used with human patients. Most FOBs have several components or tests. A typical FOB is summarized in Table 5 and evaluates several functional domains, including neuromuscular (i.e., weakness, incoordination, gait, and tremor), sensory (i.e., audition, vision, and somatosensory), and autonomic (i.e., pupil response and salivation) function. FOB data may be in the form of interval, ordinal, or continuous measurements.

The relevance of statistically significant test results from an FOB is judged according to the number of signs affected, the dose(s) at which effects are observed, and the nature, severity, and persistence of the effects and their

incidence in relation to control animals. If only a few unrelated measures in the FOB are affected, or the effects are unrelated to dose, the results are not considered evidence of a neurotoxic effect. If several neurological signs are affected but only at the high dose and in conjunction with other overt signs of toxicity, including systemic toxicity, large decreases in body weight, decreases in body temperature, or debilitation, there is no conclusive evidence of a direct neurotoxic effect. In cases where several related measures in a battery of tests are affected and the effects appear to be dose dependent, the data are considered to be evidence of a neurotoxic effect, especially in the absence of systemic toxicity. Recently, it was proposed that data from FOB studies be grouped into several neurobiological domains, including neuromuscular (i.e., weakness, incoordination, abnormal movements, gait), sensory (i.e, auditory, visual, somatosensory), and autonomic functions (Tilson and Moser, 1992). This statistical technique is useful when separating changes that occur on the basis of chance or in conjunction with systemic toxicity from those treatment-related changes indicative of neurotoxic effects. In the case of the developing organism, chemicals may alter the maturation or appearance of sensorimotor reflexes. Significant alterations in or delay of such reflexes is evidence of a neurotoxic effect.

Examples of chemicals that affect neuromuscular function are 3-acetylpyridine, acrylamide, and triethyltin. Organophosphate and carbamate insecticides produce autonomic dysfunction, while organochlorine and pyrethroid insecticides increase sensorimotor sensitivity, produce tremors, and in some cases, cause seizures and convulsions (Spencer and Schaumberg, 1980).

(2) Motor activity. Motor activity represents a broad class of behaviors involving coordinated participation of sensory, motor, and integrative processes. Assessment of motor activity is noninvasive and has been used to evaluate the effects of acute and repeated exposure to neurotoxicants (MacPhail et al., 1989). An organism's level of activity can, however, be affected by many different types of environmental agents, including nonneurotoxic agents. Motor activity measurements also have been used in humans to evaluate disease states, including disorders of the nervous system (Goldstein and Stein, 1985).

Motor activity is usually quantified as the frequency of movements over a period of time. The total counts generated during a test period will depend on the recording mechanism and size and configuration of the testing apparatus. Effects of agents on motor activity can be expressed as absolute activity counts or as a percentage of control values. In some cases, a transformation (e.g., square root) may be used to achieve a normal distribution of the data. The frequency of motor activity within a session usually decreases and is reported as the average number of counts occurring in each successive block of time. The EPA's Office of Prevention, Pesticides and Toxic Substances guidelines (U.S. EPA, 1991a), for example, call for test sessions of sufficient duration to allow motor activity to approach steady-state levels during the last 20 percent of the session for control animals. A sum of the counts in each epoch will add up to the total number of counts per session.

In the adult, neurotoxic agents generally decrease motor activity (MacPhail et al., 1989). Examples include many pesticides (e.g., carbamates, chlorinated hydrocarbons, organophosphates, and pyrethroids), heavy metals (lead, tin, and mercury), and other agents (3-acetylpyridine, acrylamide, and 2,4-dithiobiuret). Some neurotoxicants (e.g., toluene, xylene, triadimefon) produce transient increases in activity by presumably stimulating neurotransmitter release, while others (e.g., trimethyltin) produce persistent increases in motor activity by destroying specific regions of the brain (e.g., hippocampus).

Following developmental exposures, neurotoxic effects are often observed as a change in the developmental profile or maturation of motor activity patterns. Frequently, developmental exposure to neurotoxic agents will produce an increase in motor activity that persists into adulthood or that results in changes in other behaviors. This type of effect is evidence of a neurotoxic effect. Like other organ systems, the nervous system may be differentially sensitive to toxicants in groups such as the young. For example, toxicants introduced to the developing nervous system may kill stem cells and thus cause profound effects on adult structure and function. Moreover, toxicants may have greater access to the developing nervous system before the blood-brain barrier is completely formed or before metabolic detoxifying systems are functional.

Motor activity measurements are typically used with other tests (e.g., FOB) to help detect neurotoxic effects. Agent-induced changes in motor activity associated with other overt signs of toxicity (e.g., loss of body weight, systemic toxicity) or occurring in non-dose-related fashion are of less concern than changes that are dose dependent, related to structural or other functional changes in the nervous system, or occur in the absence of life-threatening toxicity.

(3) Schedule-controlled operant behavior. Schedule-controlled operant behavior (SCOB) involves the maintenance of behavior (e.g., performance of a lever-press or key-peck response) by reinforcement. Different rates and patterns of responding are controlled by the relationship between response and subsequent reinforcement. SCOB provides a measure of performance of a learned behavior (e.g., lever press or key peck) and involves training and motivational variables that must be considered in evaluating the data. Agents may interact with sensory processing, motor output, motivational variables (i.e., related to reinforcement), training history, and baseline characteristics (Rice, 1988; Cory-Slechta, 1989). Rates and patterns of SCOB display remarkable species and experimental generality.

In laboratory animals, SCOB has been used to study a wide range of neurotoxicants, including methylmercury, many pesticides, carbon disulfide, organic and inorganic lead, and triethyl and trimethyltin (MacPhail, 1985; Tilson, 1987; Rice, 1988). The primary SCOB end points for evaluation are response rate and the temporal pattern of responding. These end points may vary as a function of the contingency between responding and reinforcement presentation (i.e., schedule of reinforcement). While most chemicals decrease the efficiency of responding at some dose, some agents may increase response efficiency on schedules requiring high response rates due to a stimulant effect or an increase in central nervous system excitability. Agent-induced changes in responding between reinforcements (i.e., the temporal pattern of responding) may occur independently of changes in the overall rate of responding. Chemicals may also affect the reaction time to respond following presentation of a stimulus. Agent-induced changes in response rate or temporal patterning associated with other overt signs of toxicity (e.g., body weight loss, systemic toxicity, or occurring in a non-dose-related fashion) are of less concern than changes that are dose dependent, related to structural or other functional changes in the nervous system, or occur in the absence of life-threatening toxicity.

(4) Convulsions. Observable convulsions in animals are indicative of an adverse effect. These events can

reflect central nervous system activity comparable to that of epilepsy in humans and could be defined as neurotoxicity. Occasionally, other toxic actions of compounds, such as direct effects on muscle, might mimic some convulsion-like behaviors. In some cases, convulsions or convulsion-like behaviors may be observed in animals that are otherwise severely compromised, moribund, or near death. In such cases, convulsions might reflect an indirect effect of systemic toxicity and are less clearly indicative of neurotoxicity. As discussed in the section on neurophysiological measures, electrical recordings of brain activity could be used to determine specificity of effects on the nervous system.

(5) Specialized tests for neurotoxicity. Several procedures have been developed to measure agent-induced changes in specific neurobehavioral functions such as motor, sensory, or cognitive function (Tilson, 1987; Cory-Slechta, 1989). Table 6 lists several well-known behavioral tests, the neurobehavioral functions they were designed to assess, and agents known to affect the response. Many of these tests in animals have been designed to assess neural functions in humans using similar testing procedures.

TABLE 6.—EXAMPLES OF SPECIALIZED BEHAVIORAL TESTS TO MEASURE NEUROTOXICITY

Function	Procedure	Representative agents
Neuromuscular:		
Weakness	Grip strength; swimming endurance; suspension rod; discriminative motor function.	n-Hexane, methl n-butylketone, carbaryl.
Incoordination	Rotorod, gait measurements; righting reflex	3-Acetylpyridine, ethanol.
Tremor	Rating scale, spectral analysis	Chlordecone, Type I pyrethroids, DDT.
Myoclonia spasms	Rating scale, spectral analysis	DDT, Type II pyrethroids.
Sensory:		
Auditory	Discrimination conditioning Reflex modification	Toluene, trimethyltin.
Visual	Discrimination conditioning	Methylmercury.
Somatosensory	Discrimination conditioning	Acrylamide.
Pain sensitivity	Discrimination conditioning (titration); functional observational battery.	Parathion.
Olfactory	Discrimination conditioning	3-Methylindole, methylbromide.
Learning/Memory:		
Habituation	Startle reflex	Diisopropyl-fluorophosphate (DFP) Pre/neonatal methylmercury.
Classical conditioning	Nictitating membrane	Aluminum.
	Conditioned flavor aversion	Carbaryl.
	Passive avoidance	Trimethyltin, IDPN.
	Olfactory conditioning	Neonatal trimethyltin.
Operant conditioning	One-way avoidance	Chlordecone.
	Two-way avoidance	Pre/neonatal lead.
	Y-maze avoidance	Hypervitaminois A.
	Biel water maze	Styrene.
	Morris water maze	DFP.
	Radial arm maze	Trimethyltin.
	Delayed matching to sample	DFP.
	Repeated acquisition	Carbaryl.
	Visual discrimination	Lead.

A statistically significant chemically induced change in any measure in Table 6 is presumptive evidence of adverse effect. Judgments of neurotoxicity may involve not only the analysis of changes seen but the structure and class of the chemical and other available neurochemical, neurophysiological, and neuropathological evidence. In general, behavioral changes seen across broader dose ranges indicate more specific actions on the systems underlying those changes, i.e., the nervous system. Changes that are not dose dependent or that are confounded with body weight changes and/or other systemic toxicity may be more difficult to interpret as neurotoxic effects.

(a) Motor function: Neurotoxicants commonly affect motor function. These effects can be categorized generally into (1) weakness or decreased strength, (2) tremor, (3) incoordination, and (4) spasms, myoclonia, or abnormal motor movements (Tilson, 1987; Cory-Slechta, 1989). Specialized tests used to assess weakness include measures of grip strength, swimming endurance, suspension from a hanging rod, and discriminative motor function. Rotarod and gait assessments are used to measure incoordination, while rating scales and spectral analysis techniques can be used to quantify tremor and other abnormal movements.

(b) Sensory function: Gross perturbations of sensory function can be observed in simple neurological assessments such as the FOB. However, these tests may not be sufficiently sensitive to detect subtle sensory changes. Psychophysical procedures that study the relationship between a physical dimension (e.g., intensity, frequency) of a stimulus and behavior may be necessary to quantify agent-induced alterations in sensory function. Examples of psychophysical procedures include discriminated conditioning and startle reflex modification.

(c) Cognitive function: Alterations in learning and memory in experimental animals must be inferred from changes in behavior following exposure when compared with that either seen prior to exposure or with a nonexposed control group. Learning is defined as a relatively lasting change in behavior due to experience, and memory is defined as the persistence of a learned behavior over time. Table 6 lists several examples of learning and memory tests and representative neurotoxicants known to affect these tests. Measurement of changes in learning and memory must be separated from other changes in behavior that do not involve cognitive or associative processes (i.e., motor function, sensory capabilities, motivational factors). In addition, any apparent toxicant-induced change in learning or memory should ideally be

demonstrated over a range of stimulus and response conditions and testing conditions. In developmental exposures, it should be shown that the animals have matured enough to perform the specified task. Developmental neurotoxicants can accelerate or delay the ability to learn a response or interfere with cognitive function at the time of testing. Older animals frequently perform poorly on some types of tests, and it must be demonstrated that control animals in this population are capable of performing the procedure. Neurotoxicants might accelerate age-related dysfunction or alter motivational variables that are important for learning to occur. Further, it is not necessarily the case that a decrease in responding on a learning task is adverse while an increase in performance on a learning task is not. It is well known that lesions in certain regions of the brain can facilitate the acquisition of certain types of behaviors by removing preexisting response tendencies (e.g., inhibitory responses due to stress) that moderate the rate of learning under normal circumstances. Examples of learning and memory procedures include simple habituation, classical conditioning, and operant (or instrumental) conditioning, including tests for spatial learning and memory.

e. Developmental Neurotoxicity. Although the previous discussion of various neurotoxicity end points and tests applies to studies in which developmental exposures are used, there are particular issues of importance in the evaluation of developmental neurotoxicity studies. Exposure to chemicals during development can result in a spectrum of effects, including death, structural abnormalities, altered growth, and functional deficits (U.S. EPA, 1991b). Children are often differentially sensitive to chemical exposure. A number of agents have been shown to cause developmental neurotoxicity when exposure occurred during the period between conception and sexual maturity (e.g., Riley and Vorhees, 1986; Vorhees, 1987). Table 7 lists several examples of agents known to produce developmental neurotoxicity in experimental animals. Animal models of developmental neurotoxicity have been shown to be sensitive to several environmental agents known to produce developmental neurotoxicity in humans, including lead, ethanol, x-irradiation, methylmercury, and polychlorinated biphenyls (PCBs) (Kimmel et al., 1990; Needleman, 1990; Jacobson et al., 1985; Needleman, 1986). In many of these cases, functional deficits are observed at dose levels below those at which other indicators of developmental toxicity are evident or at minimally toxic doses in adults. Such effects may be transient, but generally are considered to be adverse effects.

TABLE 7.—EXAMPLES OF DEVELOPMENTAL NEUROTOXICANTS

Alcohols	Methanol, ethanol.
Antimitotics	X-radiation, azacytidine.
Insecticides	DDT, kepone.
Metals	Lead, methylmercury, cadmium.
Polyhalogenated hydrocarbons.	PCBs, PBBs.
Solvents	Carbon disulfide, toluene.

Testing for developmental neurotoxicity has not been required routinely by regulatory agencies in the United States, but is required by the EPA when other information indicates the potential for developmental neurotoxicity (U.S. EPA, 1986, 1988a, 1988b, 1989, 1991a, 1991b). Useful data for decision making may be derived from well-conducted adult neurotoxicity studies, standard developmental toxicity studies, and multigeneration studies, although the dose levels used in the latter may be lower than that in studies with shorter term exposure.

Important design issues to be evaluated for developmental neurotoxicity studies are similar to those for standard developmental toxicity studies (e.g., a dose-response approach with the highest dose producing minimal overt maternal or perinatal toxicity, number of litters large enough for adequate statistical power, randomization of animals to dose groups and test groups, litter generally considered as the statistical unit). In addition, the use of a replicate study design provides added confidence in the interpretation of data. A pharmacological/physiological challenge may also be valuable in evaluating neurologic function and ''unmasking'' effects not otherwise detectable. For example, a challenge with a psychomotor stimulant such as d-amphetamine may unmask latent developmental neurotoxicity (Hughes and Sparber, 1978; Adams and Buelke-Sam, 1981; Buelke-Sam et al., 1985).

Direct extrapolation of developmental neurotoxicity to humans is limited in the same way as for other end points of toxicity, i.e., by the lack of knowledge about underlying toxicological mechanisms and their significance (U.S. EPA, 1991b). However, comparisons of human and animal data for several agents known to cause developmental neurotoxicity in humans showed many similarities in effects (Kimmel et al., 1990). Comparisons at the level of functional category (sensory, motivational, cognitive, and motor function and social behavior) showed close agreement across species for the agents evaluated, even though the specific end points used to assess these functions varied considerably across species (Stanton and Spear, 1990). Thus, it can be assumed that developmental neurotoxicity effects in animal studies indicate the potential for altered neurobehavioral development in humans, although the specific types of developmental effects seen in experimental animal studies will not necessarily be the same as those that may be produced in humans. Therefore, when data suggesting adverse effects in developmental neurotoxicity studies are encountered for particular agents, they should be considered in the risk assessment process.

Functional tests with a moderate degree of background variability (e.g., a coefficient of variability of 20 percent or less) may be more sensitive to the effects of an agent on behavioral end points than are tests with low variability that may be impossible to disrupt without using life-threatening doses. A battery of functional tests, in contrast to a single test, is usually needed to evaluate the full complement of nervous system functions in an animal. Likewise, a series of tests conducted in animals in several age groups may provide more information about maturational changes and their persistence than tests conducted at a single age.

It is a well-established principle that there are critical developmental periods for the disruption of functional competence, which include both the prenatal and postnatal periods to the time of sexual maturation, and the effect of a toxicant is likely to vary depending on the time and degree of exposure (Rodier, 1978, 1990). It is also important to consider the data from studies in which postnatal exposure is included, as there may be an interaction of the agent with maternal behavior, milk composition, pup suckling behavior, as well as possible direct exposure of pups via dosed food or water (Kimmel et al., 1992).

Agents that produce developmental neurotoxicity at a dose that is not toxic to the maternal animal are of special concern. However, adverse developmental effects are often produced at doses that cause maternal toxicity (e.g., <20 percent reduction in weight gain during gestation and lactation). In these cases, the

developmental effects are still considered to represent neurotoxicity and should not be discounted as being secondary to maternal toxicity. At doses causing moderate maternal toxicity (i.e., ≥20 percent reduction in weight gain during gestation and lactation), interpretation of developmental effects may be confounded. Current information is inadequate to assume that developmental effects at doses causing minimal maternal toxicity result only from maternal toxicity; rather, it may be that the mother and developing organism are equally sensitive to that dose level. Moreover, whether developmental effects are secondary to maternal toxicity or not, the maternal effects may be reversible while the effects on the offspring may be permanent. These are important considerations for agents to which humans may be exposed at minimally toxic levels either voluntarily or involuntarily, because several agents are known to produce adverse developmental effects at minimally toxic doses in adult humans (e.g., alcohol) (Coles et al., 1991).

Although interpretation of developmental neurotoxicity data may be limited, it is clear that functional effects must be evaluated in light of other toxicity data, including other forms of developmental toxicity (e.g., structural abnormalities, perinatal death, and growth retardation). For example, alterations in motor performance may be due to a skeletal malformation rather than nervous system change. Changes in learning tasks that require a visual cue might be influenced by structural abnormalities in the eye. The level of confidence that an agent produces an adverse effect may be as important as the type of change seen, and confidence may be increased by such factors as reproducibility of the effect either in another study of the same function or by convergence of data from tests that purport to measure similar functions. A dose-response relationship is an extremely important measure of a chemical's effect; in the case of developmental neurotoxicity both monotonic and biphasic dose-response curves are likely, depending on the function being tested. The EPA Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b) may be consulted for more information on interpreting developmental toxicity studies. The endpoints frequently used to assess developmental neurotoxicity in exposed children was recently reviewed by Winneke (1995).

3. Other Considerations

a. Pharmacokinetics. Extrapolation of test results between species can be aided considerably by data on the pharmacokinetics of a particular agent in the species tested and, if possible, in humans. Information on a toxicant's half-life, metabolism, absorption, excretion, and distribution to the peripheral and central nervous system may be useful in predicting risk. Of particular importance for the pharmacokinetics of neurotoxicants is the blood-brain barrier, which ordinarily excludes ionic and nonlipid soluble chemicals from the central nervous system. The brain contains circumventricular organs whose purpose seems to be to sense the chemical composition of the peripheral circulation and activate mechanisms to bring the composition of the blood back to equilibrium if disturbed. These areas are technically inside the brain, but they lie outside of the blood-brain-barrier. Therefore, chemicals from the periphery can pass directly into the brain at these sites. The majority of these structures are located within or near the hypothalamus, an area that is crucial for maintenance of neuroendocrine function. Pharmacokinetic data may be helpful in defining the dose-response curve, developing a more accurate basis for comparing species sensitivity (including that of humans), determining dosimetry at target sites, and comparing pharmacokinetic profiles for various dosing regimens or routes of administration. The correlation of pharmacokinetic parameters and neurotoxicity data may be useful in determining the contribution of specific pharmacokinetic processes to the effects observed.

b. Comparisons of Molecular Structure. Comparisons of the chemical or physical properties of an agent with those of known neurotoxicants may provide some indication of the potential for neurotoxicity. Such information may be helpful for evaluating potential toxicity when only minimal data are available. The structure-activity relationships (SAR) of some chemical classes have been studied, including hexacarbons, organophosphates, carbamates, and pyrethroids. Therefore, class relationships or SAR may help predict neurotoxicity or interpret data from neurotoxicological studies. Under certain circumstances (e.g., in the case of new chemicals), this procedure is one of the primary methods used to evaluate the potential for toxicity when little or no empirical toxicity data are available. It should be recognized, however, that effects of chemicals in the same class can vary widely. Moser (1994), for example, reported that the behavioral effects of prototypic cholinesterase-inhibiting pesticides differed qualitatively in a battery of behavioral tests.

c. Statistical Considerations. Properly designed studies on the neurotoxic effects of compounds will include appropriate statistical tests of significance. In general, the likelihood of obtaining a significant effect will depend jointly on the magnitude of the effect and the variability obtained in control and treated groups. A number of texts are available on standard statistical tests (e.g., Siegel, 1956; Winer, 1971; Sokal and Rohlf, 1969; Salsburg, 1986; Gad and Weil, 1988).

Neurotoxicity data present some unique features that must be considered in selecting statistical tests for analysis. Data may involve several different measurement scales, including categorical (affected or not), rank (more or less affected), and interval and ratio scales of measurement (affected by some percentage). For example, convulsions are usually recorded as being present or absent (categorical), whereas neuropathological changes are frequently described in terms of the degree of damage (rank). Many tests of neurotoxicity involve interval or ratio measurements (e.g., frequency of photocell interruptions or amplitude of an evoked potential), which are the most powerful and sensitive scales of measurement. In addition, measurements are frequently made repeatedly in control and treated subjects, especially in the case of behavioral and neurophysiological end points. For example, OPPTS guidelines for FOB assessment call for evaluations before exposure and at several times during exposure in a subchronic study (U.S. EPA, 1991a).

Descriptive data (categorical) and rank order data can be analyzed using standard nonparametric techniques (Siegel, 1956). In some cases, if it is determined that the data fit the linear model, the categorical modeling procedure can be used for weighted least-squares estimation of parameters for a wide range of general linear models, including repeated-measures analyses. The weighted least-squares approach to categorical and rank data allows computation of statistics for testing the significance of sources of variation as reflected by the model. In the case of studies assessing effects in the same animals at several time points, univariate analyses can be carried out at each time point when the overall dose effect or the dose-by-time interaction is significant.

Continuous data (e.g., magnitude, rate, amplitude), if found to be normally distributed, can be analyzed with general linear models using a grouping factor of dose and, if necessary, repeated measures across time (Winer, 1971). Univariate analyses of dose, comparing dose groups to the control group at each time point, are performed when there is a significant overall dose effect or a dose-by-time interaction. Post hoc comparisons between control and treatment groups can be made following tests for overall significance. In the case of multiple end points within a series of evaluations, some type of correction for multiple observations is warranted (Winer, 1971).

d. In Vitro Data in Neurotoxicology. Methods and procedures that fall under the general heading of short-term tests include an array of in vitro tests that have been proposed as alternatives to whole-animal tests (Goldberg and Frazier, 1989). In vitro approaches use animal or human cells, tissues, or organs and maintain them in a nutritive medium. Various types of in vitro techniques produce data for evaluating potential and known neurotoxic substances, including primary cell cultures, cell lines, and cloned cells. While such procedures are important in studying the mechanism of action of toxic agents, their use in hazard identification in human health risk assessment has not been explored to any great extent.

Data from in vitro procedures are generally based on simplified approaches that require less time to yield information than do many in vivo techniques. However, in vitro methods generally do not take into account the distribution of the toxicant in the body, the route of administration, or the metabolism of the substance. It also is difficult to extrapolate in vitro data to animal or human neurotoxicity end points, which include behavioral changes, motor disorders, sensory and perceptual disorders, lack of coordination, and learning deficits. In addition, data from in vitro tests cannot duplicate the complex neuronal circuitry characteristic of the intact animal.

Many in vitro systems are now being evaluated for their ability to predict the neurotoxicity of various agents seen in intact animals. This validation process requires considerations in study design, including defined end points of toxicity and an understanding of how a test agent would be handled in vitro as compared to the intact organism. Demonstrated neurotoxicity in vitro in the absence of in vivo data is suggestive but inadequate evidence of a neurotoxic effect. In vivo data supported by in vitro data enhance the reliability of the in vivo results.

B. Dose-Response Evaluation

Dose-response evaluation is a critical part of hazard characterization and involves the description of the dose response relationship in the available data. Human studies covering a range of exposures are rarely available and therefore animal data are typically used for estimating exposure levels likely to produce adverse effects in humans. Evidence for a dose-response relationship is an important criterion in establishing a neurotoxic effect, although this analysis may be limited when based on standard studies using three dose groups or fewer. The evaluation of dose-response relationships includes identifying effective dose levels as well as doses associated with no increase in incidence of adverse effects when compared with controls. Much of the focus is on identifying the critical effect(s) observed at the lowest-observed-adverse-effect-level and the no-observed-adverse-effect-level associated with that effect. The NOAEL is defined as the highest dose at which there is no statistically or biologically significant increase in the frequency of an adverse neurotoxic effect when compared with the appropriate control group in a data base characterized as having sufficient evidence for use in a risk assessment (see section C). Although a threshold is assumed for neurotoxic effects, the existence of a NOAEL in an animal study does not prove or disprove the existence or level of a biological threshold. Alternatively, mathematical modeling of the dose-response relationship may be performed to determine a quantitative estimate of responses in the experimental range. This approach can be used to determine a BMD, which may be used in place of the NOAEL (Crump, 1994) (see Dose-Response Analysis, Section IV).

In addition to identifying the NOAEL/LOAEL or BMD, the dose-response evaluation defines the range of doses that are neurotoxic for a given agent, species, route of exposure, and duration of exposure. In addition to these considerations, pharmacokinetic factors and other aspects that might influence comparisons with human exposure scenarios should be taken into account. For example, dose-response curves may exhibit not only monotonic but also U-shaped or inverted U-shaped functions (Davis and Svendsgaard, 1990). Such curves are hypothesized to reflect multiple mechanisms of action, the presence of homeostatic mechanisms, and/or activation of compensatory or protective mechanisms. In addition to considering the shape of the dose-response curve, it should also be recognized that neurotoxic effects vary in terms of nature and severity across dose or exposure level. At high levels of exposure, frank lesions accompanied by severe functional impairment may be observed. Such effects are widely accepted as adverse. At progressively lower levels of exposure, however, the lesions may become less severe and the impairments less obvious. At levels of exposure near the NOAEL and LOAEL, the effects will often be mild, possibly reversible, and inconsistently found. In addition, the end points showing responses may be at levels of organization below the whole organism (e.g., neurochemical or electrophysiological end points). The adversity of such effects can be contentious (e.g., cholinesterase inhibition), yet it is such effects that are likely to be the focus of risk assessment decisions. To the extent possible, this document provides guidance on determining the adversity of neurotoxic effects. However, the identification of a critical adverse effect often requires considerable professional judgment and should consider factors such as the biological plausibility of the effect, the evidence of a dose-effect continuum, and the likelihood for progression of the effect with continued exposure.

C. Characterization of the Health-Related Data Base

This section describes a scheme for characterizing the sufficiency of evidence for neurotoxic effects. This scheme defines two broad categories: sufficient and insufficient (Table 8). Categorization is aimed at providing certain criteria for the Agency to use to define the minimum evidence necessary to define hazards and to conduct dose-response analyses. It does not address the issues related to characterization of risk, which requires analysis of potential human exposures and their relation to potential hazards to estimate the risks of those hazards from anticipated or estimated exposures.

Table 8.—Characterization of the Health-Related Database

Sufficient Evidence

The sufficient evidence category includes data that collectively provide enough information to judge whether or not a human neurotoxic hazard could exist. This category may include both human and experimental animal evidence.

Sufficient Human Evidence

This category includes agents for which there is sufficient evidence from

epidemiologic studies, e.g., case control and cohort studies, to judge that some neurotoxic effect is associated with exposure. A case series in conjunction with other supporting evidence may also be judged ''sufficient evidence.'' Epidemiologic and clinical case studies should discuss whether the observed effects can be considered biologically plausible in relation to chemical exposure. (Historically, much often has been made of the notion of causality in epidemiologic studies. Causality is a more stringent criterion than association and has become a topic of scientific and philosophical debate. See Susser [1986], for example, for a discussion of inference in epidemiology.)

Sufficient Experimental Animal Evidence/ Limited or No Human Data

This category includes agents for which there is sufficient evidence from experimental animal studies and/or limited human data to judge whether a potential neurotoxic hazard may exist. Generally, agents that have been tested according to current test guidelines would be included in this category. The minimum evidence necessary to judge that a potential hazard exists would be data demonstrating an effect in a single appropriate, well-executed study in a single experimental animal species, whereas the minimum evidence needed to judge that a potential hazard does not exist would include data from appropriate, well-executed laboratory animal studies that evaluated a variety of the potential manifestations of neuroxtoxicity and showed no effects at doses that were at least minimally toxic. Information on pharmacokinetics, mechanisms, or known properties of the chemical class may also strengthen the evidence.

Insufficient Evidence

This category includes agents for which there is less than the minimum evidence sufficient for identifying whether or not a neurotoxic hazard exists, such as agents for which there are no data on neurotoxicity or agents with data bases from studies in animals or humans that are limited by study design or conduct (e.g., inadequate conduct or report of clinical signs). Many general toxicity studies, for example, are considered insufficient in terms of the conduct of clinical neurobehavioral observations or the number of samples taken for histopathology of the nervous system. Thus, a battery of negative toxicity studies with these shortcomings would be regarded as providing insufficient evidence of the lack of a neurotoxic effect of the test material. Further, most screening studies based on simple observations involving autonomic and motor function provide insufficient evaluation of many sensory or cognitive functions. Data, which by itself would likely fall in this category, would also include information on structure-activity relationships or data from in vitro tests. While such information would be insufficient by itself to proceed further in the assessment it could be used to support the need for additional testing.

Data from all potentially relevant studies, whether indicative of potential hazard or not, should be included in this characterization. The primary sources of data are human studies and case reports, experimental animal studies, other supporting data, and in vitro and/or structure-activity relationship data. Because a complex interrelationship exists among study design, statistical analysis, and biological significance of the data, a great deal of scientific judgment, based on experience with neurotoxicity data and with the principles of study design and statistical analysis, is required to adequately evaluate the data base on neurotoxicity. In many cases, interaction with scientists in specific disciplines either within or outside the field of neurotoxicology (e.g., epidemiology, statistics) may be appropriate.

The adverse nature of different neurotoxicity end points may be a complex judgment. In general, most neuropathological and many neurobehavioral changes are regarded as adverse. However, there are adverse behavioral effects that may not reflect a direct action on the nervous system. Neurochemical and electrophysiological changes may be regarded as adverse as a function of their known or presumed relation to neuropathological and/or neurobehavioral consequences. In the absence of supportive information, a professional judgment must be made regarding the adversity of such outcomes, considering factors such as the nature, magnitude, and duration of the effects reported. Thus, correlated measures of neurotoxicity strengthen the evidence for a hazard. Correlations between functional and morphological effects, such as the correlation between leg weakness and paralysis and peripheral nerve damage from exposure to tri-ortho-cresyl phosphate, are the most common and striking example of this form of validity. Correlations support a coherent and logical link between behavioral effects and biochemical mechanisms. Replication of a finding also strengthens the evidence for a hazard. Some neurotoxicants cause similar effects across most species. Many chemicals shown to produce neurotoxicity in laboratory animals have similar effects in humans. Some neurologic effects may be considered adverse even if they are small in magnitude, reversible, or the result of indirect mechanisms.

Because of the inherent difficulty in ''proving any negative,'' it is more difficult to document a finding of no apparent adverse effect than a finding of an adverse effect. Neurotoxic effects (and most kinds of toxicity) can be observed at many different levels, so that only a single end point needs to be found to demonstrate a hazard, but many end points need to be examined to demonstrate no effect. For example, to judge that a hazard for neurotoxicity could exist for a given agent, the minimum evidence sufficient would be data on a single adverse end point from a well-conducted study. In contrast, to judge that an agent is unlikely to pose a hazard for neurotoxicity, the minimum evidence would include data from a host of end points that revealed no neurotoxic effects. This may include human data from appropriate studies that could support a conclusion of no evidence of a neurotoxic effect. With respect to clinical signs and symptoms, human exposures can reveal far more about the absence of effects than animal studies, which are confined to the signs examined.

In some cases, it may be that no individual study is judged sufficient to establish a hazard, but the total available data may support such a conclusion. Pharmacokinetic data and structure-activity considerations, data from other toxicity studies, as well as other factors may affect the strength of the evidence in these situations. For example, given that gamma diketones are known to cause motor system neurotoxicity, a marginal data set on a candidate gamma diketone, e.g., 1/10 animals affected, might be more likely to be judged sufficient than equivalent data from a member of a chemical class about which nothing is known.

A judgment that the toxicology data base is sufficient to indicate a potential neurotoxic hazard is not the end of analysis. The circumstances of expression of hazard are essential to describing human hazard potential. Thus, reporting should contain the details of the circumstances under which effects have been observed, e.g., ''long-term oral exposures of adult rodents to compound X at levels of roughly 1 mg/kg have been associated with ataxia and peripheral nerve damage.''

IV. Dose-Response Analysis

This section describes several approaches (including the LOAEL/ NOAEL and BMD) for determining the reference dose or reference concentration. The NOAEL or BMD/ uncertainty factor approach results in a RfD or RfC, which is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The dose-response analysis characterization should:

a. Describe how the RfD/RfC was calculated;

b. Discuss the confidence in the estimates;

c. Describe the assumptions or uncertainty factors used; and

d. Discuss the route and level of exposure observed, as compared to expected human exposures. (Specifically, are the available data from the same route of exposure as the expected human exposures? How many orders of magnitude do you need to extrapolate from the observed data to environmental exposures?)

A. LOAEL/NOAEL and Benchmark Dose (BMD) Determination

As indicated earlier, the LOAEL and NOAEL are determined for endpoints that are seen at the lowest dose level (so-called critical effect). Several limitations in the use of the NOAEL have been identified and described (e.g., Barnes and Dourson, 1988; Crump, 1984). For example, the NOAEL is derived from a single end point from a single study (the critical study) and

ignores both the slope of the dose-response function and baseline variability in the end point of concern. Because the baseline variability is not taken into account, the NOAEL from a study using small group sizes may be higher than the NOAEL from a similar study in the same species that uses larger group sizes. The NOAEL is also directly dependent on the dose spacing used in the study. Finally, and perhaps most importantly, use of the NOAEL does not allow estimates of risk or extrapolation of risk to lower dose levels.

Because of these and other limitations in the NOAEL approach, mathematical curve-fitting techniques (Crump, 1984; Gaylor and Slikker, 1990; Glowa, 1991; U.S. EPA, 1995a) are beginning to be used with, or as an alternative to, the NOAEL in calculating the RfD or RfC. The Agency is in the process of implementing these newer techniques and strongly encourages the calculation of BMDs for neurotoxicity and other health effect end points. These techniques typically apply a mathematical function that describes the dose-response relationship and then interpolate to a level of exposure associated with a small increase in effect over that occurring in the control group or under baseline conditions. The BMD has been defined as a lower confidence limit on the effective dose associated with some defined level of effect, e.g., a 5 percent or 10 percent increase in response (i.e., a BMD_{05} or BMD_{10} for a particular effect). Because the model is only used to interpolate within the dose range of the study, no assumptions about the existence (or nonexistence) of a threshold are needed. Thus, any model that fits the data well is likely to provide a reasonable estimate of the BMD.

Many neurotoxic end points provide continuous measures of response, such as response speed, nerve conduction velocity, IQ score, degree of enzyme inhibition, or the accuracy of task performance. Although it is possible to impose a dichotomy on a continuous effects distribution and to classify some level of response as ''affected'' and the remainder as ''unaffected,'' it may be very difficult and inappropriate to establish such clear distinctions, because such a dichotomy would misrepresent the true nature of the neurotoxic response. Alternatively, quantitative models designed to analyze continuous effect variables may be preferable. Other techniques that allow this approach, with transformation of the information into estimates of the incidence or frequency of affected individuals in a population, have been proposed (Crump, 1984; Gaylor and Slikker, 1990). Categorical regression analysis has been proposed since it can evaluate different types of data and derive estimates for short-term exposures (Rees and Hattis, 1994). Decisions about the most appropriate approach require professional judgment, taking into account the biological nature of the continuous effect variable and its distribution in the population under study.

Although dose-response functions in neurotoxicology are generally linear or monotonic, curvilinear functions, especially U-shaped or inverted U-shaped curves, have been reported as noted earlier (Section III B). Dose-response analyses should consider the uncertainty that U-shaped dose-response functions might contribute to the estimate of the NOAEL/LOAEL or BMD. Typically, estimates of the NOAEL/LOAEL are taken from the lowest part of the dose-response curve associated with impaired function or adverse effect.

B. Determination of the Reference Dose or Reference Concentration

Since the availability of dose-response data in humans is limited, extrapolation of data from animals to humans usually involves the application of uncertainty factors to the NOAEL/LOAEL or BMD. The NOAEL or BMD/uncertainty factor approach results in a RfD or RfC, which is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The oral RfD and inhalation RfC are applicable to chronic exposure situations and are based on an evaluation of all the noncancer health effects, including neurotoxicity data. RfDs and RfCs in the Integrated Risk Information System (IRIS–2) data base for several agents are based on neurotoxicity end points and include a few cases in which the RfD or RfC is calculated using the BMD approach (e.g., methylmercury, carbon disulfide). The size of the final uncertainty factor used will vary from agent to agent and will require the exercise of scientific judgment, taking into account interspecies differences, the shape of the dose-response curve, and the neurotoxicity end points observed. Default uncertainty factors are typically multiples of 10 and are used to compensate for human variability in sensitivity, the need to extrapolate from animals to humans, and the need to extrapolate from less than lifetime (e.g., subchronic) to lifetime exposures. An additional factor of up to 10 may be included when only a LOAEL (and not a NOAEL) is available from a study, or depending on the completeness of the data base, a modifying factor of up to 10 may be applied, depending on the confidence one has in the data base. Barnes and Dourson (1988) provide a more complete description of the calculation, use, and significance of RfDs in setting exposure limits to toxic agents by the oral route. Jarabek et al. (1990) provide a more complete description of the calculation, use, and significance of RfCs in setting exposure limits to toxic agents in air. Neurotoxicity can result from acute, shorter term exposures, and it may be appropriate in some cases, e.g., for air pollutants or water contaminants, to set shorter term exposure limits for neurotoxicity as well as for other noncancer health effects.

V. Exposure Assessment

Exposure assessment describes the magnitude, duration, frequency, and routes of exposure to the agent of interest. This information may come from hypothetical values, models, or actual experimental values, including ambient environmental sampling results. Guidelines for exposure assessment have been published separately (U.S. EPA, 1992) and will, therefore, be discussed only briefly here.

The exposure assessment should include an exposure characterization that:

a. Provides a statement of the purpose, scope, level of detail, and approach used in the exposure assessment;

b. Presents the estimates of exposure and dose by pathway and route for individuals, population segments, and populations in a manner appropriate for the intended risk characterization;

c. Provides an evaluation of the overall level of confidence in the estimate of exposure and dose and the conclusions drawn; and

d. Communicates the results of the exposure assessment to the risk assessor, who can then use the exposure characterization, along with the characterization of the other risk assessment elements, to develop a risk characterization.

A number of considerations are relevant to exposure assessment for neurotoxicants. An appropriate evaluation of exposure should consider the potential for exposure via ingestion, inhalation, and dermal penetration from relevant sources of exposures, including multiple avenues of intake from the same source. On-going Agency activities that support neurotoxicity exposure

assessment include characterizing cumulative risk and revising the Guidelines for the Health Risk Assessment of Chemical Mixtures.

In addition, neurotoxic effects may result from short-term (acute), high-concentration exposures as well as from longer term (subchronic), lower level exposures. Neurotoxic effects may occur after a period of time following initial exposure or be obfuscated by repair mechanisms or apparent tolerance. The type and severity of effect may depend significantly on the pattern of exposure rather than on the average dose over a long period of time. For this reason, exposure assessments for neurotoxicants may be much more complicated than those for long-latency effects such as carcinogenicity. It is rare for sufficient data to be available to construct such patterns of exposure or dose, and professional judgment may be necessary to evaluate exposure to neurotoxic agents.

VI. Risk Characterization

A. Overview

Risk characterization, the culmination of the risk assessment process, consists of an integrative analysis and a risk characterization summary. The integrative analysis (a) involves integration of the toxicity information from the hazard characterization and dose-response analysis with the human exposure estimates, (b) provides an evaluation of the overall quality of the assessment and the degree of confidence in the estimates of risk and conclusions drawn, and (c) describes risk in terms of the nature and extent of harm. The risk characterization summary communicates the results of the risk assessment to the risk manager.

This summary should include but is not limited to a discussion of the following elements:

a. Quality of and confidence in the available data;

b. Uncertainty analysis;

c. Justification of defaults or assumptions;

d. Related research recommendations;

e. Contentious issues and extent of scientific consensus;

f. Effect of reasonable alternative assumptions on conclusions and estimates;

g. Highlight reasonable plausible ranges;

h. Reasonable alternative models; and

i. Perspective through analogy.

The risk manager can then use the risk assessment, along with other risk management elements, to make public health decisions.

An effective risk characterization must fully, openly, and clearly characterize risks and disclose the scientific analyses, uncertainties, assumptions, and science policies that underlie decisions throughout the risk assessment and risk management processes. The risk characterization must feature values such as transparency in the decision-making process; clarity in communicating with each other and the public regarding environmental risk and the uncertainties associated with assessments of environmental risk; and consistency across program offices in core assumptions and science policies, which are well grounded in science and reasonable.

The following sections describe these four aspects of the risk characterization in more detail.

B. Integration of Hazard Characterization, Dose-Response Analysis and Exposure Assessment

In developing the hazard characterization, dose-response analysis and exposure portions of the risk assessment, the assessor must take into account many judgments concerning human relevance of the toxicity data, including the appropriateness of the various animal models for which data are available and the route, timing, and duration of exposure relative to expected human exposure. These judgments should be summarized at each stage of the risk assessment process (e.g., the biological relevance of anatomical variations may be established in the hazard characterization process, or the influence of species differences in metabolic patterns in the dose-response analysis). In integrating the information from the assessment, the risk assessor must determine if some of these judgments have implications for other portions of the assessment and whether the various components of the assessment are compatible.

The risk characterization should not only examine the judgments but also explain the constraints of available data and the state of knowledge about the phenomena studied in making them, including (1) the qualitative conclusions about the likelihood that the chemical may pose a specific hazard to human health, the nature of the observed effects, under what conditions (route, dose levels, time, and duration) of exposure these effects occur, and whether the health-related data are sufficient to use in a risk assessment; (2) a discussion of the dose-response characteristics of the critical effects(s), data such as the shapes and slopes of the dose-response curves for the various end points, the rationale behind the determination of the NOAEL and LOAEL and calculation of the benchmark dose, and the assumptions underlying the estimation of the RfD or RfC; and (3) the estimates of the magnitude of human exposure; the route, duration, and pattern of the exposure; relevant pharmacokinetics; and the number and characteristics of the population(s) exposed.

If data to be used in a risk characterization are from a route of exposure other than the expected human exposure, then pharmacokinetic data should be used, if available, to make extrapolations across routes of exposure. If such data are not available, the Agency makes certain assumptions concerning the amount of absorption likely or the applicability of the data from one route to another (U.S. EPA, 1992).

The level of confidence in the hazard characterization should be stated to the extent possible, including the appropriate category regarding sufficiency of the health-related data. A comprehensive risk assessment ideally includes information on a variety of end points that provide insight into the full spectrum of potential neurotoxicological responses. A profile that integrates both human and test species data and incorporates a broad range of potential adverse neurotoxic effects provides more confidence in a risk assessment for a given agent.

The ability to describe the nature of the potential human exposure is important to predict when certain outcomes can be anticipated and the likelihood of permanence or reversibility of the effect. An important part of this effort is a description of the nature of the exposed population and the potential for sensitive, highly susceptible, or highly exposed populations. For example, the consequences of exposure to the developing individual versus the adult can differ markedly and can influence whether the effects are transient or permanent. Other considerations relative to human exposures might include the likelihood of exposures to other agents, concurrent disease, and nutritional status.

The presentation of the integrated results of the assessment should draw from and highlight key points of the individual characterizations of component analyses performed under these Guidelines. The overall risk characterization represents the integration of these component characterizations. If relevant risk assessments on the agent or an analogous agent have been done by EPA or other Federal agencies, these should

be described and the similarities and differences discussed.

C. Quality of the Data Base and Degree of Confidence in the Assessment

The risk characterization should summarize the kinds of data brought together in the analysis and the reasoning on which the assessment is based. The description should convey the major strengths and weaknesses of the assessment that arise from availability of data and the current limits of our understanding of the mechanisms of toxicity.

Health risk is a function of the hazard characterization, dose-response analysis, and exposure assessment. Confidence in the results of a risk assessment is, thus, a function of confidence in the results of the analysis of these elements. Each of these elements should have its own characterization as a part of the assessment. Within each characterization, the important uncertainties of the analysis and interpretation of data should be explained, and the risk manager should be given a clear picture of consensus or lack of consensus that exists about significant aspects of the assessment. Whenever more than one view is supported by the data and choosing between them is difficult, all views should be presented. If one has been selected over the others, the rationale should be given; if not, then all should be presented as plausible alternative results.

D. Descriptors of Neurotoxicity Risk

There are a number of ways to describe risks. Several ways that are relevant to describing risks for neurotoxicity are as follows:

1. Estimation of the Number of Individuals

The RfD or RfC is taken to be a chronic exposure level at or below which no significant risk occurs. Therefore, presentation of the population in terms of those at or below the RfD or RfC (''not at risk'') and above the RfD or RfC (''may be at risk'') may be useful information for risk managers. This method is particularly useful to a risk manager considering possible actions to ameliorate risk for a population. If the number of persons in the at-risk category can be estimated, then the number of persons removed from the at-risk category after a contemplated action is taken can be used as an indication of the efficacy of the action.

2. Presentation of Specific Scenarios

Presenting specific scenarios in the form of ''what if?'' questions is particularly useful to give perspective to the risk manager, especially where criteria, tolerance limits, or media quality limits are being set. The question being asked in these cases is, at this proposed limit, what would be the resulting risk for neurotoxicity above the RfD or RfC?

3. Risk Characterization for Highly Exposed Individuals

This measure is one example of the just-discussed descriptor. This measure describes the magnitude of concern at the upper end of the exposure distribution. This allows risk managers to evaluate whether certain individuals are at disproportionately high or unacceptably high risk.

The objective of looking at the upper end of the exposure distribution is to derive a realistic estimate of a relatively highly exposed individual or individuals. This measure could be addressed by identifying a specified upper percentile of exposure in the population and/or by estimating the exposure of the highest exposed individual(s). Whenever possible, it is important to express the number of individuals who comprise the selected highly exposed group and discuss the potential for exposure at still higher levels.

If population data are absent, it will often be possible to describe a scenario representing high-end exposures using upper percentile or judgment-based values for exposure variables. In these instances caution should be used not to compound a substantial number of high-end values for variables if a ''reasonable'' exposure estimate is to be achieved.

4. Risk Characterization for Highly Sensitive or Susceptible Individuals

This measure identifies populations sensitive or susceptible to the effect of concern. Sensitive or susceptible individuals are those within the exposed population at increased risk of expressing the toxic effect. All stages of nervous system maturation might be considered highly sensitive or susceptible, but certain subpopulations can sometimes be identified because of critical periods for exposure, for example, pregnant or lactating women, infants, children.

In general, not enough is understood about the mechanisms of toxicity to identify sensitive subgroups for all agents, although factors such as nutrition, personal habits (e.g., smoking, alcohol consumption, illicit drug abuse), or preexisting disease (e.g., diabetes, sexually transmitted diseases) may predispose some individuals to be more sensitive to the neurotoxic effects of various agents.

5. Other Risk Descriptors

In risk characterization, dose-response information and the human exposure estimates may be combined either by comparing the RfD or RfC and the human exposure estimate or by calculating the margin of exposure (MOE). The MOE is the ratio of the NOAEL from the most appropriate or sensitive species to the estimated human exposure level. If a NOAEL is not available, a LOAEL may be used in calculating the MOE. Alternatively, a benchmark dose may be compared with the estimated human exposure level to obtain the MOE. Considerations for the evaluation of the MOE are similar to those for the uncertainty factor applied to the LOAEL/NOAEL or the benchmark dose. The MOE is presented along with a discussion of the adequacy of the data base, including the nature and quality of the hazard and exposure data, the number of species affected, and the dose-response information.

The RfD or RfC comparison with the human exposure estimate and the calculation of the MOE are conceptually similar but are used in different regulatory situations. The choice of approach depends on several factors, including the statute involved, the situation being addressed, the data base used, and the needs of the decision maker. The RfD or RfC and the MOE are considered along with other risk assessment and risk management issues in making risk management decisions, but the scientific issues that must be taken into account in establishing them have been addressed here.

If the MOE is equal to or more than the uncertainty factor × any modifying factor used as a basis for an RfD or RfC, then the need for regulatory concern is likely to be small. Although these methods of describing risk do not actually estimate risks per se, they give the risk manager some sense of how close the exposures are to levels of concern.

E. Communicating Results

Once the risk characterization is completed, the focus turns to communicating results to the risk manager. The risk manager uses the results of the risk characterization along with other technological, social, and economic considerations in reaching a regulatory decision. Because of the way in which these risk management factors

may affect different cases, consistent but not necessarily identical risk management decisions must be made on a case-by-case basis. These Guidelines are not intended to give guidance on the nonscientific aspects of risk management decisions.

F. Summary and Research Needs

These Guidelines summarize the procedures that the U.S. Environmental Protection Agency would use in evaluating the potential for agents to cause neurotoxicity. These Guidelines discuss the general default assumptions that should be made in risk assessment for neurotoxicity because of gaps in our knowledge about underlying biological processes and how these compare across species. Research to improve the risk assessment process is needed in a number of areas. For example, research is needed to delineate the mechanisms of neurotoxicity and pathogenesis, provide comparative pharmacokinetic data, examine the validity of short-term in vivo and in vitro tests, elucidate the functional modalities that may be altered, develop improved animal models to examine the neurotoxic effects of exposure during the premating and early postmating periods and in neonates, further evaluate the relationship between maternal and developmental toxicity, provide insight into the concept of threshold, develop approaches for improved mathematical modeling of neurotoxic effects, improve animal models for examining the effects of agents given by various routes of exposure, and address the synergistic or antagonistic effects of mixtures of chemicals and neurotoxic response. Such research will aid in the evaluation and interpretation of data on neurotoxicity and should provide methods to assess risk more precisely.

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