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Guidelines for Neurotoxicity Risk Assessment

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Risk Assessment Forum

U.S. Environmental Protection Agency
Washington, DC

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GUIDELINES FOR NEUROTOXICITY RISK ASSESSMENT

[FRL-6011-3]

AGENCY: Environmental Protection Agency

ACTION: Notice of availability of final Guidelines for Neurotoxicity Risk Assessment.

SUMMARY: The U.S. Environmental Protection Agency (EPA) is today publishing in final form a document entitled *Guidelines for Neurotoxicity Risk Assessment* (hereafter "Guidelines"). These Guidelines were developed as part of an interoffice guidelines development program by a Technical Panel of the Risk Assessment Forum. The Panel was 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. The Guidelines are based, in part, on recommendations derived from various scientific meetings and workshops on neurotoxicology, from public comments, and from recommendations of the Science Advisory Board. An earlier draft underwent external peer review in a workshop held on June 2-3, 1992, and received internal review by the Risk Assessment Forum. The Risk Assessment Subcommittee of the Committee on the Environment and Natural Resources of Office of Science and Technology Policy reviewed the proposed Guidelines during a meeting held on August 15, 1995. The Guidelines were revised and proposed for public comment on October 4, 1995 (60 FR 52032-52056). The proposed Guidelines were reviewed by the Science Advisory Board on July 18, 1996. EPA appreciates the efforts of all participants in the process, and has tried to address their recommendations in these Guidelines.

This notice describes the scientific basis for concern about exposure to agents that cause neurotoxicity, outlines the general process for assessing potential risk to humans because of environmental contaminants, and addresses Science Advisory Board and public comments on the 1995 *Proposed Guidelines for Neurotoxicity Risk Assessment* (FR 60:52032-52056). These 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 Agency.

DATES: The Guidelines will be effective April 30, 1998.

ADDRESSES: The Guidelines will be made available in several ways:

(1) The electronic version will be accessible from EPA's National Center for Environmental Assessment home page on the Internet at http://www.epa.gov/ncea.

(2) 3½" high-density computer diskettes in WordPerfect format will be available from ORD Publications, Technology Transfer and Support Division, National Risk Management Research Laboratory, Cincinnati, OH; Tel: 513-569-7562; Fax: 513-569-7566. Please provide the EPA No.: EPA/630/R-95/001Fa when ordering.

(3) This notice contains the full document. Copies of the Guidelines will be available for inspection at EPA headquarters and regional libraries, through the U.S. Government Depository Library program, and for purchase from the National Technical Information Service (NTIS), Springfield, VA; telephone: 1-800-553-6847, or 703-605-6000, fax: 703-321-8547. Please provide the NTIS PB No. [PB98-117831] when ordering.

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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" to promote consistency and technical quality in risk assessment, and to ensure that the risk assessment process is maintained as a scientific effort separate from risk management. A task force within 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, revised, reproposed, and finalized. These guidelines continue the process initiated in 1984. As with other EPA guidelines (e.g., developmental toxicity, 56 FR 63798-63826; exposure assessment, 57 FR 22888-22938; and carcinogenicity, 61 FR 17960-18011), EPA will revisit these guidelines as experience and scientific consensus evolve.

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. Policies in this document are intended as internal guidance for EPA. Risk assessors and risk managers

at EPA are the primary audience, although these Guidelines may be useful to others outside the Agency. 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 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.

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Dated: April 30,1998

signed by EPA Administrator
Carol M. Browner

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Part A: Guidelines for Neurotoxicity Risk Assessment

1. Introduction

These Guidelines describe the principles, concepts, and procedures that the U.S. Environmental Protection Agency (EPA) will 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 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. This document is not a regulation and is not intended for EPA regulations. The Guidelines set forth current scientific thinking and approaches for conducting and evaluating neurotoxic risk assessments. They are not intended, nor can they be relied upon, to create any rights enforceable by any party in litigation with the United States.

1.1. *Organization of These Guidelines*

This introduction (section 1) summarizes the purpose of these Guidelines within the overall framework of risk assessment at 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 2 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 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 that deals with recurring conceptual issues that cut across all stages of risk

assessment. These Guidelines describe a more interactive approach by organizing the process around the qualitative evaluation of the toxicity data (hazard characterization), the quantitative dose-response analysis, the exposure assessment, and the risk characterization. In these Guidelines, hazard characterization includes deciding whether a chemical has an effect by means of qualitative consideration of dose-response relationships, route, and duration of exposure. Determining a hazard often depends on whether a dose-response relationship is present (Kimmel et al., 1990). This approach combines the information important in comparing the toxicity of a chemical with potential human exposure scenarios (section 3). In addition, 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 qualitatively 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 database can be characterized as sufficient or insufficient for use in risk assessment (section 3.3). Combining hazard identification and some aspects of dose-response evaluation into hazard characterization does not preclude the evaluation and use of data for other purposes when quantitative information for setting reference doses (RfDs) and reference concentrations (RfCs) is not available.

The next step in the dose-response analysis (section 4) is the quantitative analysis, which 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, the benchmark dose approach (BMD) (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

database. 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 5) 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 6), the hazard characterization, dose-response analysis, and 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 database 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 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 in order 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 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 to categorize evidence that will identify and characterize neurotoxic hazards, as described in section 3.3, Characterization of the Health-Related Database, 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.

1.2. *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 in air, water, foods, cosmetics, household products, and drugs used therapeutically or illicitly. During daily life, a person experiences a multitude of exposures to potentially neuroactive substances, singly and in combination, both synthetic and natural. 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). EPA's TSCA inventory of chemical substances manufactured, imported, or processed in the United States includes more than 65,000 substances and is increasing yearly. An overwhelming majority of the materials in commercial use have not been tested for 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 affect the nervous system of the target species 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 1 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). The number of chemicals with neurotoxic potential has been estimated to range from 3% to 28% of all chemicals (OTA, 1990). Thus, estimating the risks of exposure to chemicals with neurotoxic potential is of concern with regard to their overall impact on human health.

1.3. *Neurotoxicity Risk Assessment*

In addition to its primary role in psychological 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 their potential to cause transient or persistent and direct or indirect effects on human health.

These Guidelines develop principles and concepts in several areas. They 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. They also discuss adverse effects on neurological development and function in infants and children following prenatal and perinatal exposure to chemical agents. They outline the methods 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 database for neurotoxicity risk assessment, and discuss the integration of exposure information with results of the dose-response assessment to characterize risks. These Guidelines do not advocate developing reference doses specific for neurotoxicity, but rather support the use of neurotoxicity as one possible endpoint 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 neurotoxicity risk assessment guidelines provide the Agency's first comprehensive guidance on the use and interpretation of neurotoxicity data, and are part of the Agency's risk assessment guidelines development process, which was initiated in 1984. As part of its neurotoxicity guidelines development program, 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 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, 1994). 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 for noncancer endpoints (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.

1.4. *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 indiscriminately. First, all available mechanistic and pharmacokinetic data should be considered. If these data indicate that an alternative assumption is appropriate or if they obviate the need for applying an assumption, such information should be used in risk assessment. For example, research in rats may determine that the neurotoxicity of a chemical is caused by a metabolite. If subsequent research finds that the chemical is metabolized to a lesser degree or not at all in humans, then this information should be used in formulating the default assumptions. The following default assumptions form the basis of the approaches taken in these Guidelines:

(1) 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.

(2) 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 endpoints of concern. Based on 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 neurotoxic effect may be different or absent. For example, certain organophosphate compounds produce a delayed-onset neuropathy in hens similar to that seen in humans, whereas rodents are characteristically insensitive to these compounds. 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.

(3) It is assumed that the neurotoxic effects seen in animal studies may not always be the same as those produced in humans. Therefore, it may be difficult to determine the most appropriate species in terms of predicting specific effects 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.

(4) It is also assumed that, in the absence of data to the contrary, the most sensitive species is used to estimate human risk. This is based on the assumption that humans are as sensitive as the most sensitive animal species tested. This provides a conservative estimate of sensitivity for added protection to the public. As with other noncancer endpoints, it is assumed that there is a nonlinear dose-response relationship for neurotoxicants. Although there may be a threshold for neurotoxic effects, these are often difficult to determine empirically. Therefore, a nonlinear relationship is assumed to exist for neurotoxicants.

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.

2. Definitions and Critical Concepts

This section defines the key terms and concepts that 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 or normal conditions 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). Functional neurotoxic effects include adverse changes in somatic/autonomic, sensory, motor, and/or cognitive function. Structural neurotoxic effects are defined as neuroanatomical changes occurring at any level of nervous system organization; functional changes are defined as neurochemical, neurophysiological, or behavioral effects. Chemicals can also be categorized into four classes: those that act on the central nervous system, the peripheral nerve fibers, the peripheral nerve endings, or muscles or other tissues (Albert, 1973). Changes in function can result from toxicity to other specific organ systems, and these indirect changes may be considered adverse. For example, exposure to a high dose of a chemical may cause damage to the liver, resulting in general sickness and a decrease in a functional endpoint such as motor activity. In this case, the change in motor activity could be considered as adverse, but not necessarily neurotoxic. A discussion concerning problems associated with risk assessment of high doses of chemicals in the context of drinking water and health was published by the National Research Council (1986).

The risk assessor should also know that there are different levels of concern based on the magnitude of effect, duration of exposure, and reversibility of some neurotoxic effects. Neurotoxic effects may be irreversible (the organism cannot return to the state prior to exposure, resulting in a permanent change) or reversible (the organism can return to the pre-exposure condition). 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 lifespan 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 that 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. Setting of exposure limits is not always associated with the determination of a reference dose, which is based on

chronic dosing. Data from acute or subacute dosing can be used for health advisories or in studies involving developmental exposures.

It should also be noted that the nervous system is known for its reserve capacity (Tilson and Mitchell, 1983). That is, repeated insult to the nervous system could lead to an adaptation. There are, however, limits to this capacity, and when these limits are exceeded, further exposure could lead to frank manifestations of neurotoxicity at the structural or functional level. The risk assessor should be aware 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 (those that continue to worsen even after the causal agent has been removed), delayed-onset effects (those that occur at a time distant from the last contact with the causal agent), residual effects (those that persist beyond a recovery period), or latent effects (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. Evidence for reversibility may depend on the region of the nervous system affected, the chemical involved, and organismic factors such as the age of the exposed population. Some regions of the nervous system, such as peripheral nerves, have a high capacity for regeneration, while regions in the brain such as the hippocampus are known for their ability to compensate or adapt to neurotoxic insult. For example, compensation is likely to be seen with solvents (e.g., n-hexane) that produce peripheral neuropathy because of the repair capacity of the peripheral nerve. In addition, tolerance to some cholinergic effects of cholinesterase-inhibiting compounds may be due to compensatory down-regulation of muscarinic receptors. Younger individuals may have more capacity to adapt than older individuals, suggesting that the aged may be at greater risk to neurotoxic exposure.

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 sites in the nervous system (primary neurotoxic agents) or those that require metabolism prior to interacting with their sites (secondary neurotoxic agents). Chemically induced neurotoxic effects can be direct (due to an agent or its metabolites acting directly on sites in the nervous system) or indirect (due to agents or metabolites that produce their effects primarily by interacting with sites outside the nervous system). For example, excitatory amino acids such as domoic acid damage specific neurons directly by activating excitatory amino acid receptors in the nervous system, whereas carbon monoxide decreases oxygen availability, which can indirectly kill neurons. Other examples of indirect effects 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 database. 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 endpoints (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.

3. Hazard Characterization

3.1. *Neurotoxicological Studies: Endpoints and Their Interpretation*

The qualitative characterization of neurotoxic hazard can be based on either human or animal data (Anger, 1984; Reiter, 1987; U.S. EPA, 1994). 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 3.3. 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 endpoints).

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 endpoints);
- 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).

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

g. Discuss other health endpoints of concern.

h. Comment on any nonpositive 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
- Highlights of major assumptions.

3.1.1. 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 past 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, epidemiologic studies, and human laboratory exposure studies. A more extensive description of issues concerning human neurotoxicology and risk assessment has been published elsewhere (U.S. EPA, 1993). A review of the types of tests used to assess cognitive and neurological function in children, in addition to a discussion of methodological issues in the design of prospective, longitudinal studies of developmental neurotoxicity in humans, has recently been published (Jacobson and Jacobson, 1996). Stanton and Spear (1990) reviewed assessment measures used in developmental neurotoxicology for their comparability in humans and laboratory animals and their ability to detect

comparable adverse effects across species. At the level of the various functional assessments for sensory, motivational, cognitive and motor function, and social behavior, there was good agreement across species among the neurotoxic agents reviewed.

3.1.1.1. Clinical Evaluations

Clinical methods are used extensively in neurology and neuropsychology to evaluate patients suspected of having neurotoxicity. An 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 neurophysiological studies and medical history to derive a working diagnosis. Brain functional imaging techniques based on magnetic resonance imaging or emission tomography may also be useful in helping diagnose neurodegenerative disorders following chemical exposures in humans (Omerand 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 chemical- or drug-induced changes in nervous system processes.

Neurological and neuropsychological 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 neurological examination approach limit its usefulness for neurotoxicological risk assessment. Information obtained from the neurological 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 endpoints are therefore the absolute and relative amount of muscle load sensed by the examiner in his or her arms.

Compared with other methods, the neurological 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 neurological 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 neurological exams share two disadvantages with respect to neurotoxicity risk assessment. First, neurological 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, neurological exams and neuropsychological 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 neurological 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.

It should be noted that alternative approaches are available that avoid many of the limitations of clinical and neurological and traditional neuropsychological methods. Computerized behavioral assessment systems designed for field testing of populations exposed to chemicals in the community or workplace have been developed during the past decade. The most widely used system is the Neurobehavioral Evaluation System (NES) developed by Baker et al. (1985). Advantages of computerized tests include (1) standardized administration to eliminate intertester variability and minimize subject-experimenter interaction; (2) automated data collection and scoring, which is faster, easier, and less error-prone than traditional methods; and (3) test administration requires minimal training and experience. NES tests have proven sensitive to a variety of solvents, metals, and pesticides (Otto, 1992). Computerized systems available for human neurotoxicity testing are critically reviewed in Anger et al. (1996).

3.1.1.2. 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 involving 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 can be obtained more quickly than more complex studies. Case reports of acute high-level exposure to a toxicant can be useful for identifying signs and symptoms that may also apply to lower exposure. Case reports can also be useful when corroborating epidemiological data are available.

3.1.1.3. 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 useful means of evaluating the effects of neurotoxic substances on human populations, particularly if effects of exposure are cumulative or exposures are repeated. Such

studies are less useful in cases of acute exposure, where the effects are short-term. Frequently, determining the precise dose or exposure concentration in epidemiological studies can be difficult.

3.1.1.3.1. 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.

3.1.1.3.2. 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. 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 nonexposed 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 that may invalidate 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 should 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.

3.1.1.3.3. Cohort (Prospective, Follow-Up) Studies. In a prospective study design, a healthy group of people is assembled and followed forward in time and observed for the development of dysfunction. Such studies are invaluable for determining the time course for development of dysfunction (e.g., follow-up 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 toxic 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. More credence should be given to those studies in which both observer and subject bias are carefully controlled (e.g., double-blind studies).

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 or negative results from a properly controlled prospective study should weigh heavily in the risk assessment process.

3.1.1.4. Human Laboratory Exposure Studies

Neurotoxicity assessment has an advantage not afforded to the evaluation of other toxic endpoints, 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 sometimes offer advantages over epidemiologic field studies. Combined with appropriate sampling of biological 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 in-depth 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 methodological 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 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 chemical-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 can be difficult. Chemical-naive participants are more easily recruited but may differ significantly in important characteristics from a representative sample of exposed persons. Chemical-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, whereas 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. In all cases, the potential for participant bias should be as carefully controlled for as possible. Even the consent form can lead to participant bias, as toxic effects have been reported in some individuals who were warned of such effects in an informed consent form. In addition, double-blind studies have been shown to provide some control for observer bias that may occur in single-blind studies. More credence should be given to those studies in which both observer and subject bias are carefully controlled (Benignus, 1993).

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

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 another 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.

3.1.2. Animal Studies

This section provides an overview of the major types of endpoints 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 past 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 endpoints included in the 1991 document serve as a convenient focus for this section, there are many other endpoints for which there are no current EPA guidelines. The goal of the current document is to provide a framework for interpreting data collected in tests frequently used by neurotoxicologists.

Five categories of endpoints will be described: structural or neuropathological, neuro-physiological, neurochemical, behavioral, and developmental. Table 1 lists a number of endpoints in each of these categories. It is imperative for the risk assessor to understand that the interpretation of the indicators listed in Table 1 as neurotoxic effects is dependent on the dose at which such changes occur and the possibility that damage to other organ systems may contribute to or cause such changes indirectly.

Table 1. Examples of possible indicators of a neurotoxic effect

Structural or neuropathological endpoints
Gross changes in morphology, including brain weight
Histologic changes in neurons or glia (neuronopathy, axonopathy, myelinopathy)
Neurochemical endpoints
Alterations in synthesis, release, uptake, degradation of neurotransmitters
Alterations in second-messenger-associated signal transduction
Alterations in membrane-bound enzymes regulating neuronal activity
Inhibition and aging of neuropathy enzyme
Increases in glial fibrillary acidic protein in adults
Neurophysiological endpoints
Change in velocity, amplitude, or refractory period of nerve conduction
Change in latency or amplitude of sensory-evoked potential
Change in electroencephalographic pattern
Behavioral and neurological endpoints
Increases or decreases in motor activity
Changes in touch, sight, sound, taste, or smell sensations
Changes in motor coordination, weakness, paralysis, abnormal movement or posture, tremor, ongoing performance
Absence or decreased occurrence, magnitude, or latency of sensorimotor reflex
Altered magnitude of neurological measurement, including grip strength, hindlimb splay
Seizures
Changes in rate or temporal patterning of schedule-controlled behavior
Changes in learning, memory, and attention
Developmental endpoints
Chemically induced changes in the time of appearance of behaviors during development
Chemically induced changes in the growth or organization of structural or neurochemical elements

3.1.2.1. Structural Endpoints of Neurotoxicity

Structural endpoints are typically defined as neuropathological changes evident by gross observation or light microscopy, although most neurotoxic changes will be detectable only at the light microscopic level. Gross changes in morphology can include discrete or widespread lesions in nerve tissue. A change in brain weight is considered to be a biologically significant effect. This is true regardless of changes in body weight, because brain weight 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. Changes in brain weight are a more reliable indicator of alteration in brain structure than are measurements of length or width in fresh brain, because there is little historical data in the toxicology literature.

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 (Spencer and Schaumburg, 1980; WHO, 1986; Krinke, 1989; Griffin, 1990). Neurotoxicant-induced 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 nerve terminal degeneration. Nerve terminal degeneration represents a very subtle change that may not be detected by routine histopathology, but requires detection by special procedures such as silver staining or neurotransmitter-specific immunohistochemistry. 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, such as Parkinson's disease, in humans (WHO, 1986). Table 2 lists examples of such neurotoxic chemicals, their putative site of action, the type of neuropathology produced, and the disorder or condition that each typifies. Inclusion of any chemical in any of the following tables is for illustrative purposes, i.e., it has been reported that the chemical will produce a neurotoxic effect at some dose; any individual chemical listed may also adversely affect other organs at lower doses. It is important that the severity of each structural union be graded objectively and the grading criteria reported.

Table 2. Neurotoxicants and disorders with specific neurological targets

Site of action	Neurotoxic change	Neurotoxic chemical	Corresponding neurodegenerative disorder
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 (MPTP) (dopaminergic)	Parkinson's disease
Schwann cell myelin	Myelinopathy	Hexachlorophene	Congenital hypomyelinogenesis
Centra-peripheral distal axon	Distal axonopathy	Acrylamide, carbon disulfide, n-hexane	Peripheral neuropathy
Central axons	Central axonopathy	Clioquinol	Subacute myeloopticoneuro-pathy
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 their functional specialization and lack of regenerative capacity.

Within each general class of nervous system structural alteration, there are various histological changes that can result after exposure to neurotoxicants. For example, specific changes in nerve cell bodies include chromatolysis, vacuolization, and cell death. Axons can undergo swelling, degeneration, and atrophy, while myelin sheath changes include folding, edematous splitting, and demyelination. Although terminal degeneration does occur, it is not readily detectable by light microscopy. Many of these changes are a result of complex effects at specific subcellular organelles, such as the axonal swelling that occurs as a result of neurofilament accumulation in acrylamide toxicity. Other changes may be associated with regenerative or adaptive processes that occur after neurotoxicant exposure.

3.1.2.2. Neurophysiological Endpoints of Neurotoxicity

Neurophysiological studies 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 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 provoked	Acrylamide, n-hexane
Somatosensory function	Sensory-evoked potential (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)	Dithiobiuret
General central nervous system/level of arousal	Electroencephalography (EEG)	Toluene

3.1.2.2.1. 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 sensory nerves or by measuring the muscle response evoked by nerve stimulation to measure motor effects. While a number of endpoints can be recorded, the most critical variables are nerve conduction velocity, response amplitude, and refractory period. It is important to recognize that damage to nerve fibers may not be reflected in changes in these endpoints if the damage is not sufficiently extensive. Thus, the interpretation of data from such studies may be enhanced if evaluations such as nerve pathology and/or other structural measures are also included.

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 will 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 is 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 reflects 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.

3.1.2.2.2. 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 should be interpreted in light of the behavioral status of the subject and would generally be considered evidence of a neurotoxic effect.

3.1.2.2.3. Seizures/Convulsions. Some neurotoxicants (e.g., lindane, pyrethroids, trimethyltin, dichlorodiphenyltrichloroethane [DDT]) produce observable convulsions. When convulsionlike behaviors are observed, as described in the behavioral section on convulsions, neurophysiological recordings can provide additional information to help interpret the results. Recordings of brain electrical activity that demonstrate seizurelike activity are indicative of a neurotoxic effect.

In addition to producing seizures directly, chemicals may also alter the frequency, severity, duration, or threshold for eliciting seizures through other means by a phenomenon known as “kindling.” Such alterations can occur after acute exposure or after repeated exposure to dose levels below the acute threshold. In experiments demonstrating changes in sensitivity following repeated exposures to the test compound, information regarding possible changes in the pharmacokinetic distribution of the compound is required before the seizure susceptibility changes can be interpreted as evidence of neurotoxicity. Increases in susceptibility to seizures are considered adverse.

3.1.2.2.4. 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 anesthetic drugs and stimuli producing arousal (e.g., lights, sounds). In studies with toxicants, changes in EEG pattern can sometimes precede alterations in other objective signs of neurotoxicity (Dyer, 1987).

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

3.1.2.3. Neurochemical Endpoints of Neurotoxicity

Many different neurochemical endpoints 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; Silbergeld, 1993). 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
Neurotoxicants acting on ionic balance:	
Inhibit sodium entry	Tetrodotoxin
Block closing of sodium channel	p,p'-DDT, pyrethroids
Increase permeability to sodium	Batrachotoxin
Increase intracellular calcium	Chlorodecone
Synaptic neurotoxicants	MPTP
Uptake blockers	Hemicholinium
Metabolic poisons	Cyanide
Hyperactivation of receptors	Domoic acid
Blocks transmitter release	Botulinum toxin
Inhibition of transmitter degradation	Pesticides of the organophosphate and carbamate classes
Blocks axonal transport	Acrylamide

As stated previously, any neurochemical change is potentially neurotoxic. 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 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 in either the central or peripheral nervous system prolongs the action of the acetylcholine at the neuron's synaptic receptors and is thought to be responsible for the range of effects these chemicals produce, although it is possible that these compounds have other modes of action (Eldefrawi et al., 1992; Greenfield et al., 1984; Small, 1990).

There is agreement that objective clinical measures of cholinergic overstimulation (e.g., salivation, sweating, muscle weakness, tremor, blurred vision) can be used to evaluate dose-response and dose-effect relationships and define the presence and absence of effects. A given depression in peripheral and central cholinesterase activity may or may not be accompanied by clinical manifestations. A depression in RBC and/or plasma cholinesterase activity may or may not be accompanied by clinical manifestations. It should be noted, however, that reduction in cholinesterase activity, even if the anticholinesterase exposure is not severe enough to precipitate clinical signs or symptoms, may impair the organism’s ability to adapt to additional exposures to anticholinesterase compounds. Inhibition of RBC and/or plasma cholinesterase activity is a biomarker of exposure, as well as a reflection of cholinesterase inhibition in other peripheral tissues (e.g., neuromuscular junction, peripheral nerve, or ganglia) (Maxwell et al., 1987; Nagymajtenyi et al., 1988; Padilla et al., 1994), thereby contributing to the overall hazard identification of cholinesterase-inhibiting compounds.

The risk assessor should also be aware that tolerance to the cholinergic overstimulation may be observed following repeated exposure to cholinesterase-inhibiting chemicals. It has been reported, however, that although tolerance can develop to some effects of cholinesterase inhibition, the cellular mechanisms responsible for the development of tolerance may also lead to the development of other effects, i.e., cognitive dysfunction, not present at the time of initial exposure (Bushnell et al., 1991).

These adaptive biochemical changes in the tolerant animal may render it supersensitive to subsequent exposure to cholinergically active compounds (Pope et al., 1992).

In general, the risk assessor should understand that assessment of cholinesterase-inhibiting chemicals should be done on a case-by-case basis using a weight-of evidence approach in which all of the available data (e.g., brain, blood, and other tissue cholinesterase activity, as well as the presence or absence of clinical signs) is considered in the evaluation. Generally, the toxic effects of anticholinesterase compounds are viewed as reversible, but there is human and experimental animal evidence indicating that there may be residual, if not permanent, effects of exposure to these compounds (Steenland et al., 1994; Tandon et al., 1994; Stephens et al., 1995). A subset of organophosphate agents also produces organophosphate-induced delayed neuropathy (OPIDN) after acute or repeated exposure. Inhibition and aging of neurotoxic esterase (or neuropathy enzymes) are associated with agents that produce OPIDN (Johnson, 1990; Richardson, 1995). The conclusion that a chemical may produce OPIDN should be based on at least two of three factors: (1) evidence of a clinical syndrome, (2) pathological lesions, and (3) neurotoxic esterase (NTE) inhibition. NTE inhibition is necessary, but not sufficient, evidence of the potential to produce OPIDN when there is at least 55%-70% inhibition after acute exposure (Ehrich et al., 1995) and at least 45% inhibition following repeated exposure.

Chemically induced injury to the central nervous system may be accompanied by hypertrophy of astrocytes. In some cases, these astrocytic changes can be seen light microscopically with immunohistochemical stains for glial fibrillary acidic protein (GFAP), the major intermediate filament protein in astrocytes. In addition, GFAP can be quantified by an immunoassay, which has been proposed as a marker of astrocyte reactivity (O'Callaghan, 1988). Immunohistochemical stains have the advantage of better localization of GFAP increases, whereas immunoassay evaluations are superior at detecting and quantifying changes in GFAP levels and establishing dose-response relationships. The ability to detect and quantify changes in GFAP by immunoassay is improved by dissecting and analyzing multiple brain regions. 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 as an optional test in the Neurotoxicity Screening Battery (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. There is less agreement as to how to interpret decreases in GFAP relative to an appropriate control group. The absence of a change in GFAP following exposure does not 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 may be confounded by the possibility that chemically induced increases in GFAP could 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.

3.1.2.4. Behavioral Endpoints of Neurotoxicity

Behavior reflects the integration of the various functional components of the nervous system. 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 (e.g., the relationship between liver damage and motor activity) is extremely important. The presence of systemic toxicity may complicate, but does not 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.

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). Examples of measures obtained in a typical FOB are presented in Table 5. There are many other measures of behavior, including specialized tests of motor and sensory function and of learning and memory (Tilson, 1987; Anger, 1984).

Table 5. Examples of measures in a representative functional observational battery

Home cage and open field	**Manipulative**	**Physiological**
Arousal	Approach response	Body temperature
Autonomic signs	Click response	Body weight
Convulsions, tremors	Foot splay	
Gait	Grip strength	
Mobility	Righting reflex	
Posture	Tail pinch response	
Rearing		
Stereotypy		
Touch response		

Table 6. Examples of specialized behavioral tests to measure neurotoxicity

Function	Procedure	Representative agents
Motor function		
Weakness	Grip strength, swimming endurance, suspension rod, discriminative motor function	n-Hexane, methyl n-Butylketone, carbaryl
Incoordination	Rotorod, gait assessments, righting reflex	3-Acetylpyridine, ethanol
Tremor	Rating scale, spectral analysis	Chlordecone, Type I pyrethroids, DDT
Myoclonic spasms	Rating scale	DDT, Type II pyrethroids
Sensory function		
Auditory	Discrimination conditioning Reflex modification	Toluene, trimethyltin
Visual	Discrimination conditioning	Methylmercury
Somatosensory	Discrimination conditioning	Acrylamide
Pain sensitivity	Discrimination conditioning	Parathion
Olfactory	Discrimination conditioning	3-Methylindole, methylbromide
Cognitive function		
Habituation	Startle reflex	Diisopropylfluorophosphate Pre/neonatal methylmercury
Classical conditioning	Nictitating membrane Conditioned flavor aversion Passive avoidance Olfactory conditioning	Aluminum Carbaryl Trimethyltin, IDPN Neonatal trimethyltin

Instrumental conditioning	One-way avoidance	Chlordecone
	Two-way avoidance	Pre/neonatal lead
	Y-maze avoidance	Hypervitaminosis A
	Biel water maze	Styrene
	Morris water maze	DFP
	Radial arm maze	Trimethyltin
	Delayed matching to sample	DFP
	Repeated acquisition	Carbaryl

Table 6. Examples of specialized behavioral tests to measure neurotoxicity (cont.)

At the present time, there is no clear consensus concerning the use of specific behavioral tests to assess chemical-induced sensory, motor, or cognitive dysfunction in animal models. The risk assessor should also know that the literature is clear that a number of other behaviors besides those listed in Tables 1, 5, and 6 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 endpoints.

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

3.1.2.4.1. Functional Observational Battery (FOB). An FOB 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.

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. In general, if only a few unrelated measures in the FOB are affected, or the effects are unrelated to dose, the results may not be 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 less persuasive 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. The risk assessor should be aware of

the potential for a number of false positive statistical findings in these studies because of the large number of endpoints customarily included in the FOB.

FOB data can be grouped into one or more of 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 may be 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 Schaumburg, 1980).

3.1.2.4.2. 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 the 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. In these cases, the transformed data and not raw data should be used for risk assessment purposes. 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.

Motor activity can be altered by a number of experimental factors, including neurotoxic chemicals. Decreases in activity could occur following high doses of non-neurotoxic agents (Kotsonis and Klaassen, 1977; Landauer et al., 1984). Examples of neurotoxic agents that decrease motor activity 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 ontogenetic 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 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, are related to structural or other functional changes in the nervous system, or occur in the absence of life-threatening toxicity.

3.1.2.4.3. 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 should 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). Qualitatively, rates and patterns of SCOB display cross-species

generality, but the quantitative measures of rate and pattern of performance can vary within and between species.

In laboratory animals, SCOB has been used to study a wide range of neurotoxicants, including methylmercury, many pesticides, organic and inorganic lead, triethyltin, and trimethyltin (MacPhail, 1985; Tilson, 1987; Rice, 1988). The primary SCOB endpoints for evaluation are response rate and the temporal pattern of responding. These endpoints may vary as a function of the contingency between responding and reinforcement presentation (i.e., schedule of reinforcement). Schedules of reinforcement that have been used in toxicology studies include fixed ratio and fixed interval schedules. Fixed ratio schedules engender high rates of responding and a characteristic pause after delivery of each reinforcement. Fixed interval schedules engender a relatively low rate of responding during the initial portion of the interval and progressively higher rates near the end of the interval. For some schedules of reinforcement, the temporal pattern of responding may play a more important role in defining the performance characteristics than the rate of responding. For other schedules, the reverse may be true. For example, the temporal pattern of responding may be more important than rate of responding for defining performance on a fixed interval schedule. For a fixed ratio schedule, more importance might be placed on the rate of responding than on the post-reinforcement pause.

The overall qualitative patterns are important properties of the behavior. Substantial qualitative changes in operant performance, such as elimination of characteristic response patterns, can be evidence of an adverse effect. Most chemicals, however, can disrupt operant behavior at some dose, and such adverse effects may be due either to neurotoxic or non-neurotoxic mechanisms. Unlike large qualitative changes in operant performance, small quantitative changes are not adverse. Some changes may actually represent an improvement, e.g., an increase in the index of curvature with a decrease in fixed interval rate of responding. Assessing the toxicological importance of these effects requires considerable professional judgment and evaluation of converging evidence from other types of toxicological endpoints. While most chemicals decrease the efficiency of responding at some dose, some agents may increase response efficiency on schedules requiring high response rates because of 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.

3.1.2.4.4. 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 convulsionlike behaviors. In some cases, convulsions or convulsionlike 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.

3.1.2.4.5. 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 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.

A statistically or biologically significant chemically induced change in any measure in Table 6 may be evidence of an adverse effect. However, 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.

3.1.2.4.5.1. 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 strength include measures of grip strength, swimming endurance, suspension from a hanging rod, and discriminative motor function. Rotorod and gait assessments are used to measure coordination, while rating scales and spectral analysis techniques can be used to quantify tremor and other abnormal movements.

3.1.2.4.5.2. Sensory Function. Gross perturbations of sensory function can be observed in simple neurological assessments such as the hot plate or tail flick test. 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.

3.1.2.4.5.3. Cognitive Function. Alterations in learning and memory in experimental animals should be inferred from changes in behavior following exposure when compared with that 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 should 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 may interfere with cognitive function at the time of testing. Older animals frequently perform poorly on some types of tests, and it should 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 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.

Apparent improvement in performance is not either adverse or beneficial until demonstrated to be so by converging evidence with a variety of experimental methods. Examples of procedures to assess cognitive function include simple habituation, classical conditioning, and operant (or instrumental) conditioning, including tests for spatial learning and memory.

3.1.2.4.5.4. Developmental Neurotoxicity. Although the previous discussion of various neurotoxicity endpoints and tests applies to studies in which developmental exposures are used, there are particular issues of importance in the evaluation of developmental neurotoxicity studies. This section underscores the importance of detecting neurotoxic effects following developmental exposure because an NRC (1993) report has indicated that infants and children may be differentially sensitive to environmental chemicals such as pesticides. 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). 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 adverse. Developmental exposure to a chemical could result in transient or reversible effects observed during early development that could reemerge as the individual ages (Barone et al., 1995).

Table 7. Examples of compounds or treatments producing developmental neurotoxicity

Alcohols	Methanol, ethanol
Antimitotics	X-radiation, azacytidine
Insecticides	DDT, chlordecone
Metals	Lead, methylmercury, cadmium
Polyhalogenated hydrocarbons	PCBs, PBBs

Testing for developmental neurotoxicity has not been required routinely by regulatory agencies in the United States, but is required by 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 those 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, with number of litters large enough for adequate statistical power, with randomization of animals to dose groups and test groups, with 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 neurological 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 endpoints 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). As evidenced primarily by observations in laboratory animals, comparisons at the level of functional category (sensory, motivational, cognitive, motor function, and social behavior) showed close agreement across species for the agents evaluated, even though the specific endpoints 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 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% or less) may be more sensitive to the effects of an agent on behavioral endpoints 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, or 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 mild maternal toxicity (e.g., 10%-20% reduction in weight gain during gestation and lactation). At doses causing moderate maternal toxicity (i.e., 20% or more 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 (e.g., alcohol) are known to produce adverse developmental effects at minimally toxic doses in adult humans (Coles et al., 1991).

Although interpretation of developmental neurotoxicity data may be limited, it is clear that functional effects should 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 have been reviewed by Winneke (1995).

3.1.3. Other Considerations

3.1.3.1. 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. The vast majority of the central nervous system is served by blood vessels with blood-brain barrier properties, which exclude most ionic and nonlipid-soluble chemicals from the brain and spinal cord. The brain contains several structures called circumventricular organs (CVOs) that are served by blood vessels lacking blood-brain barrier properties. Brain regions adjacent to these CVOs are thus exposed to relatively high levels of many neurotoxicants. 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 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.

3.1.3.2. 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 (1995), for example, reported that the behavioral effects of prototypic cholinesterase-inhibiting pesticides differed qualitatively in a battery of behavioral tests.

3.1.3.3. 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. The risk assessor should be aware that some neurotoxicants may induce a greater variability in biologic response, rather than a clear shift in mean or other parameters (Laties and Evans, 1980; Glowa and MacPhail, 1995). 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 should 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 endpoints. 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, can be 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 endpoints within a series of evaluations, some type of correction for multiple observations is warranted (Winer, 1971).

3.1.3.4. 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, including primary cell cultures, cell lines, and cloned cells, produce data for evaluating potential and known neurotoxic substances. 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 endpoints, 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 endpoints 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.

3.1.3.5. Neuroendocrine Effects

Neuroendocrine dysfunction may occur because of a disturbance in the regulation and modulation of neuroendocrine feedback systems. One major indicator of neuroendocrine function is secretion of hormones from the pituitary. Hypothalamic control of anterior pituitary secretions is also involved in a number of important bodily functions. Many types of behaviors (e.g., reproductive behaviors, sexually dimorphic behaviors in animals) are dependent on the integrity of the hypothalamic-pituitary system, which could represent a potential site of neurotoxicity. Pituitary secretions arise from a number of different cell types in this gland, and neurotoxicants could affect these cells directly or indirectly. Morphological changes in cells mediating neuroendocrine secretions could be associated with adverse effects on the pituitary or hypothalamus and could ultimately affect behavior and the functioning of the nervous system. Biochemical changes in the hypothalamus may also be used as indicators of potential adverse effects on neuroendocrine function. Finally, the development of the nervous system is intimately associated with the presence of circulating hormones such as thyroid hormone (Porterfield, 1994). The nature of the nervous system deficit, which could include cognitive dysfunction, altered neurological development, or visual deficits, depends on the severity of the thyroid disturbance and the specific developmental period when exposure to the chemical occurred.

3.2. *Dose-Response Evaluation*

Dose-response evaluation is a critical part of the qualitative characterization of a chemical's potential to produce neurotoxicity 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 adverse effects when compared with controls. The lack of a dose-response relationship in the data may suggest that the effect is not related to the putative neurotoxic effect or that the study was not appropriately controlled. Much of the focus is on identifying the critical effect(s) observed at the LOAEL and the NOAEL 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 database characterized as having sufficient evidence for use in a risk assessment (see section 3.3). The risk

assessor should be aware of possible problems associated with estimating a NOAEL in studies involving a small number of test subjects and that have a poor dose-response relationship.

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 endpoints showing responses may be at levels of organization below the whole organism (e.g., neurochemical or electrophysiological endpoints). The adversity of such effects can be disputed (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.

3.3. *Characterization of the Health-Related Database*

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 in order to estimate the risks of those hazards from anticipated or estimated

exposures. Several examples using a weight-of-evidence approach similar to that described in these Guidelines have been described elsewhere (Tilson et al., 1995; Tilson et al., 1996).

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, often much 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 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 adverse neurotoxic effect in a single appropriate, well-executed study in a single experimental animal species. The minimum evidence needed to judge that a potential hazard does not exist would include data from an appropriate number of endpoints from more than one study and two species showing no adverse neurotoxic effects at doses that were minimally toxic in terms of producing an adverse effect. 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 databases 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 SAR or data from in vitro tests. Although such information would be insufficient by itself to proceed further in the assessment it could be used to support the need for additional testing.

Table 8. Characterization of the health-related database (cont.)

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 SAR 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 database 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 endpoints 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 because of their known or presumed relation to neuropathological and/or neurobehavioral consequences. In the absence of supportive information, a professional judgment should 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 neurological 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 only a single endpoint needs to be found to demonstrate a hazard, but many endpoints 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 endpoint 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 endpoints 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, or 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 database is sufficient to indicate a potential neurotoxic hazard is not the end of analysis. The circumstances of expression of the 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."

4. Quantitative Dose-Response Analysis

This section describes several approaches (including the LOAEL/NOAEL and BMD) for determining the reference dose (RfD) or reference concentration (RfC). The NOAEL or BMD/uncertainty factor approach results in an 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:

- Describe how the RfD/RfC was calculated;
- Discuss the confidence in the estimates;
- Describe the assumptions or uncertainty factors used; and
- Discuss the route and level of exposure observed, as compared to expected human exposures.

4.1. *LOAEL/NOAEL and 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 endpoint from a single study (the critical study) and ignores both the slope of the dose-response function and baseline variability in the endpoint 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, it has been proposed that mathematical curve-fitting techniques (Crump, 1984; Gaylor and Slikker, 1990; Glowa, 1991; Glowa and MacPhail, 1995; U.S. EPA, 1995a) be compared with the NOAEL procedure in calculating the RfD or RfC. 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% or 10% increase in response. These guidelines suggest that the use of the BMD should be

explored in specific situations. The Agency is currently developing guidelines for the use of the BMD in risk assessment.

Many neurotoxic endpoints 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. The risk assessor should be aware of the importance of trying to reconcile findings from several studies that seem to report widely divergent results. 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; Glowa and MacPhail, 1995). Categorical regression analysis has been proposed because 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 3.2). 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.

4.2. *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 an 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) database for several agents are based on neurotoxicity endpoints 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 endpoints observed. 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 database, a modifying factor of up to 10 may be applied, depending on the confidence one has in the database. Uncertainty factors of less than 10 can be used, depending upon the availability of relevant information. 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.

5. 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:

- Provides a statement of the purpose, scope, level of detail, and approach used in the exposure assessment;
- 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;
- Provides an evaluation of the overall level of confidence in the estimate of exposure and dose and the conclusions drawn; and
- Communicates the results of the exposure assessment to the risk assessor, who can then use the exposure characterization, along with the hazard and dose/response characterizations, 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 exposure, including multiple avenues of intake from the same source.

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.

6. Risk Characterization

6.1. *Overview*

Risk characterization is the summarization step of the risk assessment process and consists of an integrative analysis and a 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 in a complete, informative, and useful format.

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

- Quality of and confidence in the available data;
- Uncertainty analysis;
- Justification of defaults or assumptions;
- Related research recommendations;
- Contentious issues and extent of scientific consensus;
- Effect of reasonable alternative assumptions on conclusions and estimates;
- Highlights of reasonable plausible ranges;
- Reasonable alternative models; and
- Perspectives through analogy.

The risk manager can then use the derived risk to make public health decisions.

An effective risk characterization should 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 should feature values such as transparency in the decision-making process; clarity in communicating with the scientific community 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.

6.2. *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 risk assessor should 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 should 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, data such as the shapes and slopes of the dose-response curves for the various endpoints, 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 endpoints 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 in order 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.

6.3. *Quality of the Database 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.

A health risk assessment is only as good as its component parts, i.e., 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.

6.4. *Descriptors of Neurotoxicity Risk*

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

6.4.1. 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.

6.4.2. 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 exposure limit, what would be the resulting risk for neurotoxicity above the RfD or RfC?

6.4.3. 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 in order not to compound a substantial number of high-end values for variables if a "reasonable" exposure estimate is to be achieved.

6.4.4. 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, or children. The aged population is considered to be at particular risk because of the limited ability of the nervous system to regenerate or compensate to neurotoxic insult.

In general, not enough is understood about the mechanisms of toxicity to identify sensitive subgroups for all agents, although factors such as nutrition (e.g., vitamin B), personal habits (e.g., smoking, alcohol consumption, illicit drug abuse), or preexisting disease (e.g., diabetes, neurological diseases, sexually transmitted diseases, polymorphisms for certain metabolic enzymes) may predispose some individuals to be more sensitive to the neurotoxic effects of specific agents. Gender-related differences in response to neurotoxicants have been noted, but these appear to be related to gender-dependent toxicodynamic or toxicokinetic factors.

In general, it is assumed that an uncertainty factor of 10 for intrapopulation variability will be be able to accommodate differences in sensitivity among various subpopulations, including children and the elderly. However, in cases where it can be demonstrated that a factor of 10 does not afford adequate protection, another uncertainty factor may be considered in conducting the risk assessment.

6.4.5. 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 database, 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 database 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 should be taken into account in establishing them have been addressed here.

If the MOE is equal to or more than the uncertainty factor multiplied by 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.

6.5. *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 should be made on a case-by-case basis. These Guidelines are not intended to give guidance on the nonscientific aspects of risk management decisions.

6.6. *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, determine the effects of recurrent exposures over prolonged periods of time, and address the synergistic or antagonistic effects of mixed exposures 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. Additional research is needed to determine the most appropriate dose-response approach to be used in neurotoxicity risk assessments.

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Part B: Response to Science Advisory Board and Public Comments

1. Introduction

A notice of availability for public comments of these Guidelines was published in the Federal Register in October 1995. Twenty-five responses were received. These Guidelines were presented to the Environmental Health Committee of the Science Advisory Board (SAB) on July 18, 1996. The report of the SAB was provided to the Agency in April 1997. The SAB and public comments were diverse and represented varying perspectives. Many of the comments were favorable and expressed agreement with positions taken in the proposed Guidelines. Some comments addressed items that were more pertinent to testing guidance than risk assessment guidance or were otherwise beyond the scope of these Guidelines. Some of the comments concerned generic points that were not specific to neurotoxicity issues. Others addressed topics that have not been developed sufficiently and should be viewed as research issues. There were conflicting views about the need to provide additional detailed guidance about decision making in the evaluation process as opposed to promoting extensive use of scientific judgment. Many public comments provided specific suggestions for clarification of details and corrections of factual material in the Guidelines.

2. Response to Science Advisory Board Comments

The SAB found the Guidelines "...to be quite successful, and, all things considered, well suited to its intended task." However, recommendations were made to improve specific areas.

The SAB recommended that EPA keep hazard identification as an identifiable qualitative step in the risk assessment process and that steps should be taken to decouple the qualitative step of hazard identification from the more quantitatively rigorous steps of exposure evaluation and dose-response assessment. These Guidelines now include a hazard characterization step that clearly describes a qualitative evaluation of hazard within the context of the dose, route, timing and duration of exposure. This step is clearly differentiated from the quantitative dose-response analysis, which describes approaches for determining a RfD or RfC.

The SAB supported the presumption that what appears to be reversible neurotoxicity, especially when arising from gestational or neonatal exposure and observed before adulthood, should not be dismissed as of little practical consequence. They may be indices of silent toxicity that emerge later in life or may suggest more robust and enduring responses in aged individuals. These Guidelines explain the concept of functional reserve and advise caution in instances where reversibility is seen and

in cases where exposure to a chemical may result in delayed-onset neurotoxicity. These Guidelines also indicate that reversibility may vary with the region of the nervous system damaged, the neurotoxic agent involved, and organismic factors such as age.

The SAB restated previous positions concerning cholinesterase-inhibiting chemicals. Agent-induced clinical signs of cholinergic dysfunction could be used to evaluate dose-response and dose-effect relationships and define the presence and absence of given effects in risk assessment. The SAB also indicated that inhibition of RBC and plasma cholinesterase activity could serve as a biomarker of exposure to cholinesterase-inhibiting agents and thereby corroborate observations concerning the presence of clinical effects associated with cholinesterase inhibition. The SAB also indicated that reduced brain cholinesterase activity should be assessed in the context of the biological consequences of the reduction. These Guidelines indicate that inhibition of cholinesterase in the nervous system reduces the organism's level of "reserve" cholinesterase and, therefore, limits the subsequent ability to respond successfully to additional exposures and that prolonged inhibition could lead to adverse functional changes associated with compensatory neurochemical mechanisms. In general, an attempt was made to coordinate these Guidelines with the views of a recently convened Scientific Advisory Panel regarding the risk assessment of cholinesterase-inhibiting pesticides (Office of Pesticide Programs, Science Policy on the Use of Cholinesterase Inhibition for Risk Assessments of Organophosphate and Carbamate Pesticides, 1997).

The SAB indicated that the Guidelines were inclusive of the major neurotoxicity endpoints of concern. No additional neurochemical, neurophysiological, or structural endpoints were suggested. Comments indicated that there was no need to consider endocrine disruptors differently from other potential neurotoxic agents.

The SAB found that the descriptions of the endpoints used in human and animal neurotoxicological assessments were thorough and well documented. Several sections, particularly concerning some of the neurochemical and neurobehavioral measures, were corrected for factual errors or supported with more detailed descriptions.

The SAB recommended that the use of the threshold assumption should occur after an evaluation of likely biological mechanisms and available data to provide evidence that linear responses would be expected. A strict threshold is not always clear in the human population because of the wide variation in background levels for some functions. Cumulative neurotoxicological effects might also alter the response of some individuals within a special population, which might allow the Agency to characterize the risk to the sensitive population. Although the SAB did not disagree with the Guidelines'

assumption of a threshold as a default for neurotoxic effects, it was suggested that the term "nonlinear dose-response curve for most neurotoxicants" be substituted for the term "threshold." The Neurotoxicity Risk Assessment Guidelines have been amended to harmonize their treatment of the issue of threshold with the presentation and position taken with other guidelines.

The SAB also recommended that the topic of susceptible populations be expanded to include the elderly and other groups. The elderly could be at increased risk of toxic effects for a number of reasons, including a decline in the reserve capacity with aging, changes in the ability to detoxify or excrete xenobiotics with age, and the potential to interact with medicines or other compounds that could synergize interactions with toxic chemicals. The SAB also indicated that other populations should be considered, including those with chronic and debilitating conditions, groups of workers with potential exposure to chemicals that may be neurotoxic, individuals with genetic polymorphisms that could affect responsiveness to certain neurotoxicants, and individuals that may experience differential exposure because of their proximity to chemicals in the environment or diet. The Guidelines have been modified to emphasize the possible presence of all of these susceptible populations. When specific information on differential risk is not available, the Agency will continue to apply a default uncertainty factor to account for potential differences in susceptibility.

The SAB recommended that the benchmark dose (BMD) was not ready for immediate incorporation into adjustment-factor-based safety assessment or to serve as a substitute or replacement for the more familiar NOAEL or LOAEL. The SAB also recommended that research and development on the BMD should be aggressively encouraged and actively supported. The BMD could be a replacement for the NOAEL or LOAEL after the appropriate research has been conducted.

3. Response to Public Comments

In addition to numerous supportive statements, several issues were indicated, although each issue was raised by only a few commentators. The public comment supported the SAB recommendation that there was no clear consensus concerning replacing the NOAEL approach with the BMD to calculate RfDs and RfCs for neurotoxicity endpoints. There was also support for ensuring that dose-response and other experimental design information be considered in interpreting the results of hazard identification studies before proceeding to quantitative dose-response analysis. Public comment also supported the position that reversibility cannot be ignored in neurotoxicity risk assessment and that the risk assessor should exert caution in interpreting reversible effects, especially where an apparent transient effect is cited to support evidence for relatively benign effects. The public

comment also supported the use of clinical signs in the risk assessment of cholinesterase-inhibiting compounds and the finding that inhibition of brain cholinesterase was an adverse effect. The Guidelines emphasize the importance of brain cholinesterase inhibition, particularly in cases of repeated exposure. The public comment agreed with the SAB that RBC and plasma cholinesterase activity are biomarkers of exposure. It was recommended that the Guidelines incorporate additional information addressing the neuroendocrine system as a potential target site, and a section has been added that defines the vulnerable components of the neuroendocrine system and the behavioral, hormonal, and physiological endpoints that may be indicative of a direct or indirect effect on the neuroendocrine system.

Public comment strongly endorsed the default assumption that there is a threshold for neurotoxic effects. The Guidelines, however, reflect the argument of the SAB that the term "nonlinear dose-response curve for most neurotoxicants" be substituted for "threshold" in order to be consistent with the presentation and positions taken by other risk assessment guidelines.

The public comments made a number of recommendations to improve the Guidelines with regard to consistency of language between text and tables, improve the clarity of some of the tables, and improve the description of some of the endpoints used in animal studies. A number of factual errors were corrected, including the description of the blood-brain barrier and the degree of inhibition of neurotoxic esterase associated with organophosphate-induced delayed-onset neuropathy. Therefore, a number of changes have been made in the Guidelines to clarify and correct specific passages, but every effort was made to maintain the original intent concerning the use and interpretation of results from various neurotoxicological endpoints. Finally, the public comment agreed with the SAB that factors such as nutrition, personal habits, age, or preexisting disease may predispose some individuals to be differentially sensitive to neurotoxic chemicals. The risk characterization section has been expanded to reflect these potentially sensitive subpopulations.