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Final Report: Principles of Neurotoxicity Risk Assessment; Notice

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ENVIRONMENTAL PROTECTION AGENCY

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Final Report: Principles of Neurotoxicity Risk Assessment

AGENCY: U.S. Environmental Protection Agency.

ACTION: Final Document.

SUMMARY: The U.S. Environmental Protection Agency is publishing a document entitled *Final Report: Principles of Neurotoxicity Risk Assessment*, which was prepared by the Working Party on Neurotoxicology under the auspices of the Subcommittee on Risk Assessment of the Federal Coordinating Council for Science, Engineering, and Technology (FCCSET). The purpose of this report is to articulate a view of neurotoxicology that scientists generally hold in common today and to draw on this understanding to generate a series of general principles that can be used to establish guidelines for assessing neurotoxicity risk. It is not the intent of this report to provide specific directives for how neurotoxicity risk assessment should be performed. The intent of this document is to provide the scientific basis for the development of a cogent strategy for neurotoxicity risk assessment.

SUPPLEMENTARY INFORMATION: This document is the result of the combined efforts of senior scientists of 13 Federal agencies comprising the ad hoc Interagency Committee on Neurotoxicology, including the Agency for Toxic Substances and Disease Registry, Center for Food Safety and Applied Nutrition, Center for Biologics Evaluation and Research, Center for Drug Evaluation and Research, Consumer Product Safety Commission, Department of Agriculture, Department of Defense, Environmental Protection Agency, National Center for Toxicological Research, National Institutes of Health, National Institute for Occupational Safety and Health, and National Toxicology Program. Discussions were held under the auspices of the Working Party on Neurotoxicology of the Subcommittee on Risk Assessment of the Federal Coordinating Council for Science, Engineering, and Technology. The draft report, a product of the Working Party on Neurotoxicology, contains six chapters: an introduction, an overview of the discipline of neurotoxicology, a review of methods for assessing human neurotoxicity, a review of methods for assessing animal neurotoxicity, an overview of principles of neurotoxicity risk assessment, and a general summary.

The draft report was prepared in view of the decision-making processes currently used by many regulatory agencies relating to neurotoxicity risk assessment. It is intended that the principles reviewed in this document will serve as the basis for consistent regulatory neurotoxicity guidelines to be used by Federal agencies to meet their respective legislative mandates. This document is not meant to be used to perform risk assessment nor does it recommend one approach or strategy. The document reviews the science of neurotoxicology and attempts to formulate general assumptions and principles that could lead to such approaches or strategies.

The draft report has undergone interagency review under the auspices of the Subcommittee on Risk Assessment of FCCSET. Public comments received were used in the preparation of the final report by the Working Party on Neurotoxicology.

Dated: August 9, 1994.

Ken Sexton,

Director, Office of Health Research.

Final Report: Principles of Neurotoxicology Risk Assessment

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Tables

1. Introduction

1.1. Background

Over the years, agencies and programs have been established to deal with hazardous substances, with a focus on deleterious long-term effects, including noncancer endpoints such as neurotoxicity (Reiter, 1987). Recent evidence indicates that exposure to neurotoxic agents may constitute a significant health problem (WHO, 1986; OTA, 1990; chapter 2). Table 1–1 lists the four Federal regulatory agencies with authority to regulate either exposure to or use of chemicals and that require data reporting on assessment of hazards. Regulatory bodies vary greatly in their mandate to require approval of chemicals prior to entering the marketplace and to regulate subsequent exposure (Fisher, 1980) (Table 1–2). The Occupational Safety and Health Administration (OSHA) cannot require chemical testing by the manufacturer whereas all other agencies can. Only the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) have authority for premarketing testing of chemicals (i.e., FDA for drugs and food additives and EPA for pesticides). EPA can, under some circumstances, require premarket testing of industrial and agricultural chemicals. The Consumer Product Safety Commission (CPSC) regulates a number of consumer products including household chemicals and fabric treatments. Laws administered by CPSC require cautionary labeling on all hazardous household products whether the hazard is based on acute or chronic effects. These laws also provide the authority to ban hazardous products and to ask for data in support of product labeling.

TABLE 1–1.—MAJOR REGULATORY AGENCIES

Agency	Statute and sources covered
Food and Drug Administration (FDA) ..... A unit of the Department of Health and Human Services with authority over the regulation of medical and veterinary drugs; foods and food additives; cosmetics.	Food, Drug, and Cosmetics Act for food additives; color in cosmetics; medical devices; animal drugs of medical and feed additives.
Occupational Safety and Health Administration (OSHA) ..... A unit of the Department of Labor that regulates workplace conditions ..	Occupational Safety and Health Act covers toxic chemicals in the workplace.
Environmental Protection Agency (EPA). Independent agency (i.e., not part of a Cabinet department); administers a number of diverse laws concerned with human health and the environment.	Toxic Substances Control Act requires premanufacture evaluation of all new chemicals (other than foods, food additives, drugs, pesticides, alcohol, tobacco); allows EPA to regulate existing chemical hazards not sufficiently controlled under other laws. Clean Air Act requires regulation of hazardous air pollutants. Federal Water Pollution Control Act governs toxic water pollutants. Safe Drinking Water Act covers drinking water contaminants. Federal Insecticide, Fungicide, and Rodenticide Act covers pesticides. Resource Conservation and Recovery Act covers hazardous wastes. Marine Protection Research and Sanctuaries Act covers ocean dumping.
Consumer Product Safety Commission (CPSC). Regulates a variety of consumer products including household chemicals and fabric treatments.	Federal Hazardous Substances Act covers "toxic" household products. Consumer Product Safety Act covers dangerous consumer products. Poison Prevention Packaging Act covers packaging of dangerous children's products. Lead-Based Paint Poison Prevention Act covers use of lead paint in federally assisted housing.

TABLE 1–2.—AUTHORITIES FOR TOXICITY TESTING

Agency	Law	Coverage	Authorities		
			Premarketing approval	Testing by manufacturer	Reporting of data
FDA	Food, Drug, and Cosmetics Act	Drugs and foods	x	x	x
		Food additives and cosmetics.	x	x	
EPA	Federal Insecticide, Fungicide, and Rodenticide Act.	Pesticides	x	x	x
	Toxic Substances Control Act	Industrial chemicals	[1] x	x	x
	Clean Air Act	Air pollutants			
	Resource Conservation and Recovery Act.	Industrial waste		x	x
OSHA	Occupational Safety and Health Act	Occupational exposure.			x
CPSC	Federal Hazardous Substances Act	Consumer products		x	
	Consumer Product Safety Act	Consumer products			x

[1] Can require testing based on available data.

1.2. Purpose of This Report

The purpose of this document is to: (1) articulate a view of neurotoxicity that scientists generally hold in common today and (2) draw upon this understanding to compose, as was done here by senior scientists from a number of Federal agencies, a series of general principles that can be used to establish general guidelines for assessing neurotoxicity risk. It is not the intent of this report to provide specific directives to agencies with respect to their own approach for neurotoxicity risk assessment. This document is intended to provide the scientific basis for the development of a cogent strategy for neurotoxicology risk assessment as needed by each agency.

Because of present gaps in understanding, the principles contained in this document are based on the best judgment of those involved in writing this document, as well as statements of what is generally accepted as fact. There has been, however, an attempt to distinguish where possible between the different types of information presented.

The principles contained in this document can serve as the basis for consistent regulatory neurotoxicology guidelines that the Federal agencies can tailor to meet the requirements of the legislative acts they are charged to implement. This document should be viewed broadly as part of an ongoing process within the Federal Government to periodically update and review the current scientific understanding and regulatory utility of neurotoxicity risk assessment.

This document is the result of the combined efforts of senior scientists from the following Federal health-related units, operating under the direction of the Office of Science and Technology Policy (OSTP):

Agency for Toxic Substances and Disease Registry (ATSDR)
Center for Biologics Evaluation and Research (CBER), FDA
Center for Drug Evaluation and Research (CDER), FDA
Center for Food Safety and Applied Nutrition (CFSAN), FDA
Consumer Product Safety Commission
Department of Agriculture (USDA)
Department of Defense (DoD)
Environmental Protection Agency
National Center for Toxicological Research (NCTR), FDA
National Institutes of Health (NIH)
National Institute for Occupational Safety and Health
National Toxicology Program (NTP)

1.3. Context of This Report

This document was prepared in light of a decision-making process used by many regulatory agencies pertaining to the assessment of neurotoxicity risks posed by chemical agents. The scientific basis for such assessment can be best understood by examining the decision-making process in some detail.

Risk can be thought of as being composed of two aspects, each of which can be addressed by science, i.e., hazard and exposure assessment. Although other definitions have been used historically, this document conforms to present usage. Hazard generally refers to the toxicity of a substance and is deduced from a wide array of data, including those from epidemiological studies or controlled clinical trials in humans, short- and long-term toxicological studies in animals, and studies of mechanistic information and structure-activity relationships. Exposure generally refers to the amount of a substance with which people come in contact. The risk in a quantitative risk assessment is estimated by considering the results of the exposure and hazard assessments. As either the hazard or exposure approaches zero, the risk also approaches zero.

As a first step in assessing the neurotoxic risk associated with the use of a particular chemical substance, the qualitative evidence that a given chemical substance is likely to be a human neurotoxicant must be evaluated. In this step, as in the whole process, a number of assumptions and approximations must be made in order to deal with inherent limitations found in the existing data bases. Then, estimates of human exposure and distribution of exposures likely to be encountered in the population are made. In the absence of dose-response relationships in humans, one or more methods for estimating the dose-response relationship including doses below those generally used experimentally must also be evaluated. Finally, the exposure assessment is combined with the dose-response relationship to generate an estimate of risk. The various ways in which these steps are conducted and combined and their attendant uncertainties constitute what is generally referred to as "neurotoxicity risk assessment."

Some legislation calls for action in the presence of any risk. Other forms of legislation use the concept of unreasonable risk, defined in some acts as a condition in which the risks outweigh the benefits. A spectrum of regulatory responses, from simply informing the public of a risk through restricted use to a complete ban, may be available to bring the risks and benefits into appropriate balance.

This document does not perform a risk assessment nor does it suggest that one method of neurotoxicology risk assessment is better than another. Rather, it attempts to review the science

of chemical neurotoxicology and develops from this review a set of general principles. It is not a comprehensive review nor a document written for the lay public; this document is a semitechnical review that evaluates the impact of scientific findings of the last decade on general assumptions or principles important to risk assessment. This is based on the belief that elucidation of the basic mechanisms underlying neurotoxicity and the identification of neurotoxic agents and conditions, when coupled to research aimed at identifying and characterizing the problems caused by such agents, should provide the best scientific bases for making sound and reasonable judgments. These overlapping approaches to evaluating the problems of neurotoxicology should form a strong foundation for decision-making.

1.4. Content of This Report

Including the Introduction (chapter 1), this document contains six chapters. Chapter 2 provides an overview of the discipline of neurotoxicology. It is important to understand the scope of the problem as it relates to neurotoxicology, including: (1) Definitions of neurotoxicity and adverse effect, (2) examples of neurotoxicity and incidents of exposure, and (3) Federal response to neurotoxicology. Chapter 2 also discusses the basic principles of toxicology that apply generally to the evaluation of neurotoxicity. Issues such as dose, exposure, target site, and the intended use of the chemical are discussed, as are principles of pharmacodynamics, chemical interactions, and the concept of threshold. Chapter 2 also lays the neurobiological basis for understanding how and where chemicals can affect the nervous system and provides examples of such chemical types. Finally, chapter 2 discusses special considerations for neurotoxicology including the issue of susceptible populations, the blood brain barrier, and the limited capability of the nervous system to repair following chemical insult.

Chapter 3 examines methods for assessing human neurotoxicity. Neurologic evaluations, neuropsychological testing, and applicability of methods used in clinical evaluations and case studies are discussed in this chapter. Epidemiologic study designs, endpoints, and methods are also discussed, as well as problems of causal inference and applications and limitations of epidemiologic and field study methods for risk assessment. Chapter 3 also describes human laboratory exposure studies, including methods for assessing neurobehavioral function, self-report methods for assessing subjective states, and a number of other methodological issues. This chapter also discusses the comparability of human and animal laboratory methods and special considerations in human neurotoxicity assessments.

Chapter 4 assesses methods for evaluating animal neurotoxicity. Discussed in this chapter is the role that animal models play in the assessment of chemicals for neurotoxicity, the validity of animal models, and experimental design considerations in animal neurotoxicological studies. Also included in this chapter is a discussion of tier-testing approaches in chemical evaluations. Specific endpoints used in animal neurotoxicological studies are also discussed, including methods for neurobehavioral, neurophysiological, neuroanatomical, and neurochemical assessments. Developmental neurotoxicology and in vitro neurotoxicology are also described in this chapter.

Chapter 5 of this document discusses principles of neurotoxicity risk assessment. This chapter evaluates the generic assumptions in neurotoxicity risk assessment, ending with a discussion of uncertainty reduction and identification of knowledge gaps.

Chapter 6 is a general summary of the material presented in the first five chapters.

2. Overview of Neurotoxicology

2.1. Scope of the Problem

2.1.1. Introduction

Chemicals are an integral part of our lives, with the capacity to both improve as well as endanger our health. The general population is exposed to chemicals with neurotoxic properties in air, water, foods, cosmetics, household products, and drugs used therapeutically or illicitly. Naturally occurring neurotoxins, such as fish and plant toxins, present other hazards. During the daily life of an ordinary person, there is a multitude of exposures, both voluntary and unintentional, to neuroactive substances. Under conditions of multiple exposures, identifying the substance responsible for an adverse response may be difficult. The EPA's inventory of toxic chemicals is greater than 65,000 and increasing yearly. Concerns have been raised about the toxicological data available for many compounds used commercially (NRC, 1984).

It is not known how many chemicals are neurotoxic to humans. However, estimates have been made for subsets of substances. A large percentage of the more than 500 registered active pesticide ingredients are neurotoxic to varying degrees. Of 588 chemicals listed by the American Conference of Government and Industrial Hygienists (ACGIH), 167 affected the nervous system or behavior (Anger, 1984; CDC, 1986). Using a generally broad definition of neurotoxicity, Anger (1990a) 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 at some level of exposure. Anger (1984) also recognized neurotoxic effects as one of the ten leading workplace disorders. In addition, a number of therapeutic substances, including some anticancer and antiviral agents and abused drugs, can cause adverse or neurotoxicological side effects (OTA, 1990). It has been estimated that there is inadequate toxicological information available for more than three-fourths of the 12,860 chemicals with a production volume of 1 million pounds or more (NRC, 1984). It should be noted, however, that estimates concerning the number of neurotoxicants vary widely. O'Donoghue (1989), for example, reported that of 488 compounds assessed in his chemical evaluation process, only 2.7% had effects on the nervous sytem.

2.1.2. Examples of Neurotoxicity and Incidents of Exposure

There is a long-standing history associating certain neurological and psychiatric disorders to exposure to a toxin or chemical of an environmental origin (OTA, 1990) (Table 2–1). Lead is one of the earliest examples of a neurotoxic chemical with widespread exposure. This metal is widely distributed with major sources of inorganic lead including industrial emissions, lead-based paints, food, beverages, and the burning of leaded gasolines. Organic lead compounds such as tetraethyl lead have been reported to produce a toxic psychosis (Cassells and Dodds, 1946). If exposure occurs at relatively low levels during development, lead can cause a variety of neurobehavioral problems, learning disorders, and altered mental development (Bellinger et al., 1987; Needleman, 1990). Over the years, Federal Government regulations have been developed to decrease human exposure to lead, and as a goal an intervention level of 10 μg/dcl whole blood has been recommended (CDC, 1991). Lead exposure in the United States has decreased significantly during the last several years.

TABLE 2–1.—HUMAN NEUROTOXIC EXPOSURES

Year(s)	Location	Substance	Comments
370 B.C.	Greece	Lead	Lead toxicity recognized in mining industry.
1st century A.D.	Rome	Lead	Vapors recognized as toxic.
1837	Scotland	Manganese	Chronic manganese poisoning described.
1924	United States (New Jersey).	Tetraethyl lead	Workers suffer neurologic symptoms.
1930	United States (Southeast).	Tri-o-cresylphosphate (TOCP).	Chemical contaminant added to Ginger Jake, an alcoholic beverage substitute; more than 5,000 paralyzed, 20,000 to 100,000 affected.
1930's	Europe	Apiol	Drug containing TOCP causes 60 cases of neuropathy.
1932	United States (California).	Thallium	Contaminated barley laced with thallium sulfate poisons family, causing neurologic symptoms.
1937	South Africa	TOCP	Paralysis develops after use of contaminated cooking oil.
1946	England	Tetraethyl lead	Neurologic effects observed in people cleaning gasoline tanks.
1950's	Japan (Minamata)	Methylmercury	Fish and shellfish contaminated with mercury are ingested, causing neurotoxicity.
1950's	France	Organotin	Medication (Stalinon) containing diethyltin diiodide results in poisoning.
1950's	Morocco	Manganese	Miners suffer chronic manganese intoxication.
1950's	Guam	Cycad	Ingestion of plants associated with amyortrophic lateral sclerosis and Parkinson-like syndrome.
1956	Turkey	Hexachlorobenzene	Hexachlorobenzene causes poisoning.
1956	Japan	Clioquinol	Drug causes neuropathy.
1959	Morocco	TOCP	Cooking oil contaminated with lubricating oil causes poisoning.
1960	Iraq	Methylmercury	Mercury-treated seed grain causes neurotoxicity.
1964	Japan	Methylmercury	Methylmercury neurotoxicity.
1968	Japan	PCBs	Polychlorinated biphenyls are leaked into rice oil, causing neurotoxicity.
1969	Japan	n-Hexane	Neuropathy due to n-hexane exposure.
1969	United States (New Mexico).	Methylmercury	Fungicide-treated grain results in alkyl mercury poisoning.
1971	United States	Hexachlorophene	Hexachlorophene-containing disinfectant is found to be toxic to nervous system.
1971	Iraq	Methylmercury	Methylmercury used as fungicide to treat seed grain causes poisoning.
1972	France	Hexachlorophene	Hexachlorophene poisoning of children.
1973	United States (Ohio)	Methyl n-butylketone	Fabric production plant employees exposed to MnBK solvent suffer polyneuropathy.
1974–1975	United States (Virginia)	Chlordecone (Keptone)	Chemical plant employees exposed to insecticide suffer severe neurologic problems.
1976	United States (Texas)	Leptophos (Phosvel)	At least nine employees suffer serious neurologic problems after exposure to insecticide.
1977	United States (California).	Dichloropropene (Telone II).	People hospitalized after exposure to pesticide.
1979–1980	United States (Texas)	2-t-Butylazo-2-hydroxy-5-methylhexane (BHMH) (Lucel-7).	Employees of manufacturing plant experience serious neurologic problems.
1980's	United States	Methylphenyltetrahydropyridine (MPTP).	Impurity in synthesis of illicit drug causes Parkinson's disease-like effects.
1981	Spain	Toxic oil	People ingesting toxic substance in oil suffer severe neuropathy.
1983–84	United States	Vitamin B_6	Excessive intake, causes sensory neuropathy, numbness, parathesia, and motor dysfunction.
1985	United States and Canada.	Aldicarb	People experience neuromuscular deficits after ingestion of contaminated melons.
1987	Canada	Domoic acid	Ingestion of mussels contaminated with domoic acid causes illnesses.
1988	India	TOCP	Ingestion of adulterated rapeseed oil cause polyneuritis.
1989	United States	L-tryptophan-containing products.	Ingestion of a chemical contaminant associated with the manufacture of L-tryptophan results in eosinophilia-myalgia syndrome.
1991	Nigeria	Scopoletin	Natural component of gari caused neuropathy associated with optic atrophy and ataxia.

Mercury compounds are potent neurotoxic substances and have caused a number of human poisonings, with symptoms of vision, speech, and coordination impairments (Chang, 1980). Erethism, a syndrome with such neurologic features as tremor and behavioral symptoms as anxiety, irritability, and pathologic shyness, is seen in people exposed to elemental mercury (Bidstrup, 1964). One major incidence of human exposure occurred in the mid-1950's when a chemical plant near Minamata Bay, Japan, discharged mercury as part of waste sludge. An epidemic of mercury poisoning developed when the local inhabitants consumed contaminated fish and shellfish. Congenitally affected children displayed a progressive neurological disturbance resembling cerebral palsy and manifested other neurological problems as well. In 1971, an epidemic occurred in Iraq from methylmercury used as a fungicide to treat grain (OTA, 1990).

Manganese is used in metal alloys and has been proposed to replace lead in gasoline. It is an essential dietary substance for normal body functioning yet parenteral exposure to manganese can be neurotoxic, producing a dyskinetic motor syndrome similar to Parkinson's disease (Cook et al., 1974).

Exposed miners in several countries have suffered from "manganese madness" characterized by hallucinations, emotional instability, and numerous neurological problems. Long-term manganese toxicity produces muscle rigidity and staggering gait similar to that seen in patients with Parkinson's disease (Politis et al., 1980).

A Parkinsonian-like syndrome was also observed in people who accidentally ingested 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Langston et al., 1983). MPTP was a byproduct of a meperidine derivative sold illicitly as "synthetic heroin."

Organic solvents are encountered frequently in occupational settings. Most solvents are volatile, i.e., they can be converted from a liquid to a gaseous state and readily inhaled by the worker. They are also lipid soluble and readily accumulate in the fat deposits of the exposed organism. An example of a solvent exposure in humans is carbon disulfide. Workers exposed to high levels of this solvent were found to have an increased frequency of depression and suicide (Seppalainen and Haltia, 1980). Furthermore, repeated exposure to organic solvents is suspected of producing chronic encephalopathy. Workers exposed to methyl-n-butyl ketone, a dye solvent and cleaning agent, displayed peripheral nervous system neuropathy involving degeneration of nerve fibers (Spencer and Schaumburg, 1980). Solvents including ether, ketones, alcohols, and various combinations are commonly used in glues, cements, and paints and when inhaled can be neurotoxic. Repeated abuse of such solvents can lead to permanent neurological effects due to severe and permanent loss of nerve cells (OTA, 1990).

Pesticides are one of the most commonly encountered classes of neurotoxic substances. These can include insecticides (used to control insects), fungicides (for blight and mildew), rodenticides (for rodents such as rats, mice, and gophers), and herbicides (to control weeds). Active ingredients are combined with so-called inert substances to make thousands of different pesticide formulations. Workers who are overexposed to pesticides may display obvious signs of poisoning, including tremors, weakness, ataxia, visual disturbances, and short-term memory loss (Ecobichon and Joy, 1982). Chlordecone exposure results in nervousness and tremors (Cannon et al., 1978). The organophosphorous insecticides have neurotoxic properties and account for approximately 40 percent of registered pesticides. A delayed neurotoxicity can be seen as a result of exposure to certain organophosphate pesticides, producing irreversible loss of motor function and an associated neuropathology (Ecobichon and Joy, 1982). Organophosphate and carbamate insecticides are known to interfere with a specific enzyme, acetylcholinesterase (AChE) (Davis and Richardson, 1980). Paralysis has also been reported following consumption of nonpesticide organophosphate products such as tri-o-cresylphosphate (TOCP).

Neurotoxicities in humans, domestic livestock, and poultry associated with fungal toxins (mycotoxins) have been well documented (Kurata, 1990; Aibara, 1986; Wyllie and Morehouse, 1978). Mycotoxins not only have a negative economic effect on animal production, but they also represent a definite threat to human health. Mycotoxins occur in forages, field crops, and grains used for livestock; they also are incorporated into cereals, grains, and grain-based products used for human consumption. Therefore, human exposure may occur either through direct consumption of these products or secondarily through consumption of meat, milk, or eggs. An example of human exposure to fungal toxins is *Claviceps purpurea*- or *C. paspali*-infected wheat, barley, and oats used for bread and as a dietary supplement for livestock. These fungal toxins are notorious for producing the gangrenous and convulsive forms of the disease known as "ergotism" (Bove, 1970). These fungi are in the family *Clavicipitaceae* and produce a group of compounds known as ergot alkaloids, which have neurotropic, uterotonic, and vasoconstrictive activities. They may act as dopamine agonists or serotonin antagonists, and also block alpha-adrenergic receptors. Since there are numerous naturally occurring ergot alkaloids, this represents only part of their pharmacopoeia (Berde and Schield, 1978). These alkaloids are highly toxic and cause both acute and chronic poisonings. Although guidelines now limit the amount of *Claviceps*-contaminated, or "ergot"-contaminated, grains, these compounds may enter human food sources through secondary mechanisms. Other fungi associated with ergot-like syndromes in livestock include *Acremonium lolii* (Gallagher et al., 1984) and *A. coenophialum* (Thompson and Porter, 1990).

Cyclopiazonic acid (CPA) is an indole tetramic acid produced by *Aspergillus flavus, A. oryzae, Penicillium cyclopium*, and *P. camemberti*. This mycotoxin is suspected of causing "kodua poisoning" in humans who consumed kodo millet seed in India (Rao and Husain, 1985). *Fusarium moniliforme* is a common fungal infection in corn (Bacon et al., 1992) and directly related to neurotoxic syndrome in horses known as equine leukoencephalomalaisia (ELEM).

Natural plant toxins also represent a health risk to both livestock and humans. Movement toward limited uses of herbicides, fungicides, and no-till agricultural practices increases the possibility of noxious weeds and weed seeds being incorporated into food products. Ergot alkaloids also are produced by morning glories *(Ipomea violacea)* and may be incorporated into soybeans, corn, peas, etc., during harvest. Export regulations limit morning glory-contaminated soybeans because of the hallucinogenic and other effects produced by ergot alkaloids. Jimson weed *(Datura stramonium)*, another weed incorporated into agricultural commodities, produced scopolamine, hyocyamine, and stropine, all of which have parasympatholytic (anticholinergic) activities.

Recently, an outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid, an excitotoxin, was reported (Perl et al., 1990).

2.1.3. Federal Response

In the United States, several agencies, including EPA, FDA, OSHA, CPSC, NIOSH, and ATSDR, have been given the mandate to regulate or evaluate public exposure to toxic chemicals (Tilson, 1989).

2.1.3.1. Food and Drug Administration.

The FDA has the authority to regulate the use of food and color additives as well as to determine whether or not various foods are unsafe for human consumption because of adulteration by environmental contaminants. The manufacturer must supply adequate data to establish the safety of the food additives. Before marketing approval, the potential toxicity of proposed food and color additives is established in a battery of animal toxicity studies. During all of these studies, clinical signs of toxicity, including abnormal behavior, are monitored and abnormalities recorded. At the termination of these studies, tissues from all organs, including the brain, are sectioned and evaluated for both gross and histopathological changes, in addition to being evaluated for their clinical chemistry and hematology. None of the routinely required tests is specifically designed to assess neurotoxicity. If neurotoxic effects are detected during any of the standard

toxicity tests, however, they must be reported. Specific neurotoxicity testing may then be required. The FDA is currently revising its guidelines for the safety assessment of direct food and color additives to include neurotoxicity as a routine element in toxicological testing.

The FDA is also authorized to regulate substances in food considered to be poisonous or deleterious. Unavoidable environmental contaminants in food fall into this category. The FDA determines a level at which the risks from realistically possible intakes are negligible or acceptable. Based on this risk assessment, an action level or tolerance is established. Once the action level or tolerance is formally established, the FDA may take appropriate action to restrict adulterated food from the market if these standards are exceeded.

The FDA is responsible for assessing the toxicity of human therapeutic products. Many products have been shown to produce adverse effects on the nervous system at standard therapeutic doses as well as at higher doses. Before marketing approval is given, the toxicity of potential new products is assessed. A battery of animal toxicity study parameters relevant to the nervous system, including gross behavioral observation and gross and histopathological examination of the nervous tissue, are evaluated. This information is used to help guide the surveillance of human subjects for adverse effects that are assessed during clinical trials.

2.1.3.2. Occupational Safety and Health Administration.

OSHA has been given the responsibility to ensure that the working environment is a safe and healthy place of employment. In the early 1970's, OSHA adopted the existing Federal standards, most of which were developed under the Walsh-Healy Act (including the 1968 ACGIH Threshold Limit Values), and approximately 20 consensus standards of the American National Standards Institute (ANSI) as Permissible Exposure Limits (PELs). Of the 393 remaining original PELs, 145 were set in part to protect the individual from neurotoxic effects.

Since the adoption of the initial standards, OSHA has issued new or revised health standards or work practices for 23 substances. Of these, the one concerning lead was based in part on nervous system effects. Four other compounds, inorganic arsenic, acrylonitrile, ethylene oxide, and 1,2-dibromo-3-chloropropane, were cited as causing various disturbances in the nervous system, but the standards for these were based primarily on carcinogenic effects.

In 1989, OSHA updated 428 exposure limits for air contaminants. Of these, 25 substances were categorized by OSHA as "substances for which limits are based on avoidance of neuropathic effects." In addition, 24 substances were included in the category "substances for which limits are based on avoidance of narcosis." However, OSHA stated that the categorization was intended as a tool to manage the large number of substances being regulated and not to imply that the category selected identified the most sensitive or the exclusive adverse health effects of that substance.

2.1.3.3. National Institute for Occupational Safety and Health.

The Occupational Safety and Health Act established NIOSH as a Public Health Service (PHS) agency to develop and recommend criteria for prevention of disease and hazardous conditions in the workplace. NIOSH also performs research on occupational health issues and conducts worksite evaluations of suspected hazards. OSHA and the Mine Safety and Health Administration (MSHA) use NIOSH recommendations in the promulgation of new or revised health and safety standards.

In establishing recommended exposure limits (RELs) for chemicals, NIOSH examines all relevant scientific information about a given compound and attempts to identify exposure limits that will protect all workers from adverse effects. NIOSH has recommended standards for approximately 644 chemicals or classes of chemicals. For 214 (33 percent) of these, neurotoxicity was cited as a health effect considered when formulating the REL (NIOSH, 1992).

2.1.3.4. Environmental Protection Agency.

The Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) provide the legislative authority for EPA to require data collection for premarket approval of chemicals. Under section 5 of TSCA, after a manufacturer has notified EPA of its plans to produce a "new" chemical that has not yet been listed on the inventory, EPA has the responsibility to assess possible health hazards. Potential neurotoxicity is included in the health hazards assessment. If there are reasons to suspect neurotoxicologic effects (e.g., from structure-activity analysis, information in the literature, or data submitted by the manufacturer), EPA can issue a test rule requiring the manufacturer to develop data directed toward these effects. At the same time, EPA can restrict the chemical or prohibit it entirely from entering commerce until the required data are submitted and reviewed. In addition, for "old" chemicals (under section 4 of TSCA), if EPA suspects neurotoxicity, a test rule would be the mechanism used for obtaining the data. Many other statutes provide authority to regulate chemicals through the setting of standards, including the Clean Air Act, Clean Water Act, and Safe Drinking Water Act.

Neurotoxicity is recognized as a health effect of concern under FIFRA, and there are neurotoxicity testing requirements for premarketing submission of data to EPA for registration of a pesticide under FIFRA.

2.1.3.5. Consumer Product Safety Commission.

The CPSC is an independent Federal regulatory agency with jurisdiction over most consumer products. Most chemical hazards are regulated under the Federal Hazardous Substances Act (FHSA) administered by CPSC. The FHSA requires appropriate cautionary labeling on all hazardous household products (hazards include chronic toxicity such as neurotoxicity). While the FHSA does not require premarket registration, a manufacturer is required to assess the hazards of a product prior to marketing and assure that it is labeled with all necessary cautionary information. The FHSA also bans children's products that are hazardous and provides the CPSC with the authority to ban other hazardous products.

2.1.3.6. Agency for Toxic Substances and Disease Registry.

ATSDR has a mission to prevent or mitigate adverse effects to both human health and the quality of life resulting from exposure to hazardous substances in the environment. The ATSDR publishes a National Priority List (NPL) of hazardous substances that are found at National Priority Waste Sites. The order of priority is based on an algorithm, taking into consideration frequency with which substances are found at NPL sites, toxicity, and potential for human exposure; this list is reranked on a yearly basis. So far, 129 toxicological profiles have been developed for the priority hazardous substances, and 92 substances have a profile with a neurological health effect endpoint (HAZDAT, 1992). Neurotoxicity has been selected by the ATSDR to be one of the seven high-

priority health conditions resulting from exposure to environmental toxicants.

2.2. Basic Toxicological Considerations for Neurotoxicity

2.2.1. Basic Toxicological Principles

A chemical must enter the body, reach the tissue target site(s), and be maintained at a sufficient concentration for a period of time in order for an adverse effect to occur. Not all chemicals have the same level of toxicity; some may be very toxic in small amounts while others may have little effect even at extremely high amounts. Thus, the dose-response relationship is a major concept in determining the toxicity of a specific substance. Other factors in determining toxicity include the physical and chemical properties of the substance, the route and level of exposure, the susceptibility of the target tissue, and the health, gender, and age of the exposed individual.

Once the toxic substance has entered the body, usually through the lungs (inhalation), the skin (absorption), or the gastrointestinal tract (ingestion), it is partitioned into various body tissues where it can act on its target sites. The substance is eliminated from the bloodstream by the process of accumulation into the various sites in the body, with the liver and kidney being major sites of accumulation of toxic substances. This is thought to be associated with these organs' large blood capacity and major role in elimination of substances from the body. Lipophilic chemicals accumulate in lipid-rich areas of the body and present a significant potential problem for the nervous system. The nervous system is unique in its high percentage content of lipid (50 percent of dry weight) and may be particularly vulnerable to such chemicals. The site or sites of accumulation for a specific toxic substance may or may not be the primary sites of action. Examples include two known neurotoxicants, carbon monoxide in the red blood cells and lead in the bone. It must be noted that some substances are not distributed throughout the body, partially as a function of their insolubility, polarity, or molecular weight.

The effect that a substance has will generally depend on the body burden or level in the tissue and duration of exposure. The time course of the levels is determined by several factors, including the amount at time of exposure, duration of exposure, and metabolic fate of the chemical. The study of such metabolic processes, pharmacokinetics, has demonstrated complex patterns in the absorption, distribution, possible biotransformation, and elimination of various substances (Klaassen, 1980).

Many substances are removed by the kidney and excreted through the urine. The liver can detoxify substances like organic lead, which are excreted from the liver into the bile and then the small intestines, bypassing the blood and kidney. Lipophilic toxic substances are primarily removed from the body through feces and bile, and water-soluble metabolites are removed in the urine, through the skin, and through expiration into the air.

Biotransformation is a biochemical process that converts a substance into a different chemical compound, allowing it to be excreted more easily. Substances are more easily removed if they are biotransformed into a more hydrophilic compound. Biotransformation can either aid in the detoxification of a substance or produce a more toxic metabolite. Therefore, the original substance may not be the substance that is producing the toxicity on the nervous system or any other system. Thus, several factors must be taken into consideration when evaluating the potential neurotoxicity of a chemical. They include the pharmacokinetics of the parent compound, the target tissue concentrations of the parent chemical or its bioactivated proximate toxicant, the uptake kinetics of the parent chemical or metabolite into the cell and/or membrane interactions, and the interaction of the chemical or metabolite with presumed receptor sites.

2.2.2. Basic Neurotoxicological Principles

Neurotoxicity can be manifest as a structural or functional adverse response of the nervous system to a chemical, biological, or physical agent (Tilson, 1990b). It is a function of both the property of the agent and a property of the nervous system itself. Neurotoxicity refers broadly to the adverse neural responses following exposure to chemical or physical agents (e.g., radiation) (Tilson, 1990b). Adverse effects include any change that diminishes the ability to survive, reproduce, or adapt to the environment. Neuroactive substances may also impair health indirectly by altering behavior in such a way that safety is decreased in the performance of numerous activities. Toxicity can occur at any time in the life cycle, from conception through senescence, and its manifestations can change with age. The range of responses can vary from temporary responses following acute exposures to delayed responses following acute or chronic exposure to persistent responses. Neurotoxicity may or may not be reversible following cessation of exposure. The responses may be graded from transient to fatal and there may be different responses to the same neurotoxicant at different dose levels but similar responses to exposure to different agents. Displays of a neurotoxic response may be progressive in nature, with small deficits occurring early in exposure and developing to become more severe over time. Expression of neurotoxicity can encompass multiple levels of organization and complexity including structural, biochemical, physiological, and behavioral measurements.

Caution must be exercised in labeling a substance neurotoxic. The intended use and effect of the chemical, the dose, exposure scenario and whether or not the chemical acts directly or indirectly on the nervous system, must be taken into consideration. A substance that may be neurotoxic at a high concentration may be safe and beneficial at a lower concentration. For example, vitamin A, vitamin B6, are required in the diet in trace amounts, yet all result in neurotoxicity when consumed in large quantities. Pharmaceutical agents may also have adverse effects at high dose levels or where the beneficial effects outweigh the adverse side effects. For example, antipsychotic drugs have allowed many people suffering from schizophrenia to lead relatively normal lives; however, chronic prescribed use of some of these drugs may result in severe tardive dyskinesia characterized by involuntary movements of the face, tongue, and limbs. Other examples include toxic neuropathies induced by chemotherapeutic agents like cis-platinum, toxic anticholinergic effects of high doses of tricyclic antidepressants, disabling movement disorders in patients treated with anti-Parkinsonian agents and major tranquilizers, and hearing loss and balance disruption triggered by certain antibacterials (Sterman and Schaumburg, 1980). Drugs of abuse such as ethanol also have neurotoxic potential. Opiates such as heroin may lead to dependence, which is considered to be a long-term adverse alteration of nervous system functioning. Simultaneous exposure to drugs or toxic agents may produce toxic interactions either in the environment or occupational settings. For example, exposure to noise and certain antibiotics can exacerbate the loss of hearing function (Boettcher et al., 1987; Lim, 1986; Bhattacharyya and Dayal, 1984).

The nervous system is a highly complex and integrated organ. It is

possible that nonlinear dose-response relationships or a threshold effect could exist for some agents. It has been hypothesized that the nervous system has a reserve capacity that masks subtle damage and any exposure that does not overcome this reserve capacity may not reach the threshold and no observable impairment will be evident (Tilson and Mitchell, 1983). However, the functional reserve may be depleted over time and the manifestations of toxicity may be delayed in relationship to the exposure. The reserve may be depleted by a number of factors including aging, stress, or chronic exposure to an environmental insult, in which case functioning will eventually be impaired and toxicity will become apparent. If a number of events occur simultaneously, the response is progressive in nature, or there is a long latency between exposure and manifestation of toxicity, the identification of a single cause of the functional impairment may not be possible.

2.3. Basic Neurobiological Principles

2.3.1. Structure of the Nervous System

The nervous system is composed of two parts: the central nervous system (CNS) and the peripheral nervous system (PNS) (Spencer and Schaumburg, 1980). Within the nervous system, there exist predominantly two general types of cells—nerve cells (neurons) and glial cells. Neurons have many of the same structures found in every cell of the body; they are unique, however, in that they have axons and dendrites, extensions of the neuron along which nerve impulses travel. The structure of the neuron consists of a cell body, 10 to 100 μm in diameter, containing a nucleus and organelles for the synthesis of various components necessary for the cell's functioning, e.g., proteins and lipids. There are numerous branch patterns of elongated processes, the dendrites, that emanate from the cell body and increase the neuronal surface area available to receive inputs from other sources. Neurons communicate with each other by releasing chemical signals onto specific surface regions, receptors, of the other neuron. The axon is a process specialized for the conduction of nerve impulses away from the cell toward the terminal synapses and eventually toward other cells (neurons, muscle cells, or gland cells).

Neurons are responsible for the reception, integration, transmission, and storage of information (Raine, 1989). Certain nerve cells are specialized to respond to particular stimuli. For example, chemoreceptors in the mouth and nose send information about taste and smell to the brain. Cutaneous receptors in the skin are involved in the sensation of pressure, pain, heat, cold, and touch. In the retina, the rods and cones sense light. In general, the length of the axon is tens to thousands of times greater than the cell body diameter. For example, the cell body whose processes innervate the muscles in the human foot is found in the spinal cord at the level of the middle back. The axons of these cells are more than a meter in length. Many, but not all, axons are surrounded by the layers of membrane from the cytoplasmic process of glial cells. These layers are called myelin sheaths and are composed mostly of lipid. In the PNS, the myelin sheaths are formed by Schwann cells, while in the CNS the sheaths are formed by the oligodendroglia. The myelin sheath formed by one glial cell covers only a short length of the axon. The entire length of the axon is ensheathed in myelin by numerous glial cells. Between adjacent glial sheaths, a very short length of bare axon exists called the node of Ranvier. In unmyelinated axons, a nerve impulse must travel in a continuous fashion down the entire length of the nerve. The presence of myelin accelerates the nerve impulse by up to 100 times by allowing the impulse to jump from one node to the next in a process called "saltatory conduction."

The nerve cells of the PNS are generally found in aggregates called ganglia. The brain and spinal cord make up the CNS and the neurons are segregated into functionally related aggregates called nuclei. They synthesize and secrete neurotransmitters, which are specialized chemical messengers that interact with receptors of other neurons in the communication process. Various nuclei together with the interconnecting bundles of axonal fibers are functionally related to one another to form higher levels of organization called systems. For example, there is the motor system, the visual system, and the limbic system. At the base of the brain, several small nuclei in the hypothalamus form the neuroendocrine system, which plays a critical role in the control of the body's endocrine (hormone-secreting) glands. Nerve cells in the hypothalamus secrete chemical messengers into a short loop of blood vessels that carries the messengers to the pituitary gland which, in turn, releases chemical messengers into the general circulation. These pituitary messengers regulate other glands (e.g., the thymus and the gonads). The entire system maintains a state of optimal physiological function for all of the body's organ systems.

2.3.2. Transport Processes

All types of cells must transport proteins and other molecular components from their site of production near the nucleus to the other sites in the cell (Hammerschlag and Brady, 1989). Neurons are unique in that the neuronal cell body must maintain not only the functions normally associated with its own support, but it must also provide support to its various processes. This support may require transport of material over relatively vast distances. Delivery of necessary substances by intracellular transport down the axon (axonal transport) represents a supply line that is highly vulnerable to interruption by toxic chemicals. In addition, the integrity of the function of the neuronal cell body is often dependent on a supply of trophic factors from the cells that it innervates. These factors are continually supplied to the neural cells by the process of retrograde axonal transport, often as a process of normal exchange between two or more cells. They play a significant factor in the normal growth and maintenance of the neural cells, and a continual supply of certain trophic factors is necessary for cell functioning.

The majority of axonal transport occurs along longitudinally arranged fiber tracks called neurofilaments. This movement along neurofilaments requires energy in the form of oxidative metabolism. Toxicants that interfere with this metabolism or that disrupt the spatial arrangement or production of neurofilaments may block axonal transport and can produce neuropathy (Lowndes and Baker, 1980). This can be seen following exposure to many substances, such as n-hexane and methyl n-butyl ketone as well as the drugs vincristine, vinblastine, and taxol. Acrylamide produces a dying-back axonopathy but by an alternative mechanism involving altered axonal transport.

2.3.3. Ionic Balance

The axonal membrane is semipermeable to positively and negatively charged ions (mostly potassium, sodium, and chloride) within and outside of the axon. There are several enzyme systems that maintain an ionic balance that changes following depolarization of the membrane (Davies, 1968). This is maintained only by the continual active transport of ions across the membrane, which requires an expenditure of energy. The nerve impulse is a traveling

wave of depolarization normally originating from the cell body; however, in sensory neurons it originates at the terminal receptive end of specialized axons (Davies, 1968). The wave is continued by openings in the membrane that allow ions to rush into the axon. This sudden change in the charge across the axon's membrane is the nerve impulse. It is an amplified depolarization that reaches the threshold value and spreads down the axon from one length to another until the next length of membrane reaches the threshold value. It continues in this fashion until it reaches the synaptic terminal regions. There are a number of varieties of membrane channels (e.g., calcium) that rapidly open and close during impulse generation; the common ones are the sodium and potassium channels. They are very small and allow only ions of a certain size to pass. Several classes of drugs (e.g., local anesthetics) and natural toxins (e.g., tetrodotoxin) inhibit nerve impulse conduction by blocking these channels.

2.3.4. Neurotransmission

The terminal branches of the axon end in small enlargements called synaptic "boutons." It is from these boutons that chemical messengers will be released in order to communicate with the target cell at the point of interaction, the synapse (Hammerschlag and Brady, 1989). When the nerve impulse reaches the terminal branches of the axon, it depolarizes the synaptic boutons. This depolarization causes the release of the chemical messengers (neurotransmitters and neuromodulators) stored in vesicles in the axon terminal (Willis and Grossman, 1973). Classical neurotransmitters include serotonin, dopamine, acetylcholine, and norepinephrine and are typically secreted by one neuron into the synaptic cleft where they are on the postsynaptic membrane. Neuropeptides, however, may travel long distances through the bloodstream to receptors on distant nerve cells or in other tissues. Following depolarization, the amount of secretion is dependent on the number of nerve impulses that reach the synaptic bouton, i.e., the degree of depolarization. The chemical messengers diffuse across the synaptic cleft or into the intraneuronal space and bind to receptors on adjacent nerve cells or effector organs, thus triggering biochemical events that lead to electrical excitation or inhibition.

When information is transmitted from nerves to muscle fibers, the point of interaction is called the neuromuscular junction and the interaction leads to contraction or relaxation of the muscle. When the target is a gland cell, the interaction leads to secretion. Synaptic transmission between neurons is slightly more complicated, but still dependent on the opening and closing of ion channels in the membrane. The binding of the messenger to the receptor of the receiving cell can lead to either the excitation or inhibition of the target cell. At an excitatory synapse, the neurotransmitter-receptor interaction leads to an opening in certain ion-specific channels. The charged ions that move through these opened chambers carry a current that serves to depolarize the cell membranes. At inhibitory synapses, the interaction leads to an opening in a different type of ion-specific channel that produces an increase in the level of polarization (hyperpolarization). The sum of all the depolarizing and hyperpolarizing currents determines the transmembrane potential and when a threshold level of depolarization is reached at the axon's initial segment, a nerve impulse is generated and begins to travel down the axon.

The duration of neurotransmitter action is primarily a function of the length of time it remains in the synaptic cleft. This duration is very short due to specialized enzymes that quickly remove the transmitter either by degrading it or by reuptake systems that transport it back into the synaptic bouton. A toxic substance may disrupt this process in several different ways. It is important that the duration of the effect of synaptically released chemical messengers be limited. Some neurotoxicants, e.g., cholinesterase-inhibiting organophosphorous pesticides, inhibit the enzyme (AChE), which serves to terminate the effect of the neurotransmitter (acetylcholine) on its target. The result is an overstimulation of the target cell. Other substances, particularly biological toxins, are able to interact with the receptor molecule and mimic the action of the neurotransmitter. Some toxic substances, like neuroactive pharmaceuticals, may interfere with the synthesis of a particular neurotransmitter, while others may block the neurotransmitter's access to its receptor molecule.

2.4. Types of Effects on the Nervous System

The normal activity of the nervous system can be altered by many toxic substances. A variety of adverse health effects can be seen ranging from impairment of muscular movement to disruption of vision and hearing to memory loss and hallucinations (WHO, 1986; Anger, 1984, 1990). Toxic substances can alter both the structure and the function of cells in the nervous system. Structural alterations include changes in the morphology of the cell and its subcellular structures. In some cases, agents produce neuropathic conditions that resemble naturally occurring neurodegenerative disorders in humans (Calne et al., 1986). Cellular alterations can include the accumulation, proliferation, or rearrangement of structural elements (e.g., intermediate filaments, microtubules) or organelles (mitochondria) as well as the breakdown of cells. By affecting the biochemistry and/or physiology of a cell, a toxic substance can alter the internal environment of any neural cell. Intracellular changes can result from oxygen deprivation (anoxia) because neurons require relatively large quantities of oxygen due to their high metabolic rate.

Many times the response of the nervous system to a toxic substance can be a slow degeneration of the nerve cell body or axon that may result in permanent neuronal damage. Substances can act as a cytotoxicant after having been transported into the nerve terminal. A complete loss of nerve cells can occur following exposure to a number of toxic substances. Sensory nerve cells may be lost following treatment with megavitamin doses of vitamin B6; hippocampal neurons undergo degeneration with trimethyltin and trimethyl lead poisoning; motor nerve cells are affected in cycad toxicity, which has been loosely linked to Guam-ALS-Parkinsonism dementia. Acute carbon monoxide poisoning can produce a delayed, progressive deterioration over a period of weeks of portions of the nervous system that may lead to psychosis and death. Substances such as mercury and lead can cause central nervous system dysfunction. In children, mercury intoxication can cause degeneration of neurons in the cerebellum and can lead to tremors, difficulty in walking, visual impairment, and even blindness. Lead affects the cortex of the immature brain, resulting in mental retardation.

At the cellular level, a substance might interfere with cellular processes like protein synthesis, leading to a reduced production of neurotransmitters and brain dysfunction (Bondy, 1985). Nicotine and some insecticides mimic the effects of the neurotransmitter acetylcholine. Organophosphorous compounds, carbamate insecticides, and nerve gases act by inhibiting AChE, the enzyme that inactivates the neurotransmitter acetylcholine. This results in a buildup

of acetylcholine and can lead to loss of appetite, anxiety, muscle twitching, and paralysis. Amphetamines stimulate the nervous system by releasing and blocking reuptake of the neurotransmitters norepinephrine and dopamine from nerve cells. Cocaine affects the release and reuptake of norepinephrine, dopamine, and serotonin. Both drugs can cause paranoia, hyperactivity, aggression, high blood pressure, and abnormal heart rhythms. Opium-related drugs such as morphine and heroin act at specific opioid receptors in the brain, producing sedation, euphoria, and analgesia. They also tend to slow the heart rate and cause nausea, convulsions, and slow breathing patterns. Other substances can alter the synthesis and release of specific neurotransmitters and activate their receptors in specific neuronal pathways. They may perturb the system by overstimulating receptors, blocking transmitter release and/or inhibiting transmitter degradation, or blocking reuptake of neurotransmitter precursors.

Also at the cellular level, the flow of ions such as calcium, sodium, and potassium across the cell membrane may be changed and the transmission of information between nerve cells altered. A substance may interfere with the ionic balance of a neuron. Organophosphate and carbamate insecticides produce autonomic dysfunction and organochlorine insecticides increase sensorimotor sensitivity, produce tremors and in some cases cause seizures and convulsions (Ecobichon and Joy, 1982). Lindane, DDT, pyrethroids, and trimethyltin also produce convulsions. Conversely, solvents act to raise the threshold for eliciting seizures or act to reduce the severity or duration of the elicited convulsions.

The role of excitatory amino acid (EAA)-mediated synaptic activation is critical for normal function of the CNS. Because endogenous EAA-mediated synaptic transmission is a widespread excitatory system in the brain and is involved in the process of learning and memory, the issue of the effects of endogenous and exogenous EAA-related toxicity has broad implications for both CNS morbidity and mortality in humans. Much of the injury and neuronal death associated with toxicity is mediated by receptors for excitatory amino acids, especially glutamic acid. When applied in sufficient excess from either endogenous or exogenous sources, EAAs have profound neurotoxic effects that can result in the destruction of neurons and, as a consequence, lead to acute phase confusion, seizures, and generalized weakness or to persistent impairments such as memory loss (Choi, 1988).

A final common path in the activation of these receptor classes is an increase in free cytosolic Ca^{++} that can result in the release and activation of intracellular enzymes (which break down the cytoskeleton) and in further release of glutamate, both of which can be cytotoxic (Choi, 1988). Critical to an understanding of the etiopathology associated with at least some of the neurotoxic degeneration may be the link that impaired energy metabolism could have with excitotoxic neuronal death. It is likely that reduced oxidative metabolism results in the partial depolarization of resting membrane potential, the activation of ionotropic membrane receptor/channels, and the influx of Ca^{++} or its release from intracellular stores.

The nervous system is dependent on an extensive system of blood vessels and capillaries to deliver large quantities of oxygen and nutrients as well as to remove toxic waste products. Damage to the capillaries in the brain can lead to the swelling characteristic of encephalopathy. This can be seen following exposure to higher concentrations of lead. Other metals (e.g., cadmium, thallium, and mercury) and organotin (e.g., trimethyltin) cause rupturing of vessels that can also result in encephalopathy.

One large aspect of function that may be affected by neurotoxicants is behavior, which is the product of various sensory, motor, and associative functions of the nervous system. Neurotoxic substances can adversely affect sensory or motor functions, disrupt learning and memory processes, or cause detrimental behavioral effects; however, the underlying mechanisms for these effects have yet to be determined. Although changes may be subtle, the assessment of behavior may serve as a robust means of monitoring the well-being of the organism (Tilson and Cabe, 1978).

2.5. Special Considerations

2.5.1. Susceptible Populations

Everyone is at a certain level of risk of being adversely affected by neurotoxic substances. Individuals of certain age groups, health states, and occupations, however, may be at a greater level of risk. Fetuses, children, the elderly, workers in occupations involving exposure to relatively high levels of toxic chemicals, and persons who abuse drugs are among those in high-risk groups. Neurotoxic substances may exacerbate existing neurological or psychiatric disorders in a population. Although controversial (Waddell, 1993), recent evidence suggests that there may be a subpopulation of people who have become sensitive to chemicals and experience adverse reactions to low-level exposures to environmental chemicals (Bell, et al., 1992). Confounded in all of these groups is the role that nutrition plays in the response of the organism to exposure. Both general nutritional status and specific nutritional deficiencies (for example, protein, iron, and calcium) can significantly influence the response to a toxic substance.

It is widely accepted that during development adverse effects can result from exposure to some chemicals at lower levels than would be necessary for the average adult (Suzuki, 1980). The developing nervous system appears to be differentially sensitive to some kinds of damage (Cushner, 1981; Pearson and Dietrich, 1985; Annau and Eccles, 1986; Hill and Tennyson, 1986; Silbergeld, 1986). During the developmental period, the nervous system is actively growing and establishing intricate cellular networks. Both the blood-brain and blood-nerve barriers that will eventually protect much of the adult brain, spinal cord, and peripheral nerves are incomplete. The protective mechanisms by which the organism deals with toxic substances, such as the detoxification systems, are not fully developed. Exposure to chemicals during development can result in a range of effects. At the highest exposure, effects include death, gross structural abnormalities, or altered growth. Larger populations are generally exposed to more moderate levels resulting in more subtle functional impairments. The qualitative nature of some injuries during development may differ from those seen in the adult, such as changes in tissue volume, misplaced or misoriented neurons, or delays or acceleration of the appearance of functional or structural endpoints (Rodier, 1986). In many cases, the results of early injuries may become evident only as the nervous system matures and ages (Rodier, 1990). There are several instances in which functional alterations have resulted from exposure during the period between conception and sexual maturity (Riley and Vorhees, 1986; Vorhees, 1987).

Early exposure to relatively low levels of lead can result in reduced scores on tests of mental development (Bellinger et al., 1987; Needleman, 1990). Early gestational exposure to neurotoxicants such as cocaine can produce long-term neurobehavioral abnormalities (Anderson-Brown et al., 1990;

Hutchings et al., 1989); heavy alcohol exposure produces craniofacial abnormalities and mental retardation (Jones and Smith, 1973), while moderate levels of alcohol consumption during gestation can delay motor development (Little et al., 1989).

With aging, the level of risk for a number of health-related factors increases; it has been hypothesized that the risk for toxic perturbations to the nervous system also increases with age (Weiss, 1990). It is generally believed that with increasing age comes a decreased ability of the nervous system to respond to adverse events or to compensate for either biological, physical, or toxic effects. At the tissue and cellular level, the aging process can result in nerve cell loss, formation of neurofibrillary tangles (abnormal accumulation of certain filamentous proteins) and neuritic plaques (abnormal clusters of proteins and other substances near synapses). As cells die, the complex neuronal circuitry of the brain becomes impaired. Neurotransmitter concentrations and the enzymes involved in their synthesis may be altered. Some axons can gradually lose their myelin sheath, resulting in a slowed conduction of nerve impulses along the axon. It has been postulated that with age, not only might the nervous system become more susceptible to new insults, but the effects of previous exposures also may become evident, with a diminished capacity for compensation (Weiss, 1990). The increased incidence of multiple drug-taking in the elderly population might also lead to interactions, either drug/drug or drug/chemical, which can adversely affect the nervous system. Nutritionally, the aged experience increased incidences of both general undernutrition and deficits of specific nutrients such as iron or calcium, which might influence the response to toxic substances.

In the geriatric population, the clinical manifestation of neurodegenerative disorders may have a contributing component of past exposures to environmental chemical agents. Calne et al. (1986) hypothesized that various agents contribute to Alzheimer's disease, Parkinson's disease, or amyotrophic lateral sclerosis (ALS, motoneurone disease, or Lou Gehrig's disease) by depleting neuronal reserves to an extent that perturbations become observable in the context of the natural aging process. B-N-methylamino-L-alanine, from the seed of the false sago palm (*Cycas circinalis* L.), has been reported to induce a form of amyotrophic lateral sclerosis (Spencer et al., 1987). Alzheimer-type syndromes have been reported in individuals occupationally exposed to organic solvents or metal vapors (Freed and Kandel, 1988). Severe cognitive dysfunction has been noted in Alzheimer's disease and aluminum intoxication (Yokel et al., 1988).

At any age, preexisting physical as well as mental disorders of the individual may play a significant role in the manifestation of a toxic response following exposure to a potentially toxic substance. Both types of disorders compromise the system in some way so that either the defense mechanisms of the organism are not able to deal with the toxic substance or are not able to repair themselves quickly. In addition to the basic altered biology, for individuals with a physical or mental disorder who are under some form of medical intervention, the combination of therapeutic drugs and toxic substances may have an interactive effect on the nervous system. For example, due to the delicate electrochemical balance of the nervous system, mental disorders may be exacerbated by exposure to a toxic substance.

2.5.2. Blood-Brain and Blood-Nerve Barriers

The bioavailability of a specific chemical to the nervous system is a function of both the target tissue and the chemical. The brain, spinal cord, and peripheral nerves are surrounded by a series of semipermeable tissues referred to as the blood-brain and blood-nerve barriers (Katzman, 1976; Peters et al., 1991). In the central nervous system, the blood-brain barrier is composed of tight junctions formed by endothelial cells and astrocytes. These tight junctions and cellular interactions forming the barrier restrict the free passage of most bloodborne substances. By doing this, they create a finely controlled extracellular environment for the nerve cells. Certain regions of the brain and nerves are directly exposed to chemicals in the blood because the barrier is not present in some areas of the nervous system. For example, it is absent in the circumventricular area, around the dorsal root ganglion in the peripheral nervous system, and around the olfactory nerve, which may allow chemicals to penetrate directly from the nasal region to the frontal cortex.

The existence of these blood-brain and blood-nerve barriers suggests that proper functioning of the nervous system is dependent on control of the substances to which nerve cells are exposed. The term "barrier," however, is somewhat of a misnomer. Although water-soluble and polar compounds enter the brain poorly, lipophilic substances readily cross the barrier. In addition, a series of specific transport mechanisms exist through which required nutrients (hormones, amino acids, peptides, proteins, fatty acids, etc.) reach the brain (Pardridge, 1988). If toxicants are lipid soluble or if they are structurally similar to substances that are normally transported into the brain, they can achieve high concentrations in brain tissue. It has been proposed that one reason why the developing nervous system may be differentially sensitive to some toxicants is that the blood-brain barrier is less effective than in an adult. The effectiveness of the blood-brain barrier may also be changed by chemical-induced physiological events such as metabolic acidosis and nutritional deprivation.

2.5.3. Metabolism

The central nervous system has a very high metabolic rate and, unlike other organs, the brain depends almost entirely on glucose as a source of energy and raw material for the synthesis of other molecules (Damstra and Bondy, 1980). The absence of an alternative energy source makes the CNS critically dependent on an uninterrupted supply of oxygen as well as the proper functioning of enzymes that metabolize glucose. Substances can be toxic to the nervous system if they perturb neuronal metabolism. Without glucose, nerve cells usually begin to die within minutes. Despite its relatively small size, the energy demands of the brain require 14 percent of the heart's output and consumes about 18 percent of the oxygen absorbed by the lungs.

2.5.4. Limited Regenerative Ability

The nervous system has a combination of special features not found in other organ systems. It is composed of a variety of metabolically active neurons and supporting cell types that interact through a multitude of complex chemical mechanisms. Each cell type has its own functions and vulnerabilities. At the time of puberty, the system is fully developed and neurogenesis (the birth of new neurons from cell division of precursor cells called neuroblasts) ceases. This is in marked and significant contrast to almost all other tissues, where cell replacement is continual.

It is this loss of neurogenesis that limits the nervous system's ability to recover from damage and influences the plasticity of the system. Neurons are unable to regenerate following damage; therefore, they are no longer able to perform their normal functions. Toxic damage to the brain or spinal cord that results in cell loss is usually permanent.

If nerve cell loss is concentrated in one of the CNS's functional subsystems, the outcome could be debilitating; for example, a relatively small loss of neurons that use acetylcholine as their neurotransmitter may produce a profound disturbance of memory. A relatively minor insult concentrated in a subsystem that relies on dopamine as its neurotransmitter may drastically impair motor coordination. However, in response to injury, neurons are able to show considerable plasticity both during development and after maturation. Damage to the nervous system alters connectivity between the surviving neurons, permitting functional adjustments to occur to compensate for the damage. Such responsiveness may, in and of itself, have profound consequences for neurological, behavioral, and related body functions.

After damage to axons in the peripheral nerves, if the neurons are not damaged, the axons have the ability to regenerate and to attempt to reach their original target site. This is the basis, for example, of the eventual return of sensation and muscle control in a surgically reattached limb. Neurons in the CNS also have the ability to regenerate interrupted axons; however, they have a much more difficult task in reaching their original targets due to both the presence of scar tissue formed by proliferating glia and to the increased complexity of the connectivity in the CNS.

3. Methods for Assessing Human Neurotoxicity

3.1. Introduction

This chapter outlines and discusses current methods for detecting neurotoxicity in humans. In contrast to studies of neurotoxicity in animals where functional changes readily can be correlated with neuroanatomic and neurochemical alterations, there are ethical and technical barriers to the direct observation of neuronal damage in humans. Neurotoxicity in humans is most commonly measured by relatively noninvasive neurophysiologic and neurobehavioral methods that assess cognitive, affective, sensory, and motor function. The evaluation of human neurotoxicity and the relevance to risk assessment will be discussed within the context of clinical evaluation, epidemiologic/worksite studies, and human laboratory exposure studies.

3.2. Clinical Evaluation

Neurobehavioral assessment methods are used extensively in clinical neurology and neuropsychology to evaluate patients suspected of having neurologic disease. An extensive array of examiner-administered and paper and pencil tasks are used to assess sensory, motor, cognitive, and affective functions and personality states/traits. Neurobehavioral data are synthesized with information from neurophysiologic studies, imaging techniques, medical history, etc., to derive a working diagnosis. Clinical diagnostic approaches have provided a rich conceptual framework for understanding the functions (and malfunctions) of the central and peripheral nervous systems and have formed the basis for the development of methods for measuring the behavioral expression of nervous system disorders. Human neurobehavioral toxicology has borrowed heavily from neurology and neuropsychology for concepts of nervous system impairment and functional assessment methods. Neurobehavioral toxicology has adopted the neurologic/neuropsychologic model, using adverse changes in behavioral function to assist in identifying chemically or drug-induced changes in nervous system processes.

3.2.1. Neurologic Evaluation

Assessment of neurobehavioral function by the clinical examination of a patient has long been used as a primary tool in neurologic diagnosis. The domains of cognitive function, motor function, sensation, reflexes, and cranial nerve function are a standard part of the clinical neurologic exam. Movement and gait, speech fluency and content, verbal memory, deep tendon reflexes, muscle strength, symmetry of movement and strength, ocular movements, sensory function (pressure, vibration, visual, auditory), motor coordination, and logical reasoning are only a few of the functions assessed by neurologists (Denny-Brown et al., 1982).

Trained and experienced clinicians gather these data by observation, verbal exchange, and direct examination. Neurologic exams are sensitive indicators of neurologic disease; the data have predictive value for the diagnosis of underlying nervous system disease, and the methods have been extensively validated against other diagnostic procedures (e.g., imaging, neurophysiologic testing), the course of the illness, and autopsy findings. Examination of the patient in a semistructured procedure can yield a wealth of information and insights about functional impairment and the underlying neuropathology.

3.2.2. Neuropsychological Testing

Neuropsychologists have developed quantitative methods to supplement clinical neurologic exam and laboratory data for the diagnosis of neurologic disease. Currently, two assessment batteries, the Luria-Nebraska and the Halstead-Reitan, and shorter versions are used in clinical practice. The batteries consist of subtests that quantify a wide spectrum of cognitive, motor, sensory, intellectual, affective, and personality functions. The pattern of relative performance on the subtests can be interpreted along with historical and medical data to suggest the presence or absence of neurologic disease and the possible anatomic location of any focal lesions or degeneration. Clinical interpretation of the data is enhanced by data on age-related population norms for many subtests and by the systematic observation of the patient during testing.

Several neurotoxicity assessment batteries use components of neuropsychological tests and have adapted and shortened analogs of some subtests. Tests derived from the Wechsler Adult Intelligence Scale—Revised (WAIS–R) have been used frequently to assess neurobehavioral impairment from chemical agents, and other abbreviated variations of neuropsychological battery subtests have been incorporated into neurobehavioral toxicity batteries and used in field and laboratory studies.

3.2.3. Applicability of Clinical Methods to Neurotoxicology Risk Assessment

Neurologic and neuropsychologic methods have long been employed to identify the adverse health effects of environmental workplace exposures. 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 using these clinical methods. It is very important to make external/internal dose measurements in humans in order to determine the actual dose(s) which can cause unwanted effects.

Aspects of the clinical neurologic examination approach limit its usefulness for neurotoxicologic risk assessment. Information obtained from the neurologic exam is mostly qualitative and descriptive rather than quantitative. Estimates of the severity of functional impairment can be reliably

placed into only three or four categories (for example, mild, moderate, severe). Much of the assessment depends on the subjective judgment of the examiner; the magnitude and symmetry of muscle strength are often judged by having the patient push against the resistance of the examiner's hands. The datum is therefore the absolute and relative amount of muscle load sensed by the examiner in his or her arms.

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

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

In addition to logistical problems in administration and interpretation, neuropsychological batteries and neurologic exams share two disadvantages with respect to neurotoxicity risk assessment. First, neurologic exams and neuropsychological test batteries are designed to confirm and classify functional problems in individuals selected on the basis of signs and symptoms identified by the patient, family, or other health professionals. Their usefulness in detecting low-base rate impairment in workers or the general population maybe generally thought to be limited, decreasing the usefulness of clinical assessment approaches for epidemiologic risk assessment.

Second, neurologic exams and neuropsychologic test batteries were largely 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 primarily validated against these neurologic disease states. There has been insufficient research to demonstrate which tests designed to assess functional expression of neurologic disease are most useful in characterizing the modes of CNS impairment produced by chemical agents and drugs. More research is needed to validate the usefulness of neuropsychologic test methods in neurotoxicology.

3.3. Current Neurotoxicity Testing Methods

3.3.1. Neurobehavioral Methods

Chemical agents directly or indirectly affect a wide range of nervous system activities. Many of these chemical actions are expressed as alterations of behavior; Anger (1990a) lists 35 neurobehavioral effects of chemical exposure that illustrate alterations in sensory, motor, cognitive, affective, and personality function. Professional judgment is important in the interpretation of data from studies using neurobehavioral methods since some endpoints can be subjective.

Dozens of tests of neurobehavioral function have been proposed or used in field or laboratory studies to assess the neurotoxicity of chemical agents. Table 3–1 lists some frequently used tests of motor, sensory, cognitive, and affective neurobehavioral function.

TABLE 3–1.—NEUROBEHAVIORAL METHODS

Neurobehavioral function	Test
Sensation	Flicker Fusion.
	Lanthony (color vision).
Motor/Dexterity	Pursuit Aiming.
	Finger Tapping.
	Postural Stability.
	Reaction Time.
	Santa Ana Peg Board.
Cognition	Benton Visual Retention.
	Continuous Performance Task.
	Digit-Symbol.
	Digit Span.
	Dual Tasks.
	Paired-Associate.
	Symbol-Digit Task.
	Wechsler Adult Intelligence Scale—Revised© (Components).
	Wechsler Memory Scale.©
Affect	Profile of Mood States© (POMS).

In contrast to the individual focus in clinical evaluation, neurobehavioral tests primarily have been used to evaluate differences between groups, comparing unexposed groups with persons environmentally or occupationally exposed to a suspected neurotoxic agent. An ideal evaluation of groups for quantitative evidence of chemically induced neurobehavioral impairment would involve the assessment of a wide variety of functions, but testing all possible neurobehavioral functions that might be affected in a group of exposed workers, for example, would be impossible. Therefore, a testing strategy has been to use limited number tests that sample representative neurobehavioral functional domains such as dexterity, visual memory, and reaction time.

3.3.1.1. Test batteries.

Many field and laboratory studies have selected neurobehavioral methods according to available information about the spectrum of effects of the suspected neurotoxic agent(s). This focused strategy is useful for answering specific questions about known neurotoxins. To identify unspecified neurotoxic effects in groups of workers or to characterize the effects of less well-studied chemicals or mixtures of chemicals, several tests that sample a representative range of functional domains have been grouped into test batteries. The advantage of a standardized battery is that data from different study populations and chemical classes can be compared, and similarities in effects observed (Johnson, 1987). Standardized batteries can be categorized into investigator-administered and computer-administered types.

3.3.1.2. Investigator-administered test batteries.

The WHO-recommended Neurobehavioral Core Test Battery (NCTB) (Johnson, 1987), the Finnish Institute of Occupational Health (FIOH) (Hanninen, 1990), and the Pittsburgh Occupational Exposures Test Battery (POET) (Ryan et al., 1987) are three commonly used batteries. The NCTB is frequently used in field studies worldwide and can be fit inside a

medium-sized suitcase for transport. The NCTB consists of the following tests: simple reaction time task, digit-symbol coding task, timed motor coordination test (Santa Ana pegboard), digit span memory test, Benton Visual Retention test, pursuit aiming test, and the Profile of Mood States (POMS). Based on factor-analytic studies (Hooisma et al., 1990), these tests are believed to measure the functional domains of immediate memory, attention, dexterity/hand-eye coordination, reaction time, and mood. Long-term memory, verbal and language functions, auditory sensation, judgment, and so forth are not assessed.

3.3.1.3. Computerized test batteries.

Computerized tests and batteries have been developed for field and laboratory use. The Neurobehavioral Evaluation System (NES) (Baker et al., 1985), MicroTox (Eckerman et al., 1985), the SPES (Iregren et al., 1985), and the NCTR Operant Battery (Paule et al., 1990) are computerized systems developed for neurotoxicity assessment. Current versions of the NES, for example, consist of about 15 different neurobehavioral tests, and the battery has been used in epidemiologic studies of groups exposed to solvent, pesticide, and mercury, and in laboratory studies of NO_2, ethanol, and toluene (Letz, 1990).

Although many computerized tests appear to tap similar neurobehavioral domains as noncomputerized batteries, the visual mode of presentation, the manual mode of response, and the emphasis on speed of responding are believed to have led to significant differences in results obtained from computerized versus noncomputerized forms of similar tests. Attempts to clarify the differences between computerized and noncomputerized test batteries have met with difficulty. Although some tests are similar in each type of battery, size and duration of stimuli, presentation and response modality, number of trials, and scoring vary arbitrarily, preventing direct comparison. An example is the digit-symbol test on the NCTB and the symbol-digit test on the NES. Although almost identical in task requirements, procedural and scoring differences prevent direct comparison of the results from these two tests.

Postural stability is an aspect of integrated sensory and motor function that increasingly is being evaluated in clinical, epidemiologic, and laboratory investigations of effects of pesticides and solvents, and would be useful for assessing therapeutic drug-induced movement disorders such as neuroleptics. Measurement of postural stability requires a computer, special software, monitor, and a force transduction platform on which the subjects must stand (Dick et al., 1990). Mechanical and capacitive field methods for assessing the amplitude and frequency of tremor also are seeing more frequent use.

An advantage of computerized testing is the standardization of test presentation, but a disadvantage is the need for delicate, expensive computers and measurement devices that require transport for field studies. Noncomputerized test batteries may be less costly to purchase and easier to transport, enhancing their desirability in field studies, but test administrators require training and small differences in test administration may affect the data.

3.3.2. Neurophysiologic Methods

With improvements in the capabilities and size of equipment, quantitative neurophysiologic measurement of sensory and motor function will be increasingly useful in human neurotoxicity evaluations. A major advantage of these methods for risk assessment is that they can be assessed in both human and animal subjects and the data can be interpreted in an homologous manner.

Electromyographic responses (EMG) and nerve conduction velocity (NCV) have been used in the assessment of peripheral nerve neurotoxicity. Some techniques require that needle electrodes be placed beneath the skin for stimulation and recording and are therefore somewhat uncomfortable for the subject. However, the methods are quantitative, provide multiple endpoints of PNS function, and have clinical relevance.

The adverse effects of solvents, pesticides, and metals have been identified with EMG/NCV neurophysiologic measures. Although not reduced as a function of duration of employment, maximum nerve conduction velocity (MCV) has been reported to vary systematically with cumulative exposure to carbon disulfide (Johnson et al., 1983), suggesting that this measure may be particularly valuable for quantitative risk assessment of some types of peripheral motor nerve toxicity.

Noninvasive neurophysiologic test methods used in neurotoxicity evaluations include the electroencephalogram (EEG), visually evoked response (VER), somatosensory evoked potential (SEP), and the brainstem auditory evoked response (BAER). The EEG is the summed electrical activity of neurons measured with scalp electrodes; voltage and frequency are primary measures. Evoked methods employ specific eliciting stimuli applied to the sense organs to measure nervous system electrical response. Visual patterns, sounds, and cutaneous stimuli are presented to the subject, and "evoked" voltage changes in the nervous system are measured with skin electrodes.

While EEGs were developed as a tool in the neurologic diagnosis of seizure disorders and other brain diseases, dose-related EEG changes in chemically exposed (especially solvents and styrene) individuals have been noted (Seppalainen and Harkonen, 1976). EEG measurement requires large recording devices that can be used in the laboratory or clinic, but are difficult to use in field studies. However, compact computerized recording equipment has been developed, and automated spectral analyses of EEGs have recently been applied to neurotoxicity evaluation (Piikivi and Tolonen, 1989).

In contrast to EEGs, evoked response technology is improving, and equipment, while expensive, is becoming more portable. VERs have been used to detect the sensory toxicity of solvents and carbon monoxide in human subjects, and a relationship has been suggested between BAER and blood lead levels in children exposed to lead-containing dust in the environment (Otto and Hudnell, 1990). Evoked potentials also may be conditioned, allowing the use of sensory methods to investigate associative processes.

Dose-response functions have been found with evoked methods. A curvilinear relationship was found between BAER and blood lead concentrations in children (Otto and Hudnell, 1990), and a biphasic function described visual evoked potential (VEP) latency and visual contrast sensitivity and perchloroethylene exposure concentration in a laboratory study (Altmann et al., 1991). In the latter study, the direction of the response was jointly dependent on dose and stimulus parameters. In addition, changes over time in the effect of the solvent on VEP were dose and stimulus parameter dependent.

Two important methodologic considerations are illustrated by BAER and VEP data. One is that low concentrations of some chemical agents may produce effects (shorter latencies in these examples) that could be inaccurately interpreted as facilitation rather than impairment. Changes in neuronal latencies in either direction could be a result of a neurotoxic process. The second is that the detection of neurotoxic effects is dependent on

dose-time-testing parameter interactions. A thorough understanding of the effects of testing parameters on the dose-response relationship and the time course of chemical effect will be necessary for interpreting neurotoxicity studies.

The development of neurophysiologic methods, such as evoked and conditioned potentials, for neurotoxicity risk assessment should be encouraged. These methods provide relatively unambiguous quantitative data on sensory function that may have clear implications for health, are influenced by fewer extraneous variables than are self-report and neurobehavioral performance tests, and allow relatively direct extrapolation of effects between animals and humans.

3.3.3. Neurochemical Methods

One of the major difficulties in risk assessment is estimating exposure parameters and the dose or body burden actually absorbed by the individual. In epidemiologic studies, the actual absorption and bioavailability of a chemical from an exposure are frequently unknown.

Measurement of chemical concentrations in biologic fluids or tissues is one way to measure more precisely the concentration at the site(s) of toxic effect. In epidemiologic studies, this has been possible only for chronic exposure and for acute exposure to chemicals with long biologic half-lives in the body, such as lead, other metals, and bromides. Blood lead levels show correlations with neurobehavioral impairment, but blood lead levels are representative correlates of toxicity only for relatively acute doses. In children, for example, the majority of lead-related impairment is the result of chronic, rather than acute, absorption. The cumulative amount of lead sequestered in tissues (such as deciduous teeth) may be a more representative indicator of the area under the time-concentration curve.

For chemicals with half-lives in the body too short for estimating absorbed dose, the biochemical products from the chemical or from the physiologic effects of the chemical may serve as an index of exposure. Serum enzyme concentrations (cholinesterase) and esterases in other tissues (lymphocyte target esterase) have been employed in field studies to detect pesticide exposure, while vanillylmandelic acid (product of catecholamine neurotransmitter biotransformation) and erythrocyte protoporphyrin concentrations have been used with varying success in differentiating between lead-exposed and control workers. The addition of similar "exposure biomarker" measures to laboratory studies may allow the development of quantitative estimates of absorbed dose under various exposure conditions.

The measurement of metabolic products of neurotoxic agents may be extremely useful in risk assessment; an example comes from cancer risk assessment. Human data from the early 1970s on saturation of microsomal methylene chloride biotransformation to carbon monoxide (Stewart et al., 1972), along with subsequent animal carcinogenesis data garnered in the 1980s, provided a quantitative basis for a physiologically based pharmacokinetic model of methylene chloride cancer risk assessment (Andersen et al., 1991). The information on human CO pathway kinetics provided the homologous key that allowed extrapolation of risk from animals to humans on a comparative physiologic basis rather than using default assumptions.

3.3.4. Imaging Techniques

A number of recently developed computerized imaging techniques for evaluating brain activity and cerebral/peripheral blood flow have added valuable information to the neurologic diagnostic process. These imaging methods include thermography, positron emission tomography, passive neuromagnetic imaging (magnetoencephalography), magnetic resonance imaging, magnetic resonance spectroscopy, computerized tomography, doppler ultrasonography, and computerized EEG recording/analysis (brain electrical activity mapping). The research application of these invasive and noninvasive quantitative methods has primarily been in neurology, schizophrenia research, drug abuse, AIDS research and toxic encephalopathy (Hagstadius et al., 1989). Although the equipment for brain imaging is expensive and not portable, neuroimaging techniques promise to be valuable clinical and laboratory research tools in human neurotoxicology.

3.3.5. Neuropathologic Methods

Neuropathologic examination of nervous system tissue has been used to confirm data from clinical testing and to contribute to the understanding of mechanisms of action of neurotoxicity. Peripheral nerve biopsies have confirmed chemically induced peripheral neuropathies and evaluated rates of recovery (Fullerton, 1969). Postmortem examination of nervous tissue also has elucidated the neuropathological effects of carbon disulfide, clioquinol, and doxorubicin (Spencer and Schaumburg, 1980).

3.3.6. Self-Report Assessment Methods

Self-report measures relevant to neurotoxicity risk assessment consist of histories of symptoms, events, behaviors, and environmental conditions. Information is obtained by face-to-face interviews, structured interviews (often conducted for diagnostic purposes), medical histories, questionnaires, and survey instruments.

Self-report instruments are the only means for measuring some symptoms and all interoceptive states, such as pain and nausea. Self-reports also are used to obtain information on behaviors and events (e.g., exposure conditions) especially when practical, legal, or ethical limitations prevent direct observation.

Subjective symptoms elucidated from self-report instruments are responsive to dose. Hanninen et al. (1979) found that subjective symptoms were positively correlated with blood lead levels in exposed workers. Subjective pain estimations are correlated with dose and type of centrally and peripherally acting analgesics, and anxiety scores on a variety of scales are responsive to the size of the anxiolytic dose.

Symptom checklists are used in epidemiologic research to identify the pattern of subjective complaints, which can be used to guide the selection of objective assessment methods. The distribution of symptoms can be correlated with indices of exposure to determine if particular symptoms are more prevalent in exposed persons (Sjogren et al., 1990).

Self-report data are notable for biases that may influence them; these biases are well known in epidemiology, clinical practice, and social science. Even in the most superficial of questions, respondents may consciously or unknowingly bias the answer to fit what they believe to be the examiner's expectations. Details of objective events or subjective states are subject to alteration; recall and reporting of remembered occurrences may be biased to fit interpretations and expectations. The socioeconomic status, gender, and affiliation of the tester also have been identified as biasing variables. Bias occurs when information is requested about behaviors, beliefs, or feelings believed by the respondent to be socially undesirable or when reinforcement contingencies (e.g., litigation) strongly favor selective reporting.

Biases in self-report data can be reduced by making the questionnaire anonymous or highly confidential;

objective data can be used to validate self-reports. Ethnographic observations, objective measurement of behavior, biologic samples, and the observations of significant others are employed to validate self-report data. Consistent descriptions of events by several persons lend credence to the reliability of the report. Many clinical interviews and self-report assessment instruments include some mechanisms for detecting self-report bias, either by looking for endorsement of improbable behaviors, or by examining the consistency of information gathered in several ways or from several sources. Concordance among biologic indices, observations, and physical examinations increases the judged validity of self-reports.

3.3.6.1. Mood scales.

Changes in mood and emotionality can be consequences of neurotoxicity. For example, case reports have identified mood changes from exposure to mercury, lead, solvents, and organophosphate insecticides. The Taylor Manifest Anxiety Scale and the Profile of Mood States (POMS) are standardized self-report assessment instruments for which there is some evidence of sensitivity to chemical insult.

The POMS, a component of the Neurobehavioral Core Test Battery, is a self-report measure that asks respondents to use a 5-point scale to rate the magnitude of 65 subjective states, such as "tense," "relaxed," "hopeless," "guilty," etc., that they have experienced within the past week. The responses are scored according to six mood factors, and a Total Mood Disturbance Score also may be calculated. Liang et al. (1990) used the POMS to evaluate lead-exposed workers (mean blood lead concentration of 41 µg/dL) from a battery plant and a control group from a fabric-weaving manufacturer. Exposed workers were significantly higher on tension, depression, anger, fatigue, and confusion scales.

Mood scales were developed to aid in assessment of psychological disorders, such as depression, and to track treatment response. In addition, mood is modulated by metabolic and endocrine variables in health and disease and can change rapidly in response to interpersonal, workplace, and environmental events. The large number of nonchemical variables and the lability of mood make inclusion of carefully selected controls essential in using affect as an endpoint in neurotoxicity research.

The validity of mood scales may be limited to the specific populations in which the validity studies were performed. As characterizations of internal states, the meaning of the descriptors in the POMS established for one culture may not be the same as the meaning of that concept or term in other cultures or in other language systems. There may be variations in interpretation of the terms by respondents across English-speaking subcultures, perhaps as a function of education or the size of the verbal community. While these differences may not impede a global clinical interpretation, the reduction in generalizability across study populations may be sufficient to decrease the usefulness of subjective scales in quantitative neurotoxicity risk assessment.

3.3.6.2. Personality scales.

The Minnesota Multiphasic Personality Inventory (MMPI), the Cattell 16 PF, and the Eysenck Personality Inventory have occasionally been used in neurotoxicity research. Exposed and nonexposed groups have differed on several scales derived from these standardized questionnaires. The diagnostic power of the MMPI, for example, is not in the individual scales but in the pattern of scores on the 10 clinical and 3 validity scales. Because interpretation of the MMPI requires a trained diagnostician with experience in the population of interest, it is less likely to be useful in quantitative neurotoxicity assessment.

3.4. Approaches to Neurotoxicity Assessment

3.4.1. 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. Epidemiologic studies are a means of evaluating the effects of neurotoxic substances in human populations, but such studies are limited because they must be performed shortly after exposure if the effect is acute. Most often these effects are suspected to be a result of occupational exposures due to the increased opportunity for exposure to industrial and other chemicals.

3.4.1.1. Case reports.

The first type of human study undertaken is 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. For example, the neurological hazards of exposure to Kepone, dimethylaminopropionitrile, and methyl-n-butyl ketone were first reported as case studies by physicians who noted an unusual cluster of diseases in persons later found to have been exposed to these chemicals (Cone et al., 1987). However, case histories where exposure involved a single neurotoxic agent, though informative, are rare in the literature; for example, farmers are exposed to a wide variety of potentially neurotoxic pesticides. Careful case histories assist in identifying common risk factors, especially when the association between the exposure and disease is strong, the mode of action of the agent is biologically plausible, and clusters occur in a limited period of time.

Case reports are inexpensive compared with other types of epidemiologic studies and can be obtained more quickly than more complex studies. They provide little information about disease frequency or population at risk, but their importance has been clearly demonstrated, particularly in accidental poisoning or acute exposure to high levels of toxicant. They remain an important source of index cases of new diseases and for surveillance in occupational settings. These studies require confirmation by additional epidemiologic research employing other study design.

3.4.1.2. 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 is 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.

3.4.1.3. 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 neurotoxins in the environment has provided a great deal of information. In his recent text, 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, and will be discussed only briefly here. Many of these biases also can be present in cross-sectional studies. For example, recall bias or faulty recall of information by study subjects in a questionnaire-based study can distort the results of the study. Analysis of the case-comparison study design assumes that the selected cases are representative persons with the disease—either all cases with the disease or a representative sample of them have been ascertained. It further assumes that the control or comparison group is representative of the nondiseased population (or that the prevalence of the characteristic under study is the same in the control group as in general population). Failure to satisfy these assumptions may result in selection bias, but violation of assumptions does not necessarily invalidate the study results.

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

The definition of exposure is critical in epidemiologic studies. In occupational settings, exposure assessment 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.

3.4.1.4. Prospective (cohort, followup) studies.

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

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

A special type of cohort study is the retrospective cohort study in which the investigator goes back in time to select the study groups and traces them over time, often to the present. The studies usually involve specially exposed groups and have provided much assistance in estimating risks due to occupational exposures. Occupational retrospective cohort studies rely on company records of past and current employees that include information on the dates of employment, age at employment, date of departure, and whether diseased (or dead in the case of mortality studies). Workers can then be classified by duration and degree of exposure. A retrospective cohort study was performed in which a cohort of 1,790 bricklayers and 2,601 men exposed to paint solvents was retrospectively identified and, if a disability pension had been awarded, the subjects were examined for evidence of presenile dementia. This study found a rate ratio of 3.4 for presenile dementia among the painters as compared with the bricklayers (Johnson, 1987).

3.4.2. Human Laboratory Exposure Studies

Neurotoxicity assessment has an advantage not afforded 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. Under certain circumstances, it is 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. These chemicals include methylene chloride, perchloroethylene, trichloroethylene, and p-xylene (Dick and Johnson, 1986).

Human exposure studies offer advantages over epidemiologic field studies. Combined with appropriate biological sampling (breath or blood), it is possible to calculate body concentrations, to examine toxicokinetics, and identify metabolites. Bioavailability, elimination, dose-related changes in metabolic pathways, individual variability, time course of effects, interactions between chemicals, interactions between chemical and environmental/biobehavioral factors (stressors, workload/respiratory rate) are some processes that can be evaluated in laboratory studies.

Other goals of laboratory studies include the indepth characterization of effects, the development of new assessment methods, and the examination of the sensitivity, specificity, and reliability of neurobehavioral assessment methods across chemical classes.

The laboratory is the most appropriate setting for the study of environmental and biobehavioral variables that affect the action of chemical agents. The effects of ambient temperature, task difficulty, the rate of ongoing behavior, conditioning variables, tolerance/

sensitization, sleep deprivation, motivation, etc., can be studied.

3.4.2.1. Methodologic aspects.

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

3.4.2.2. Human subject selection factors.

Participants in laboratory exposure studies may be recruited from populations of persons already exposed to the chemical/drug or from naive populations. Although the use of exposed volunteers has ethical advantages, can militate against novelty effects, and allows evaluation of tolerance/sensitization, finding an accessible exposed population in reasonable proximity to the laboratory is difficult. Naive participants are more easily recruited, but may differ significantly in important characteristics from a representative sample of exposed persons. Naive volunteers are often younger, healthier, and better educated than the populations exposed environmentally, in the workplace, or pharmacotherapeutically. For example, phase I drug trial data from relatively young and healthy volunteers may not adequately predict the incidence of neurotoxic side effects in older persons with chronic health problems.

3.4.2.3. Exposure conditions and chemical classes.

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

Most laboratory studies of neurobehavioral function have employed individual solvents, combinations of two solvents, or very low concentrations of chemicals released from household and office materials (volatile organic compounds). This selection is primarily because solvent effects are reversible, because there are wide margins of safety for acute effects of solvents, because solvents can be administered via inhalation methods that allow calculation of body concentrations by breath sampling methods that do not require needle sticks, because over 1 million workers may have occupational solvent exposure, and because of the extensive use of solvents in household products. Chemicals studied in the laboratory over the past 40 years have included ozone, NO_2, CO, styrene, lead, anesthetic gases, pesticides, irritants, chlorofluorocarbon compounds, and propylene glycol dinitrite. Caffeine, diazepam, and ethanol have been used in laboratory studies as positive control substances.

3.4.2.4. Test methods.

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

3.4.2.5. Controls.

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 on 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.

3.4.2.6. Ethical issues.

Most human exposure studies in the laboratory have been justified on the basis of data indicating that the chemical or drug exposure produces only temporary and reversible functional effects. The use of occupationally, environmentally, or therapeutically exposed populations as a source of participants also makes the risks from research exposure small relative to nonlaboratory sources of risk. Protection of human subjects is also provided by the informed consent process; the health risks (known and unknown) and benefits of the research are thoroughly explained to each participant, who may terminate participation in the study at any time.

Despite safeguards, several chemicals and drugs thought at the time of the exposure study to produce only temporary neurobehavioral effects are now (20 years later) suspected of being potential human carcinogens on the basis of animal and human data (e.g., methylene chloride, perchloroethylene). Other chemicals, however, are now thought to be less carcinogenic or otherwise less toxic in humans than once believed. Rapid advances in all areas of toxicology make it difficult to communicate, to potential subjects, reliable information about the likelihood of long-term, latent, or delayed adverse effects on health subsequent to the study. The communication of uncertainty about potential long-term effects to research participants is essential if human exposure studies are to be conducted ethically and are to continue their contributions to neurotoxicology and risk assessment.

3.5. Assessment of Developmental Neurotoxicity

3.5.1. Developmental Deficits

While adult neurotoxicology evaluates the effects of chemical exposure on relatively stable nervous system structure and function, developmental neurotoxicology addresses the special vulnerabilities of the young and the old. Neurobehavioral assessment of chemical neurotoxicity is complicated by having to measure functional impairment within a sequential progression of emergence, maturation, and gradual decline of nervous system capabilities. Methods in developmental neurotoxicity assessment must reflect the diversity of

neurobehavioral functions, from neonates to the elderly.

Exposure of pregnant women to alcohol, drugs of abuse, therapeutic drugs, nicotine, and environmental chemicals may result in the immediate or delayed appearance of neurobehavioral impairment in children (Kimmel, 1988; Nelson, 1991a). Postnatal exposure of children to chemical agents in the environment, such as lead, also may impair IQ and other indices of neurobehavioral function (Needleman et al., 1979). Neurotoxic effects may impair speech and language, attention, general intelligence, "state" regulation and responsiveness to external stimulation, learning and memory, sensory and motor skills, visuospatial processing, affect and temperament, and responsiveness to nonverbal social stimuli. Chemical neurotoxicity may be manifested as decreases in functional capabilities or delays in normative developmental progression.

Neurotoxic effects are not limited to direct exposure of the fetus or child to the chemical. Animal studies suggest that altered neurobehavioral development in offspring may result from exposure of males (Joffe and Soyka, 1981) and females to chemical substances prior to conception. In this case, altered postnatal development may reflect chemical influences on mechanisms of inheritance, copulatory behavior, nutritional status, hormonal status, or the uterine environment. In animals and humans, chemical exposure of parents may indirectly impair postnatal development through changes in milk composition, parenting behaviors, and other aspects of the environment.

In older adults the normal aging process alters the response to neurotoxicants. Both pharmacodynamic and pharmacokinetic changes may underlie altered sensitivities to the neurotoxic effects of drugs and chemicals. An example well known in geriatric medicine is the apparent increase in sensitivity of the elderly to the toxic effects of anxiolytics (Salzman, 1981). Decreases in biotransformation rate and renal elimination of parent drug and active metabolites, not related to disease processes, may partially account for the increased vulnerability (Friedel, 1978). Chronic disease states in older persons may result in decreased functional capabilities and increased vulnerability to neurotoxic effects. Chronic diseases also may prompt pharmacotherapy that may impair neurobehavioral function. Cardiovascular, psychopharmacologic, and antineoplastic medications may result in patterns of neurobehavioral impairment not typically seen in younger individuals.

3.5.2. Methodologic Considerations

Standardized methods are being developed for pediatric neurotoxicity assessment. Neurobehavioral functions emerge during developmental phases from neonatal stage through secondary school, and nervous system insult may be reflected not only in impairment of emergent functions, but also as delays in the appearance of new functions. Both the severity and type of deficit are affected by the dose and duration of exposure (Nelson, 1991b), and different sensitivities to chemical effects may be exhibited at different stages of nervous system development. Early episodes of exposure may produce structural damage to the nervous system that may not be developmentally expressed in behavior for several months or years.

The selection of appropriate testing methods and conditions is more important when assessing children because of shorter attention spans and increased dependence on parental and environmental supports. In addition, because of the increasing complexity of functional capabilities during early development, only a few tests appropriate for infants can be validly readministered to older children. Given the complexity of these variables, the task of devising sensitive, reliable, and valid assessment instruments or batteries for pediatric populations will be challenging.

Assessment methods in older adults must be capable of distinguishing chemical and drug effects from the effects of aging processes and chronic disease states (Crook et al., 1983). Assessment methods must be valid and reliable with repeated administration across a significant portion of the lifespan, and take into consideration the time (days, months, or years) that may intervene between exposure/insult and the expression of neurotoxicity as functional impairment. Research on nonexposed populations to develop age-appropriate normative scores for neurobehavioral functions will be important for the interpretation of assessment instruments.

Environmental exposure to neurotoxic chemicals and drugs is correlated with socioeconomic and ethnic status. Assessment methods will therefore have to be adapted to diverse ethnic, cultural, and language groups. While gender differences in early development have been noted, differential responses of males and females to neurotoxicants have been less well explored and should receive attention.

3.6. Issues in Human Neurotoxicology Test Methods

3.6.1. Risk Assessment Criteria for Neurobehavioral Test Methods

The value of human neurobehavioral test methods for quantitative risk assessment is related to the number of the following criteria that can be met:

a. Demonstrate sensitivity to the kinds of neurobehavioral impairment produced by chemicals; that is, able to detect a difference between exposed and nonexposed populations in field studies or between exposure and nonexposure periods in human laboratory research or within exposed populations over time.

b. Show specificity for neurotoxic chemical effects and not be unduly responsive to a host of other nonchemical factors, and show specificity for the neurobehavioral function believed to be measured by the test method.

c. Demonstrate adequate reliability (consistency of measurement over time) and validity (concordance with other behavioral, physiologic, biochemical, or anatomic measures of neurotoxicity).

d. Show graded amounts of neurobehavioral change as a function of exposure parameter, absorbed dose, or body burden along some ordinal or continuous metric (dose response).

e. For representative classes or subclasses of CNS/PNS-active chemicals, identify single effects or patterns of impairment across several tests or functional domains that are reasonably consistent from study to study (structure-activity).

f. Be amenable to the development of a procedurally similar counterpart that can be used to assess homologous behaviors in animals.

g. Whenever it is relevant, care must be taken to insure to the extent possible that subjects are blind to the variate of interest (Benignus, 1993).

3.6.1.1. Sensitivity.

Individual neurobehavioral tests and test batteries have detected differences between exposed and nonexposed populations in epidemiologic studies and in laboratory studies. Effects have been detected by neurobehavioral methods at concentrations thought by other kinds of evaluation not to produce neurotoxicity. Workplace exposure limits to many chemicals have been set on the basis of neurobehavioral studies. While the overall sensitivity of neurobehavioral methods is sufficient to be useful in neurotoxicology risk assessment, some methods are notably insensitive across several chemical classes while the sensitivity of other neurobehavioral tests varies according

to the spectrum of neurotoxic effects of the chemical or drug.

Sensitivity is sometimes negatively correlated with reliability; selecting for tests that show little change over time may also select for tests that are not sensitive to neurotoxic insult.

Having more control over the testing environment and using a repeated measures design may decrease variability and increase statistical power, but these tactics may introduce other problems. There is some suggestion that experience in highly structured laboratory environments with explicit stimulus conditions may reduce the sensitivity of humans and animals to the effects of drugs and chemicals, and the sensitivity of neurobehavioral measures to impairment by a chemical or drug may depend on neurobehavioral training history (Terrace, 1963; Brady and Barrett, 1986). Sensitivity may also be decreased if baseline behaviors are stable and well practiced or an escape/avoidance procedure is employed.

The systematic introduction of stimulus or response changes to induce transitional behaviors, such as in a transitional state or repeated learning paradigms, may be one way to retain the advantage of a stable baseline, have sufficient sensitivity, and avoid practice effects (Anger and Setzer, 1979).

3.6.1.2. Specificity.

There are two kinds of specificity in neurobehavioral assessment of chemical or drug neurotoxicity. Chemical specificity refers to the ability of a test to reflect chemical or drug effects and to be relatively resistant to the influence of nonchemical variables. The second type of specificity refers to the ability of a test method to measure changes in a single neurobehavioral function (e.g., dexterity) or a restricted number of functions, rather than a broad range of functions (attention, reasoning, dexterity, and vision).

The neurobehavioral expression of neurotoxic chemical or drug effects is a function of the joint interaction of ongoing nervous system processes with the chemical substance and with biopsychosocial variables that also influence nervous system activity. In laboratory exposure studies numerous environmental, behavioral, and biologic variables can influence the type or magnitude of neurotoxic effects of chemical agents and drugs (MacPhail, 1990). These variables include ambient temperature, physical workload, task difficulty, the social and tangible reward characteristics of the laboratory setting, redundancy of stimuli, the rate and form of the behavioral response, conditioning factors, and the interoceptive stimulus properties of the chemicals.

The laboratory research participant's history and habits outside the laboratory also may affect chemical-neurobehavioral interactions by influencing the baseline level of performance on neurobehavioral tests or directly affecting the response of the CNS to the exposure. Age, gender, educational level, intellectual functioning, economic status, acute and chronic health conditions (including developmental or current neurologic conditions), alcohol/drug/tobacco effects or withdrawal, emotional status or significant life events, sleep deprivation, fatigue, and cultural factors are only a few of the variables that may affect performance in laboratory studies (Williamson, 1991; Cassitto et al., 1990).

The influence of these selection and biopsychosocial variables on the neurobehavioral effects of workplace chemicals is poorly understood, although their effects on drug-behavior interactions have been more thoroughly explored. Controlling or understanding chemical and nonchemical variables will be important for ensuring adequate specificity for risk assessment purposes.

3.6.1.3. Reliability and validity.

Reliability refers to the ability of a given test to produce closely similar results when administered more than once over a period of time or in similar populations. Reliability is meaningful only with respect to the measurement of functions that would not be expected to change significantly over the time period. Test-retest reliability coefficients are between 0.6 and 0.9 (Beaumont, 1990) for most of the tests in the NCTB. With notable exceptions, other neurobehavioral tests have similar reliabilities. Reliabilities in the 0.8 to 0.9 range are usually thought acceptable. As reliability decreases, measurement error is more likely to mask neurotoxic chemical effects.

The validity of a given neurotoxicity test relies on evidence that it adequately measures the domain of interest and is not highly correlated with tests that are believed to measure unrelated functions. These convergent and divergent aspects of validity are frequently divided into construct, content, and criterion subcategories. Construct validity refers to the ability of a given test to measure the intended function or construct (e.g., attention), content to how well the test measures the major aspects of the function, and criterion to how highly the test correlates with other tests of the same function or predicts neurotoxic impairment after similar insult.

Many neurobehavioral tests purport to measure the same or similar cognitive, sensory, or motor functions, but correlations between these tests under chemical exposure or control conditions can be disappointingly low. This is not surprising given the procedural differences that exist among neurobehavioral tests. Tests intended to measure the same function often have different presentation and response modalities (visual, verbal, manual), have differing numbers of trials or a different time limit, and have different methods for scoring the results. Many tests have such large procedural differences that direct comparison is difficult. Assessment of validity for neurobehavioral tests of specific constructs, such as attention, is further complicated in that sensory input, other cognitive processes, and motor responses are unavoidable contributors to the test result.

3.6.1.4. Dose response.

Dose in this discussion refers to the measurement of chemical or metabolite concentrations in the body and to estimations of exposure. Both exposure assessment and biologic concentrations should be measured whenever possible. Dose-response relationships have been observed both in field and laboratory studies. Two recent human solvent exposure studies used lower exposure concentration that resulted in mucosal membrane effects reported by subjects as odors or irritation (Dick et al., 1992; Hjelm et al., 1990). Neurobehavioral impairment was not detected in these studies. A review of over 50 organic solvent human exposure experiments found that neurobehavioral impairment generally occurred at mean concentrations higher than those associated with irritation, although there was often overlap among the irritant and impairment concentration ranges (Dick, 1988). Defining neurotoxic dose-response relationships in humans decreases the uncertainties of extrapolation from animal data and allows a more accurate risk assessment.

Recent human solvent exposure studies have employed low concentrations under which neurobehavioral impairment was not observed. Rather, these studies have primarily detected the effects of solvents on mucosal membranes reported by subjects as odors or irritation (Dick, unpublished observation). While these data may be relevant to setting workplace and environmental exposure limits, they can be expected to provide little information about the neurobehavioral impairment that occurs at higher concentrations. The

relationship between irritant/odor concentration-effect functions and neurobehavioral impairment concentration-effect functions is not known, but it is probably not linear. Dose-dependent mechanisms of toxic effect can be expected to complicate risk extrapolation across the dose-response range in humans.

A further complication in dose-response extrapolation is that low concentrations of chemicals may appear to improve performance as measured by neurobehavioral tests, while higher doses are more likely to impair performance. Improved performance does not necessarily indicate the absence of neurotoxicity; both increases and decreases in neurobehavioral performance may result from deleterious chemical interactions with neurons. Dose-response extrapolation is further complicated by the observation that facilitative or impairment effects within a given dosage range may occur at some parameters of the test stimulus or aspects of the response (response rate-dependent) but not at others (Altmann et al., 1991). Therefore, dose extrapolations are more difficult when there is uncertainty about the shape of the dose-response function (biphasic, linear, etc.) at the relevant test stimulus and response parameters.

The risk assessment process with animal data involves extrapolation from the effects of high doses in animals to predict the effects of chronic low-dose exposure in humans. With data from laboratory studies of humans in a risk assessment, however, the extrapolation is in the other direction, from very low-dose laboratory exposure to predict the effects of chronic exposure at higher (but still low) concentrations in the environment and workplace. Low- to high-dose extrapolation within the same species may require different assumptions and risk assessment procedures. Although high-dose human exposures have occurred in accidents, those data are primarily descriptive in nature and cannot easily be plugged into a quantitative risk extrapolation process. Low dose laboratory data may be combined with data from epidemiologic studies of persons exposed to higher concentrations.

3.6.1.5. Structure-activity.

Structure-activity relationships for well-known chemicals have largely been established by clinical methods (and animal studies) and verified by neurobehavioral and neurophysiologic testing. Although an area of active research, neurobehavioral testing of humans has not yet been able to identify reliable patterns of impairment among chemical classes. This endeavor has been hampered by most laboratory research having been limited to the evaluation of low concentrations of solvents and a few other reversible toxicants and by the exposure uncertainties, biases, and confounding variables found in cross-sectional or cohort field studies.

3.6.2. Other Considerations in Risk Assessment

3.6.2.1. Mechanisms of action

Uncovering behavioral and neurophysiologic mechanisms of action is a potential contribution of human laboratory exposure studies to neurotoxicity risk assessment. For example, Stewart et al. (1972) demonstrated that methylene chloride was metabolized to carbon monoxide in humans, and further studies (Putz et al., 1979) found that CO production could account for some of the neurobehavioral impairment observed with that chemical. Recent human laboratory studies of solvents employed low concentrations that produced mucosal irritation and strong odor, but little neurobehavioral impairment (Dick, unpublished observation). The mechanisms of action that produce mucosal irritation and the neurotoxic mechanisms that are expressed in neurobehavioral impairment may be quite different. Data on mucosal irritation and odor may therefore provide limited information for a neurotoxicity risk assessment.

3.6.2.2. Exposure duration

A criticism of extrapolation from animal studies to human exposure conditions is that the effects of short-term exposure (months to 1–2 years) in animals may not accurately predict the effects of chronic exposure (>10 years) in humans. Laboratory studies rarely expose human subjects to solvents for more than 4–6 hours per day for 2–5 days while environmental and workplace exposures of concern involve 6–8 hours of exposure per day for years. The uncertainties of extrapolating from relatively acute exposures to predict the risks from chronic exposure will not be eliminated by using human laboratory exposure data in risk assessment.

3.6.2.3. Time-dependent effects

The acute exposures that are possible in human laboratory studies may provide little information on chronic time-dependent neurobehavioral effects. The effects of initial exposure may remain the same, decrease (tolerance), or increase (sensitization) with continued or repeated exposure to the chemical. All effects will not change in unison; tolerance and sensitization may be observed simultaneously on different measures of neurobehavioral function. The multiple toxicodynamic effects of chemical exposure (neurobehavioral and other) seem to follow individual time courses suggestive of multiple mechanisms of action. In addition, the processes of tolerance and sensitization can be influenced by testing conditions and the nature of the behavioral task.

One also must be concerned about latent effects that do not appear for some time after a brief exposure and "silent" cumulative neurotoxic effects that are not observable in acute human studies. Latent and silent effects not only bring up the possibility of unknown risks for human subjects, but also make more difficult the extrapolation of chronic neurotoxic risks on the basis of acute exposures.

Therefore, the acute exposure conditions possible in human laboratory studies may provide us with very limited information about the long-term effects of chronic exposure.

3.6.2.4. Multiple exposures

In the environment and the workplace, persons are seldom exposed to only a single chemical. Rather, they are most often exposed to complex mixtures of chemicals, the relative concentrations of which may vary over time. For example, one farmer had more than 50 different chemical products (pesticides, herbicides, solvents, metals, gases) with nervous system effects that he used, prepared, or stored in his work shed. Chemicals used in industrial processes may also contain impurities or contaminants that may produce neurotoxic effects or alter the neurotoxicity of the more abundant chemical species. Chemical mixtures may have additive or potentiating effects not predictable from studies of single chemicals (Strong and Garruto, 1991). Human laboratory exposure studies traditionally have employed one highly purified chemical or combinations of two chemicals (usually solvents) and thus may produce a spectrum of neurotoxic effects different from environmental and occupational exposures.

Recently volatile organic compounds (VOCs) have been used in human exposure studies (Otto and Hudnell, 1991). VOCs consist of multiple volatile compounds administered at concentrations commonly found in indoor air from emissions by laminates, carpet, plastics, and other building and decorating materials. Although VOCs are thought to produce primarily mucosal irritation and odors, reports of "sick building syndrome" and

individual sensitivity to indoor air contaminants suggest that other neurobehavioral mechanisms also may be operating.

3.6.2.5. Generalizability and individual differences

The results of field studies and laboratory exposure studies are most valuable when they can be extrapolated to the general population. Studies conducted in male workers or in young, healthy volunteers may have limited applicability to women or to people in other age ranges. It therefore is important to conduct studies that include males and females of different ages and ethnic heritage. Culture-sensitive neurobehavioral test methods are being developed and validated in the United States and other countries.

While it is important to increase the generalizability of results, it is equally important to know when results cannot be generalized. Studies should be specifically directed toward identifying subsets of individuals who are more or less sensitive to neurotoxic insult or differ in mode of expression. There are many examples of individual differences that alter response to chemicals and drugs: phenylketonurics are more sensitive to dietary tyramine and persons with variants of plasma pseudocholinesterase are more affected by some neuromuscular blocking agents.

3.6.2.6. Veracity of neurobehavioral test results

In most epidemiologic and human laboratory studies, research volunteers are highly motivated to perform well on tests of neurobehavioral function. Under voluntary conditions, actual neurobehavioral performance may serve as a reasonable index of nervous system capabilities. Some studies, however, are conducted in response to complaints of symptoms thought to be related to workplace, environmental, or therapeutic exposure to chemicals and drugs. The performance of research participants with symptoms and complaints may be significantly affected (consciously or unconsciously) by monetary rewards, emotional relief, or social gains from the validation of their complaints. Under these conditions, performance may or may not accurately reflect the capabilities of the nervous system and may lead to inaccurate conclusions about the magnitude of nervous system dysfunction or about putative chemical or drug etiologies.

In addition to suboptimal performance engendered by potential reinforcers or rewards, research participants involved in disputes over suspected neurotoxic exposures or in litigation for monetary damages are likely to be experiencing significant emotional and behavioral reactions from situational sources that can alter the outcome of neurobehavioral assessment. Anxiety, depression, sleep disturbances, fatigue, worry, obsessive thoughts, and distractibility may contribute to less than optimal performance on motor and cognitive neurobehavioral tasks, especially where speed and sustained concentration are important. Under stressful conditions, it may be extremely difficult to differentiate between neurotoxic and situational sources of observed functional impairment. Functional neurobehavioral tests are not well equipped to distinguish between impairment from neurotoxicity and from nonchemical variables. The use of functional tests in symptomatic populations requires great care in interpretation. The development of validity scales and other control procedures for assessing nonchemical influences on performance is greatly needed.

3.6.3. Cross-Species Extrapolation

Many neurobehavioral tests were developed according to constructs of human cognitive processes. The diverse measures of cognitive, sensory, and motor performance in humans are therefore not easily compared with neurobehavioral function in animals. While it may be possible to conceptually relate some animal and human neurobehavioral tests (e.g., grip strength or signal detection), many procedural differences prevent direct comparison between species.

A more direct extrapolation from animals to man might be possible if the tests were chosen on the basis of procedural similarity rather than on a conceptual basis (Anger, 1991). Stebbins and colleagues (1975) were successful in developing homologous procedures in nonhuman primates for the psychophysical evaluation of antibiotic ototoxicity. Efforts to develop comparable tests of memory and other neurobehavioral functions in animals and humans are under way (Stanton and Spear, 1990, Paule et al., 1990), and such efforts may aid in cross-species extrapolation. Other procedurally defined methods, such as Pavlovian conditioning (Solomon and Pendlebury, 1988), operant conditioning (Cory-Slechta, 1990), signal detection, and psychophysical scaling techniques (Stebbins and Coombs, 1975), could also be used to facilitate interspecies risk extrapolation. Deriving comparable neurobehavioral assessment methods in animals and humans that will allow a more straightforward extrapolation across species is of paramount importance for neurotoxicity risk assessment.

4. Methods to Assess Animal Neurotoxicity

4.1. Introduction

4.1.1. Role of Animal Models

Determining the risk posed to human health from chemicals requires information about the potential toxicological hazards and the expected levels of exposure. Some toxicological data can be derived directly from humans. Sources of such information include accidental exposures to industrial chemicals, cases of food-related poisoning, epidemiological studies, as well as clinical investigations. While human data are available from clinical trials for therapeutics and they provide the most direct means of determining effects of potentially toxic substances, for other categories of substances, it is generally difficult, expensive, and, in some cases, unethical to develop this type of information. Quite often, the nature and extent of available human toxicological data are too incomplete to serve as the basis for an adequate assessment of potential health hazards. Furthermore, for a majority of chemical substances human toxicological data are simply not available. Consequently, for most toxicological assessments it is necessary to rely on information derived from animal models, usually rats or mice. One of the primary functions of animal studies is to predict human toxicity prior to human exposure. In some cases, species phylogenetically more similar to human, such as monkeys or baboons, are used in neurotoxicological studies.

Biologically, animals resemble humans in many ways and can serve as adequate models for toxicity studies (Russell, 1991). This is particularly true with regard to the assessment of adverse effects to the nervous system, whereby animal models provide a variety of useful information that helps minimize exposure of humans to the risk of neurotoxicity. There are many approaches to testing for neurotoxicity, including whole animal (in vivo) testing and tissue/cell culture (in vitro) testing.

At present, in vivo animal studies currently serve as the principal approach to detect and characterize neurotoxic hazard and to help identify factors affecting susceptibility to neurotoxicity. Data from animal studies are used to supplement or clarify limited information obtained from clinical or epidemiological studies in humans, as well as provide specific types of information not readily

obtainable from humans due to ethical considerations. Frequently, results from animal studies are used to guide the design of toxicological studies in humans.

In vitro tests have been proposed as a means of complementing whole animal tests, which could ultimately reduce the number of animals used in routine toxicity testing. It also has been proposed that in vitro testing, when properly developed, may be less time-consuming and more cost-effective than in vivo assessments (Goldberg and Frazier, 1989; Atterwill and Walum, 1989). By understanding the biological structures or functions affected by toxic substances in vitro, it also may be possible to predict neurotoxicological effects in the whole animal. An added advantage of in vitro testing is the growing availability of human cell lines that could be used for directly assessing potential neurotoxic effects on human tissue. The currently available strategies for in vitro testing have certain limitations, including the inability to model neurobehavioral effects such as loss of memory or sensory dysfunction or to evaluate effectively the influence of organ system interactions (e.g., neuronal, endocrinological, and immunological) on the development and expression of neurotoxicity.

In using animal models to predict neurotoxic risk in humans, it is important to understand that the biochemical and physiological mechanisms that underlie human biological processes, particularly those involving neurological and psychological functions, are very complex and are sometimes difficult, if not impossible, to model exactly in a lower species. While this caveat does not preclude extrapolating the results of animal studies to humans, it does highlight the importance of using valid animal models in well-designed experimental studies.

4.1.2. Validity of Animal Models

Whether animal tests or methods actually measure what they are intended to measure, whether the data from such tests can be obtained reliably, and whether such data can be logically extrapolated to humans are problems for most disciplines in toxicology. Various proposals have been made for the standardization and validation of methods used in neurotoxicological research. It is generally agreed that validation is an ongoing process that establishes the credibility of a test, building an increasing level of confidence in the effective utility of any model of evaluation. The credibility of a method, as it applies to testing, is usually discussed within several different contexts, including construct validity, criterion validity, predictive validity, and detection accuracy.

Construct validity concerns the ability of a method to measure selectively a particular biological function and not other dimensions. Construct validity is frequently established empirically. For example, sensory dysfunction such as hearing loss is reported by humans exposed to some chemicals, and tests are designed to detect and quantify those changes. Such tests are designed to measure changes in auditory function, while other sensations are unaffected (Tilson, 1987; Moser, 1990).

Criterion validity refers to the ability of a method to measure a characteristic relative to some standard. For example, Horvath and Frantik (1973) noted that the significance of a test measurement as an index of an actual treatment effect should be validated relative to the effects of a defined reference substance or positive control. Furthermore, each specific test or type of effect may require an appropriate reference substance for which the given type of effect is a determining factor of the toxicity. Use of reference agents has obvious advantages in the assessment of unknown chemicals.

Predictive validity refers to the ability of a method to predict effects from an incomplete or partial data set. An animal model of neurotoxicity with good predictive validity would reliably predict neurotoxicity in humans, i.e., the animal to human extrapolation would be good. There are several examples in neurotoxicology where animal models have been developed based on neurotoxicological reports from humans. Presumably, the predictive validity of such models would enable detecting similar kinds of effects produced by uncharacterized chemicals having a similar mechanism of action.

It has been proposed (Tilson and Cabe, 1978) that the most logical approach to validate animal methods in neurotoxicology is to evaluate chemicals with and without known neurotoxicity in humans in tests designed for animals (predictive validity). By using such an approach, it is possible to generate a profile of effects characteristic of each type of neurotoxicant (criterion validity). This profile could then be used to assess the construct validity of various tests. That is, procedures assumed to measure the same neurobiological dimension should show similar effects; measures designed to detect changes in other functions should not be affected. This approach to test validation has been described as the multitrait-multimethod process of validation (Campbell and Fiske, 1959).

Of particular importance in establishing the credibility of a method is the accuracy of detecting a treatment-related effect (Gad, 1989). Accuracy is a function of two interacting elements, specificity and sensitivity. Specificity is the ability of a test to respond positively only when the toxic endpoint of interest is present. Sensitivity is the ability to detect a change when present. This aspect depends on the inherent design of the procedure and experiment. Increasing the specificity of a test may reduce the possibility of classifying a chemical as neurotoxic when, in fact, it is not (false positive), but it may increase the probability of missing a true neurotoxicant (false negative). Increasing sensitivity of a test may reduce the possibility of false negatives, but may increase the probability of false positives.

4.1.3. Special Considerations in Animal Models

4.1.3.1. Susceptible populations.

Like most other measures of toxicological effect, neurotoxic endpoints are subject to a number of experimental variables that may affect susceptibility to the biological effects of toxicants. In this regard, genetic variation (Festing, 1991) is a particularly important issue in neurotoxicology. For example, most neurotoxicological assessments are carried out with only one or two species. This may pose problems, however, since species may differ in sensitivity to neurotoxicants. For example, nonhuman primates are more sensitive than rats (Boyce et al., 1984) or mice (Heikkila et al., 1984) to the neurodegenerative effects of MPTP, a byproduct in the illicit synthesis of a meperidine analog (Langston et al., 1983). In the assessment of delayed neuropathology produced by some cholinesterase inhibitors, it is well known that hens are much more sensitive than rodents (Cavanagh, 1954; Abou-Donia, 1981, 1983). In addition, rat strains also may be differentially sensitive to some neurotoxicants (Moser et al., 1991). Although it is preferred that more than one species be tested, the cost required for routine multispecies testing must be considered. Whenever possible, the choice of animal models should take into account differences in species with regard to pharmacodynamic, genetic composition and sensitivity to neurotoxic agents.

In addition to species, other factors such as gender of the test animal must be taken into consideration. Some toxic substances may have a greater

neurotoxicological effect in one gender (Squibb et al., 1981; Matthews et al., 1990). Thus, screening evaluations frequently require both male and female animals. Another important variable is the age of the animal (Veronesi et al., 1990). Whether a chemical produces neurotoxicity may depend on the maturational stage of the organism (Rodier, 1986). Most preliminary assessments are designed to provide information on adults, which have the greatest probability of being exposed. However, populations undergoing rapid maturation or aged individuals may be especially vulnerable to neurotoxic agents. Longitudinal studies that assess both genders at any stage of development address many of the problems associated with differentially sensitive populations.

4.1.3.2. Dosing scenario.

The dosing strategy used in experimental studies is an important variable in the development and expression of neurotoxicity (WHO, 1986). Some neurotoxicants can produce neurotoxicity following a single exposure, while others require repeated dosing. Repeated dosing represents the typical pattern of human exposure to many chemical substances. Significant differences in response may occur when an acutely toxic quantity of material is administered over different exposure periods. For some neurotoxicants the onset of neurotoxicity can occur immediately after dosing, while others may require time after exposure for the toxicity to develop. Effects of repeated exposure may result in a progressive alteration in nervous system function or structure, while latent or residual effects may be discovered only in association with age-related changes or after suitable environmental or pharmacological challenge (Zenick, 1983; MacPhail et al., 1983). To ensure adequate assessment of neurotoxicity, study designs should include multiple dosing regimens, e.g., repeated exposure, with appropriate dose-to-response intervals of testing. Conduct of neurotoxicological evaluations in studies utilizing excessively toxic doses should be avoided.

4.1.3.3. Other factors.

There are a number of other factors that should be considered in the design and interpretation of studies using animal models (WHO, 1986). Design factors include such issues as using properly trained personnel to conduct the studies, the use of appropriate numbers of animals per group to achieve reliable statistical significance, and controlling the time-of-day variability. Time of testing relative to exposure is also important for assessing neurotoxic endpoints such as behavior, and experiments should be designed to generate a time course of effects, including recovery of function, if any. Housing is an important environmental design factor, because animals housed individually and animals housed in groups can respond differently to toxic agents. Temperature, as an experimental variable, may also affect the outcome of neurotoxicological studies. The responsiveness to some chemicals (e.g., triethyltin, methamphetamine) varies with ambient temperature (Dyer and Howell, 1982; Bowyer et al., 1992). Some neurobiological endpoints, such as sensory evoked potentials, can be influenced by the endogenous temperature of the animal (Dyer, 1987). Therefore, changes in body temperature, whether due to fluctuations in ambient temperature or to some chemically induced effect such as inhibition of sweating, can confound the interpretation of measures such as evoked responses unless proper controls are included in the experimental design.

Because a variety of other physiological changes can influence neuronal functions, it is important to recognize that chemical-related neurotoxicity could result from treatment-induced physiological changes, such as altered nutritional state (WHO, 1986). As part of a neurotoxicological profile, correlative measures, such as relative and absolute organ weights, food and water consumption, and body weight and weight gain, may be signs of physiological change associated with systemic toxicity and may be useful in determining the relative contribution of general toxicity.

4.1.3.4. Statistical considerations.

Experimental designs for neurotoxicological studies are frequently complex, with two or more major variables (e.g., gender, time of testing) varying in any single experiment. In addition, such studies typically generate varying types of data, including continuous, dichotomous, and rank-order data. Knowledge and experience in experimental design and statistical analyses are important. There are several key statistical concepts that should be understood in neurotoxicological studies (WHO, 1986; Gad, 1989). The power, or probability, of a study to detect a true effect is dependent on the size of the study group, the frequency of the outcome variable in the general population, and the magnitude of effect to be identified. Statistical evaluation of a treatment-related effect involves the consideration of two factors or types of errors to be avoided. A Type I error refers to the attribution of an exposure-related neurotoxicological effect when none has occurred (false positive), while a Type II error refers to the failure to attribute an effect when an exposure-related effect has actually occurred (false negative). In general, the probability of a Type I error should not exceed 5 percent and the probability of a Type II error should not exceed 20 percent. Power is defined as one minus the probability of a Type II error.

Determination of power also requires knowledge of the difference in magnitude of outcome measures observed between exposed and control groups and the variability of the outcome measure among subjects. The sample size required to achieve a given level of statistical power increases as variability increases or the difference between groups decreases.

Continuous data (i.e., magnitude, rate, amplitude), if found to be normally distributed, can be analyzed with a general linear model using a grouping factor of dose and, if necessary, repeated measures across time. Post hoc comparisons between control and other treatment groups can be made following tests for overall significance. In the case of multiple endpoints within a series of evaluations, correction for multiple observations (e.g., Bonferroni's) might be necessary.

Descriptive data (categorical) and rank data can be analyzed using standard nonparametric techniques. In some cases, if it is believed that the data fit the linear model, the categorical data 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.

4.2. Tiered Testing in Neurotoxicology

The utility of tiered testing as an efficient and cost-effective approach to evaluate chemical toxicity, including neurotoxicity, has been recognized (NRC, 1975). Briefly, first-tier tests are designed to determine the presence or absence of neurotoxicity, while second-tier tests characterize the neurotoxic effect (NRC, 1992). There are at least two aspects of tiered testing, one involving the type of test used (Tilson, 1990a) and the other involving the dosing regimen (Goldberg and Frazier, 1989).

4.2.1. Type of Test

Tests designed to measure the presence or absence of an effect are usually different from those used to assess the degree of toxicity or the lowest exposure level required to produce an effect (Tilson, 1990a). Screening procedures are first-tier tests that typically permit the testing of many groups of animals. Such procedures may not require extensive resources and are usually simple to perform. However, these techniques may be labor intensive, provide subjective measures, yield semiquantitative data, and may not be as sensitive to subtle effects as those designed to characterize neurotoxic effects or second-tier tests. Specialized tests are usually more sensitive and employed in studies concerning mechanisms of action or the estimation of the lowest effective dose. Such testing procedures are usually referred to as secondary tests and may require special equipment and more extensive resources. Secondary tests are usually quantitative and yield graded or continuous data amenable to routine parametric statistical analyses.

Testing at the first tier is used to determine if a chemical might produce neurotoxicity following exposure, i.e., hazard detection. In this case, there may be little existing information concerning the neurotoxic potential of an agent. Examples of first-tier tests include functional observational batteries (FOB), including an evaluation of motor activity and routine neurohistopathology. For some chemicals or types of chemicals, there may be a specific interest in screening for a particular presumed mechanism of toxicity (e.g., inhibition of cholinesterase or neurotoxic esterase) or neurobiological response (e.g., a site-specific neuronal degeneration). In these cases, specific neurochemical or neuropathological endpoints can be used in conjunction with first-tier tests. It is desirable that tests selected for use in hazard detection provide a suitable level of sensitivity using the smallest number of animals necessary.

A decision to test at the next tier is based on data suggesting that an agent produces neurotoxicity. The information used to make a decision to test a chemical at the secondary level can come from a variety of sources, including neurotoxicological data already in the literature, structure-activity relationships, data from first-tier testing, or following reports of specific neurotoxic effects in humans exposed to the agent. Testing at the secondary level includes detailed neuropathological evaluation as well as specific behavioral tests, e.g., procedures to assess learning and memory, or sensory function. Tests at the second tier usually measure the most sensitive endpoints of neurotoxicity, and are the most suitable for determining the no observable adverse effect level or benchmark dose. At this stage of testing, the use of a second species is considered to address the issue of cross-species extrapolation. At the present time, tiered testing approaches in neurotoxicology rely heavily on functional endpoints. It is possible that future testing protocols will employ a different strategy as more information concerning neurotoxic mechanisms of action become available and biologically based dose-response models are developed.

4.2.2. Dosing Regimen

Goldberg and Frazier (1989) have indicated that first-tier evaluations identify effects of substances following acute or repeated exposure over a wide range of doses. Measures are simple, focused on detection of effects, and results are used to help establish parameters for the second tier of testing. The subsequent stage(s) of tier testing are designed to characterize more fully the toxicity of repeated dosing. In this case, animals are exposed repeatedly or continuously to define the scope of toxicity, including latent or delayed effects, development of tolerance, and the reversibility of adverse effects. The subsequent stage(s) of testing also provide information about specific effects or study mechanisms of neurotoxicity. This tier uses methods appropriate to characterize the effects observed in the first tier of testing.

4.3. Endpoints of Neurotoxicity

4.3.1. Introduction

As applied to the safety assessment of chemical substances, neurotoxicity is any adverse change in the development, structure, or function of the central and peripheral nervous system following exposure to a chemical agent (Tilson, 1990b). Measures used in animal neurotoxicological studies are designed to assess these changes. Neurotoxicity can be described at multiple levels of organization, including chemical, anatomical, physiological, or behavioral levels. At the chemical level, for example, a neurotoxic substance might inhibit protein or transmitter synthesis, alter the flow of ions across cellular membranes, or prevent release of neurotransmitter from nerve terminals. Anatomical changes may include destruction of the neuron, axon, or myelin sheath. At the physiological level, neuronal responsiveness to stimulation might be enhanced by a decrease of inhibitory thresholds in the nervous system. Chemical-induced effects at the behavioral level can involve a variety of alterations in motor, sensory, or cognitive function, including increases or decreases in frequency or accuracy of responding. Although behavioral and neurophysiological endpoints may be very sensitive indicators of neurotoxicity, they can be influenced by other factors. The uncertainties associated with data from functional endpoints can be reduced if interpreted within the context of other neurotoxicological measures (neurochemical or neuropathological) and systemic toxicity endpoints, particularly if such measures are taken concurrently. Behavioral effects that reflect an indirect effect secondary to systemic toxicities may also be considered adverse. Table 4–1 provides examples of potential endpoints of neurotoxicity at the behavioral, physiological, chemical, and structural levels.

TABLE 4–1.—EXAMPLES OF POTENTIAL ENDPOINTS OF NEUROTOXICITY

Behavioral Endpoints:
Absence or altered occurrence, magnitude, or latency of sensorimotor reflex
Altered magnitude of neurological measurements, such as grip strength or hindlimb splay
Increases or decreases in motor activity
Changes in rate or temporal patterning of schedule-controlled behavior
Changes in motor coordination, weakness, paralysis, abnormal movement or posture, tremor, ongoing performance
Changes in touch, sight, sound, taste, or smell sensations
Changes in learning and memory
Occurrence of seizures
Altered temporal development of behaviors or reflex responses
Autonomic signs
Neurophysiological Endpoints:
Change in velocity, amplitude, or refractory period of nerve conduction
Change in latency or amplitude of sensory-evoked potential
Change in EEG pattern or power spectrum
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
Decreases in brain AChE
Inhibition of NTE
Altered developmental patterns of neurochemical systems
Altered proteins (c fos, substance P)
Structural Endpoints:

TABLE 4–1.—EXAMPLES OF POTENTIAL ENDPOINTS OF NEUROTOXICITY—Continued

Accumulation, proliferation, or rearrangement of structural elements
Breakdown of cells
GFAP increases (adult)
Gross changes in morphology, including brain weight
Discoloration of nerve tissue
Hemorrhage in nerve tissue

4.3.2. Behavioral Endpoints

Neurotoxicants produce a wide array of functional deficits, including motor, sensory, and learning or memory dysfunction (WHO, 1986; Tilson and Mitchell, 1984). Many procedures have been devised to assess overt as well as relatively subtle changes in those functions; hence their applicability to the detection of neurotoxicity and to hazard characterization. Many of the behavioral tests have been developed and validated with well-characterized neurotoxicants. Behavioral tests and agents that affect them have been reviewed recently (WHO, 1986; Cory-Slechta, 1989). Examples of such tests, the nervous system function being measured, and neurotoxicants known to affect these measures are listed in Table 4–2.

TABLE 4–2. EXAMPLES OF SPECIALIZED TESTS TO MEASURE NEUROTOXICITY

Function	Procedure	Representative-agents
Neuromuscular:		
Weakness	Grip strength; swimming endurance; suspension from rod; discriminative motor function; hindlimb splay.	n-hexane, methyl butylketone, carbaryl.
Incoordination	Rotorod, gait measurements	3-acetylpyridine, ethanol.
Tremor	Rating scale, spectral analysis	Chlordecone, Type I pyrethroids, DDT.
Myoclonia, spasms	Rating scale, spectral analysis	DDT, Type II pyrethroids.
Sensory:		
Auditory	Discriminated conditioning Reflex modification	Toluene, trimethyltin.
Visual toxicity	Discriminated conditioning	Methyl mercury.
Somatosensory toxicity.	Discriminated conditioning	Acrylamide.
Pain sensitivity	Discriminated conditioning (titration); functional observational battery	Parathion.
Olfactory toxicity	Discriminated conditioning	3-methylindole methylbromide.
Learning/Memory:		
Habituation	Startle reflex	Diisopropyl-flurophosphate (DFP).
Classical conditioning	Nictitating membrane	Aluminum.
	Conditioned flavor aversion	Carbaryl.
	Passive avoidance	Trimethyltin, IDPN.
	Olfactory conditioning	Neonatal trimethyltin.
Operant or instrumental conditioning.	One-way avoidance	Chlordecone.
	Two-way avoidance	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.
	Visual discrimination learning	Lead.

4.3.2.1. Functional observational batteries.

Functional observational batteries are first-tier tests designed to detect and quantify major overt behavioral, physiological, and other neurotoxic effects (Moser, 1989). A number of batteries have been used (Tilson and Moser, 1992), each consisting of tests generally intended to evaluate various aspects of sensorimotor function. Most FOB are similar to clinical neurological examinations that rate presence or absence and, in some cases, the relative degree of neurological signs. A typical FOB, as summarized in Table 4–3, 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.

TABLE 4–3.—SUMMARY OF MEASURES IN THE FUNCTIONAL OBSERVATIONAL BATTERY AND THE TYPE OF DATA PRODUCED BY EACH

Home cage and open field	Manipulative	Physiologic
Posture (D)	Ease of removal (R)	Body temperature (I)
Convulsions, tremors (D)	Handling reactivity (R)	Body weight (I)
Palpebral closure (R)		
Lacrimation (R)	Approach response (R)	
Piloerection (Q)	Click response (R)	
Salivation (R)	Touch response (R)	
Vocalizations (Q)	Tail pinch response (R)	
Rearing (C)	Righting reflex (R)	

TABLE 4–3.—SUMMARY OF MEASURES IN THE FUNCTIONAL OBSERVATIONAL BATTERY AND THE TYPE OF DATA PRODUCED BY EACH—Continued

Urination (C)	Landing foot splay (I)
Defecation (C)	Forelimb grip strength (I)
Gait (D, R)	Hindlimb grip strength (I)
Arousal (R)	Pupil response (Q)
Mobility (R)	
Stereotypy (D)	
Bizarre behavior (D)	

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

The major advantages of FOB tests are that they can be administered within the context of other ongoing toxicological tests and provide some indication of the possible neurological alterations produced by exposure. Potential problems include insufficient interobserver reliability, difficulty in defining certain endpoints, and the tendency toward observer bias. The latter can be controlled by using observers unaware of the actual treatment of the subjects. Some FOB tests may not be very sensitive to agent-induced sensory loss (i.e., vision, audition) or alterations in cognitive or integrative processes such as learning and memory. FOB data may be used to trigger experiments performed at the next tier of testing.

FOB data may be interval, ordinal, or continuous (Creason, 1989). The relevance of statistically significant test results from an FOB is judged according to the number of signs affected, the dose(s) at which neurotoxic signs are observed, and the nature, severity, and persistence of the effects. Data from the FOB may provide presumptive evidence of adverse effects and neurotoxicity. If only a few unrelated measures in the FOB are affected or the effects are unrelated to dose, there is less concern about neurotoxic potentials of a chemical. If dose is associated with other overt signs of toxicity, including systemic toxicity, large decreases in body weight, or debilitation, the data must be interpreted carefully. In cases where several related measures in a battery of tests are affected and the effects appear to be dose dependent, the level of concern about the potential of a chemical is higher.

4.3.2.2. Motor activity.

Movement within a defined environment is a naturally occurring response and can be affected by environmental agents. Motor activity represents a broad class of behaviors involving coordinated participation of sensory, motor, and integrative processes. Motor activity measurements are noninvasive and can be used to evaluate the effects of acute and repeated exposure to chemicals (MacPhail et al., 1989). Motor activity measurements have also been used in humans to evaluate disease states, including disorders of the nervous system (Goldstein and Stein, 1985). The assessment of motor activity is often included in first-tier evaluations, either as part of the FOB or as a separate quantitated measurement.

There are many different types of activity measurement devices, differing in size, shape, and method of movement detection (MacPhail et al., 1989). Because of the accuracy and ease of calibration, devices with photocells are widely used. In general, situating the apparatus to minimize extraneous noise, movements, or lights usually requires that the recording devices be placed in light- and sound-attenuating chambers during the testing period. A number of different factors, including age, gender, and time of day, can affect motor activity, and should be controlled or counterbalanced. Different strains of animals may have significantly different basal levels of activity, making comparisons across studies difficult. A major factor in activity studies is the duration of the testing session. Motor activity levels are generally highest at the beginning of the session and decrease to a low level throughout the session. The rate of decline during the test session is frequently termed "habituation."

Motor activity measurements are typically included as part of a battery of tests to detect or characterize neurotoxicity. Agent-induced alterations in motor activity associated with overt signs of toxicity (e.g., loss of body weight, systemic toxicity) or occurring in non-dose-related fashion are of less concern than changes that are dose dependent, related to structural or other functional changes in the nervous system, or occur in the absence of life-threatening toxicity and are generally convincing evidence of neurotoxicity.

4.3.2.3. Neuromotor function.

Motor dysfunction is a common neurotoxic effect, and many different types of tests have been devised to measure time- and dose-dependent effects. Anger (1984) reported 14 motor effects of 89 substances, which could be classified into four categories: weakness, incoordination, tremor, and myoclonia or spasms. Chemical-induced changes in motor function can be determined with relatively simple techniques such as the FOB. More specialized tests to assess weakness include measures of grip strength, swimming endurance, suspension from a hanging rod, discriminitive motor function, and hindlimb splay. Rotarod and gait assessments measure incoordination, while rating scales and spectral analysis techniques quantify tremor and other abnormal movements (Tilson and Mitchell, 1984).

An example of a second-tier procedure to assess motor function has been described by Newland (1988), who trained squirrel monkeys to hold a bar within specified limits (i.e., displacement) to receive positive reinforcement. The bar was also attached to a rotary device, which allowed measurement of chemical-induced tremor. Spectral analysis was used to characterize the tremor, which was found to be similar to that seen in humans exposed to neurotoxicants or with such neurologic diseases as Parkinson's disease.

Incoordination and performance changes can be assessed with procedures that measure chemical-induced alterations in force (Fowler, 1987). The accuracy of performance may reflect neuromotor function and is sensitive to the debilitating effects of many psychoactive drugs (Walker et al., 1981; Newland, 1988). Gait, an index of coordination, has been measured in rats under standardized conditions and can be a sensitive indication of specific

damage to the basal ganglia and motor cortex (Hruska et al., 1979) as well as damage to the spinal cord and peripheral nervous system.

Procedures to characterize chemical-induced motor dysfunction have been used extensively in neurotoxicology. Most require preexposure training (including alterations of motivational state) of experimental animals, but such tests might be useful, in as much as similar procedures are often used in assessing humans.

4.3.2.4. Sensory function.

Alterations in sensory processes (e.g., paresthesias and visual or auditory impairments) are frequently reported signs or symptoms in humans exposed to toxicants (Anger, 1984). Several approaches have been devised to measure sensory deficits. Data from tests of sensory function must be interpreted within the context of changes in body weight, body temperature, and other physiological endpoints. Furthermore, many tests assess the behavioral response of an animal to a specific sensory stimulus; such responses are usually motor movements that could be directly affected by chemical exposure. Thus, care must be taken to determine whether proper controls were included to eliminate the possibility that changes in response to a sensory stimulus may have been related to agent-induced motor dysfunction.

Several first-tier testing procedures have been devised to screen for overt sensory deficits. Many rely on orientation or the response of an animal to a stimulus. Such tests are usually included in the FOB used in screening (e.g., tail-pinch or click responses). Responses are usually recorded as being either present, absent, or changed in magnitude (Moser, 1989; O'Donoghue, 1989). Screening tests for sensory deficits are typically not suitable to characterize chemical-induced changes in acuity or fields of perception. The characterization of sensory deficits usually necessitates psychophysical methods that study the relationship between the physical dimensions of a stimulus and the behavioral response it generates (Maurissen, 1988).

One second-tier approach to the characterization of sensory function involves the use of reflex-modification techniques (Crofton, 1990). Chemical-induced changes in the stimulus frequency or threshold required to inhibit a reflex are taken as possible changes in sensory function. Prepulse inhibition has been used only recently in neurotoxicology (Fechter and Young, 1983) and can be used to assess sensory function in humans as well as in experimental animals.

Various behavioral procedures require that a learned response occur only in the presence of a specific stimulus (i.e., discriminated or conditioned responding). Chemical-induced changes in sensory function are determined by altering the physical characteristics of the stimulus (e.g., magnitude or frequency) and measuring the alteration in response rate or accuracy. In an example of the use of a discriminated conditional response to assess chemical-induced sensory dysfunction, Maurissen et al. (1983) trained monkeys to respond to the presence of a vibratory or electric stimulus applied to the fingertip. Repeated dosing with acrylamide produced a persistent decrease in vibration sensitivity; sensitivity to electric stimulation was unimpaired. That pattern of sensory dysfunction corresponded well to known sensory deficits in humans. Discriminated conditional response procedures have been used to assess the ototoxicity produced by toluene (Pryor et al., 1983) and the visual toxicity produced by methylmercury (Merigan, 1979).

Procedures to characterize chemical-induced sensory dysfunction have been used often in neurotoxicology. As in the case of most procedures designed to characterize nervous system dysfunction, training and motivational factors can be confounding factors. Many tests designed to assess sensory function for laboratory animals can also be applied with some adaptation to humans.

4.3.2.5. Learning and memory.

Learning and memory disorders are neurotoxic effects of particular importance. Impairment of memory is reported fairly often by adult humans as a consequence of toxic exposure. Behavioral deficits in children have been caused by lead exposure (Smith et al., 1989), and it is hypothesized (Calne et al., 1986) that chronic low-level exposure to toxic agents may have a role in the pathogenesis of senile dementia.

Learning can be defined as an enduring change in the mechanisms of behavior that results from experience with environmental events (Domjan and Burkhard, 1986). Memory is a change that can be either short-lasting or long-lasting (Eckerman and Bushnell, 1992). Alterations in learning and memory must be inferred from changes in behavior. However, changes in learning and memory must be separated from other changes in behavior that do not involve cognitive or associative processes (e.g., motor function, sensory capabilities, and motivational factors), and an apparent toxicant-induced change in learning or memory should be demonstrated over a range of stimuli and conditions. Before it is concluded that a toxicant alters learning and memory, effects should be confirmed in a second learning procedure. It is well known that lesions in the brain can inhibit learning. It is also known that some brain lesions can facilitate some types of learning by removing behavioral tendencies (e.g., inhibitory responses due to stress) that moderate the rate of learning under normal circumstances. A discussion of learning procedures and examples of chemicals that can affect learning and memory have appeared in recent reviews (Heise, 1984; WHO, 1986; Peele and Vincent, 1989).

One simple index of learning and memory, which can be measured as a first-tier endpoint, is habituation. Habituation is defined as a gradual decrease in the magnitude or frequency of a response after repeated presentations of a stimulus. A toxicant can affect habituation by increasing or decreasing the number of stimulus presentations needed to produce response decrements (Overstreet, 1977). Although habituation is a very simple form of learning, it can also be perturbed by a number of chemical effects not related to learning.

A more complicated approach to studying the effects of a chemical on learning and memory involves the pairing of a novel stimulus with a second stimulus that produces a known, observable, and quantifiable response (i.e., classical "Pavlovian" conditioning). The novel stimulus is known as the conditioned stimulus, and the second, eliciting stimulus is the unconditioned stimulus. With repeated pairings of the two stimuli, the conditioned stimulus comes to elicit a response similar to the response elicited by the unconditioned stimulus. The procedure has been used in behavioral pharmacology and, to a lesser extent, in neurotoxicology. Neurotoxicants that interfere with learning and memory would alter the number of presentations of the pair of stimuli required to produce conditioning or learning. Memory would be tested by determining how long after the last presentation of the two stimuli the conditioned stimulus would still elicit a response (Yokel, 1983). Other classically conditioned responses known to be affected by psychoactive or neurotoxic agents are conditioned taste aversion (Riley and Tuck, 1985) and conditioned suppression (Chiba and Ando, 1976).

Second-tier procedures to assess learning or memory typically involve

the pairing of a response with a stimulus that increases the probability of future response through reinforcement. Response rate can be increased by using positive reinforcement or removing negative reinforcement. Learning is usually assessed by determining the number of presentations or trials needed to produce a defined frequency of response. Memory can be defined specifically as the maintenance of a stated frequency of response after initial training. Neurotoxicants may adversely affect learning by increasing or decreasing the number of presentations required to achieve the designated criterion. Decrements in memory may be indicated by a decrease in the probability or frequency of a response at some time after initial training. Toxicant-induced changes in learning and memory should be interpreted within the context of possible toxicant-induced changes in sensory, motor, and motivational factors. Examples of instrumental learning procedures used in neurotoxicology are repeated acquisition (Schrot et al., 1984), passive and active avoidance, Y-maze avoidance, spatial mazes (radial-arm maze), and delayed matching to sample (Heise, 1984; WHO, 1986; Tilson and Mitchell, 1984).

4.3.2.6. Schedule-controlled behavior.

Another type of second-tier procedure is schedule-controlled operant behavior (SCOB), which involves the maintenance of behavior (performance) by response-dependent reinforcement (Rice, 1988). Different patterns of behavior and response rates are controlled by the relationship between response and later reinforcement. SCOB affords a measure of learned behavior and with appropriate experimental design may be useful for studying chemical-induced effects on motor, sensory, and cognitive function.

The primary endpoints for evaluation are agent-induced changes in response rate or frequency and the temporal pattern of responding. Response rate is usually related to an objective response, such as lever press or key peck, and differs according to the schedule of reinforcement. Response rates are expressed per unit of time. For some classes of chemicals, the direction of an effect on response rate can differ between low and high doses. Agent-induced changes in temporal pattern of responding can occur independently of changes in the rate and require analysis of the distribution of responses relative to reinforcement schedule.

SCOB has been used to study the effects of psychoactive drugs on behavior and is sensitive to many neurotoxicants, including methylmercury, solvents, pesticides, acrylamides, carbon monoxide, and organic and inorganic lead (Paule and McMillan, 1984; MacPhail 1985; Cory-Slechta, 1989; Rice, 1988). The experimental animal often serves as its own control, and the procedure provides an opportunity to study a few animals extensively over a relatively long period. SCOB typically requires motivational procedures, such as food deprivation, and training sessions are usually required to establish a stable baseline of responding. Because of its sensitivity to neuroactive chemicals, SCOB has great potential for use in second-tier assessments.

4.3.3. Neurophysiological Endpoints of Neurotoxicity

Neurophysiological studies are those that assess function either directly through measurements of the electrical activity of the nervous system (electrophysiology) or indirectly through measurements of peripheral organ functions controlled or modulated by the nervous system (general physiology) (Dyer, 1987). When performed properly, neurophysiological techniques provide information on the integrity of defined portions of the nervous system. Many of the endpoints used in animals have also been used in humans to determine chemical-induced alterations in neurophysiological function.

The term "electrophysiology" refers to the set of neurophysiological procedures that study neural function through the direct measurement of the electrical activity generated by the nervous system (Dyer, 1987). A variety of electrophysiological procedures are available for application to neurotoxicological problems, which 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. The latter types of procedures have historically been used in studies to detect or characterize the potential neurotoxicity of agents of regulatory interest. Several macroelectrode procedures are discussed below.

4.3.3.1. Nerve conduction studies.

Nerve conduction studies are generally performed on peripheral nerves and can be useful in investigations of possible peripheral neuropathy. Most peripheral nerves contain mixtures of both individual sensory and motor nerve fibers, which may or may not be differentially sensitive to neurotoxicants. It is possible to distinguish sensory from motor effects in peripheral nerve studies by measuring activity in purely sensory nerves such as the sural to study sensory effects or by measuring the muscle response evoked by nerve stimulation to measure motor effects. While a number of endpoints can be recorded, the most commonly used variables are (1) Nerve conduction velocity, and (2) response amplitude. In well-controlled studies, decreases in nerve conduction velocity typically are evidence of neurotoxicity (Dyer, 1987). While a decrease in nerve conduction velocity is a reliable measure of demyelination, it frequently occurs rather late 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 necessarily rule out peripheral axonal degeneration if other signs of peripheral nerve dysfunction are present. Increases in conduction velocity of adult organisms following treatment with neurotoxic compounds, in the absence of hypothermia, are atypical responses and may, in fact, reflect experimental or statistical errors. Decreases in response amplitude reflect a loss of active nerve fibers, and may occur prior to decreases in conduction velocity in the course of peripheral neuropathy. Hence changes in response amplitude may be more sensitive measurements of axonal degeneration than conduction velocity. Measurements of response amplitude, however, are more variable and require careful experimental techniques, a larger sample size, and greater statistical power than measurements of velocity to detect changes. Alterations in peripheral nerve function are associated with abnormal peripheral sensations such as numbness, tingling, or burning or with motor impairments such as weakness. Examples of compounds that alter peripheral nerve function in humans or experimental animals at some level of exposure include acrylamide, carbon disulfide, hexacarbons, lead, and some organophosphates.

4.3.3.2. Sensory evoked potentials.

Sensory evoked potentials are electrophysiological procedures that involve measuring the response elicited by the presentation of a defined sensory stimulus such as a tone, a light, or a brief electrical pulse to the skin. Sensory evoked potentials reflect sensory function, and can be used to

investigate visual, auditory, or somatosensory (body sensation) systems (Rebert, 1983; Mattsson and Albee, 1988). The data are in the form of a voltage record over time, which can be quantified in several ways. Commonly, the positive and negative voltage peaks are identified and measured as to their latency (time from stimulus onset) and amplitude (voltage).

Changes in peak amplitudes or equivalent measures reflect changes in the magnitude of the neural population that is responsive to stimulation. Both increases and decreases in amplitude are possible following exposure to neurotoxicants because (1) The brain normally operates in a careful balance between excitatory and inhibitory systems, and disruption of this balance can produce either positive or negative shifts in the voltages recorded in evoked potential experiments, and (2) excitatory or inhibitory neural activity is translated into a positive or negative deflection in the sensory evoked potential depending on the physical orientation of the electrode with respect to the tissue generating the response, which is frequently unknown. Within any given sensory system, the neural circuits that generate the different evoked potential peaks differ as a function of peak latency. In general, early latency peaks reflect the transmission of afferent sensory information, and changes in either the latency or amplitude of these peaks generally indicate a neurotoxic change 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. Thus, the neurotoxicological significance of changes in later latency evoked potential peaks must be interpreted in light of the behavioral status of the subject.

4.3.3.3. Convulsions.

Observable behavioral convulsions in animals may be indicative of central nervous system seizure activity. However, behavioral convulsions that occur only at lethal or near lethal dose levels may reflect an indirect effect secondary to systemic toxicity and not directly on the nervous system. Convulsions occurring at dose levels that are clearly sublethal, and in the absence of apparent systemic toxicity, are more likely due to a direct effect on the nervous system. In such cases, neurophysiological recordings of electrical activity in the brain that are indicative of seizures may provide additional evidence of direct neurotoxicity. In addition to producing seizures, chemicals may also affect seizure susceptibility, altering the frequency, severity, duration, or threshold for eliciting seizures produced through other means. Such changes can occur after acute exposure or after repeated exposure to dose levels below the acute threshold, and are considered neurotoxic. Agents that produce convulsions include lindane, DDT, pyrethroids, and trimethyltin (WHO, 1986). Some agents, including many solvents, act to raise the threshold for eliciting seizures through other means or otherwise act to reduce the severity or duration of the elicited convulsions. These agents are difficult to classify as neurotoxic based on such data, but frequently have other effects on which a determination of neurotoxic potential can be based.

4.3.3.4. 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 the use of EEG in either setting is the relationship between specific patterns of EEG waveforms and specific behavioral states. Because states of alertness and the 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 following exposure to certain chemical classes (e.g., organophosphates). Changes in the pattern of the EEG can be elicited by stimuli producing arousal (e.g., lights, sounds) and neuroactive drugs. In studies with toxicants, changes in EEG pattern can sometimes precede alterations in other objective signs of neurotoxicity. EEG experiments must be done under highly controlled conditions, and the neurotoxicological significance of chemical-induced changes in the EEG in the absence of other signs of neurotoxicity must be considered on a case-by-case basis. Many chemicals, including metals, solvents, and pesticides, would be expected to affect the EEG.

4.3.3.5. Electromyography (EMG).

EMG involves making electrical recordings from muscle and has been used extensively in human clinical studies in the diagnosis of certain diseases of the muscle (WHO, 1986). Changes in the EMG include amplitude and firing frequency of spontaneous firing; evoked muscle responses to nerve stimulation can be used to study alterations in the neuromuscular junction. EMG has been used to study toxicant-induced changes in neuromuscular function, including organophosphate insecticides, methyl n-butyl ketone, and botulinum and tetanus toxin.

4.3.3.6. Spinal reflex excitability.

Segmental spinal monosynaptic and polysynaptic reflexes are relatively simple functions in the central nervous system that can be evaluated by quantitative techniques (WHO, 1986). Many of the procedures used in animals are similar to procedures used clinically to perform neurological tests in humans. One approach infers the functional state of a reflex arc from either the latency and magnitude of the reflex response evoked by stimuli of predetermined intensity or from the stimulus intensity required to elicit a detectable response (i.e., the threshold). This approach is used best in a screening context and the significance of effects in this test should be considered on a case-by-case basis.

A second more involved approach records electrophysiologically the time required for a stimulus applied to a peripheral nerve to reach the spinal cord and return to the site of the original stimulation. Data from this procedure can indicate the excitability of the motoneuron pool, an effect seen with many volatile solvents. Although this approach is more invasive and time-consuming than the noninvasive procedure, it provides better data concerning the possible site of action. In addition, the manner in which the invasive procedure is carried out (i.e., in decerebrated animals) precludes repeated testing on the same animal. The significance of effects in this procedure should also be considered on a case-by-case basis.

4.3.4. Neurochemical Endpoints of Neurotoxicity

Neuronal function within the nervous system is dependent on synthesis and release of specific neurotransmitters and activation of their receptors in specific neuronal pathways. With few exceptions, neurochemical measurements are invasive and therefore used infrequently in human risk assessment. There are many different neurochemical endpoints that could be measured in neurotoxicological studies (Bondy, 1986; Mailman, 1987; Morell and

Mailman, 1987). Neurotoxicants can interfere with the ionic balance of a neuron, act as a cytotoxicant after being transported into a nerve terminal, block uptake of neurotransmitter precursors, act as a metabolic poison, overstimulate receptors, block transmitter release, and inhibit transmitter degradation. Table 4–4 lists several chemicals with known neurochemical effects. Many neuroactive agents can increase or decrease neurotransmitter levels in the brain. Dose-related changes on these endpoints may indicate a chemical effect on the nervous system, but the neurotoxicological significance of such changes must be interpreted in the context of other signs of neurotoxicity.

TABLE 4–4.—NEUROTOXICANTS WITH KNOWN NEUROCHEMICAL MECHANISMS

Site of attack	Examples
1. Neurotoxicants acting on ionic balance	
A. Inhibit sodium entry	Tetrodotoxin.
B. Block closing of sodium channel	p,p¹—DDT, pyrethroids (I).
C. Increase permeability to sodium	Batrachotoxin.
D. Increase intracellular calcium	Chlordecone.
2. Cytotoxicants—depend on uptake into nerve terminal	MPTP.
3. Uptake blockers	Hemicholinium.
4. Metabolic poisons	Cyanide.
5. Receptor hyperactivators	Domoic acid.
6. Transmitter release (ACh) blockers	Botulinum toxin.
7. Transmitter degradation (ACh) inhibitors	Organophosphates, carbamates.
8. Microtubule disruptors	Vincristine.

Some chemicals, such as the organophosphate and carbamate insecticides, are known to interfere with a specific enzyme, acetylcholinesterase (AChE) (Costa, 1988). Inhibition of this enzyme in brain may be considered evidence of neurotoxicity, whereas decreases in AChE in the blood, which can be easily determined in humans, are only suggestive of a neurotoxic effect. A subset of organophosphate agents produces organophosphate-induced delayed neuropathy (OPIDN) after acute or repeated exposure. Neurotoxic esterase (or neuropathy target enzyme, NTE) has been associated with agents that produce OPIDN (Johnson, 1990).

The ultimate functional significance of many biochemical changes is not known; therefore it may be difficult to determine if a specific biochemical change can be considered adverse or convincing evidence of neurotoxicity. Any such change, however, is potentially adverse and each determination of adversity requires a judgment to be made. Likewise, the absence of specific biochemical testing protocols does not mean biochemical changes are of no concern, but instead reflects a lack of understanding of the significance of changes at the biochemical level.

4.3.5. Structural Endpoints of Neurotoxicity

The central nervous system (brain and spinal cord) comprises nerve cells or neurons, which consist of a neuronal body, axon, and dendritic processes. Various types of neuropathological lesions may be classified according to their nature or the site where they are found (WHO, 1986; Krinke, 1989; Griffin, 1990). Lesions may be classified as neuropathy (changes in the neuronal body), axonopathy (changes in the axons), myelinopathy (changes in the myelin sheaths), neurodegeneration (changes in the nerve terminals), and peripheral neuropathy (changes in the peripheral nerves). For axonopathies, a more precise location of the changes should be described (i.e., proximal, central, or distal axonopathy). In some cases, agents produce neuropathic conditions that resemble naturally occurring neurodegenerative disorders in humans (WHO, 1986). Table 4–5 lists examples of such chemicals, their known site of action, the type of neuropathology produced, and the disease or condition that each typifies.

TABLE 4–5.—EXAMPLES OF KNOWN NEUROPATHIC AGENTS

Site of attack	Neuropathology	Corresponding neurotoxicant	Disease or neurodegenerative condition
Neuron cell body	Neuronopathy	Methylmercury	Minamata disease.
		A.E.T.T.	Ceroid lipofuscinoses.
		Quinolinic acid	Huntington's disease.
		3-acetylpridine	Cerebellar ataxia.
		Aluminum	Alzheimer's disease.
Nerve terminal	Neurodegeneration	MPTP	Parkinson's disease.
Schwann cell myelin	Myelinopathy	Lead Buckthorn toxin.	Neuropathy of metachromatic leukodystrophy.
Central-peripheral distal axon	Distal axonopathy	Acrylamide Hexacarbons Carbon disulfide.	Vitamin deficiency.
Central axons	Central axonopathy	Clioquinol	Subacute myelooptico-neuropathy.
Proximal axon	Proximal axonopathy	B,B'-imminodiproprionitrile.	Motor neuron disease.

In general, chemical effects lead to two types of primary cellular alteration: (1) the accumulation, proliferation, or rearrangement of structural elements (e.g., intermediate filaments, microtubules) or organelles (mitochondria) and (2) the breakdown of cells, in whole or in part. The latter can be associated with regenerative processes that may occur during chemical exposure. Such changes are considered to be neurotoxic.

While most neurotoxic damage is evident at the microscopic level, gross changes in morphology can be reflected by a significant change in the weight of the brain. Weight changes (absolute or relative to body weight), discoloration, discrete or massive cerebral hemorrhage, or obvious lesions in nerve tissue are generally considered neurotoxic effects.

Chemical-induced injury to the central nervous system is associated with astrocytic hypertrophy at the site of damage. Assays of glial fibrillary acidic protein (GFAP), the major intermediate filament protein of astrocytes, has been proposed as a biomarker of this response (O'Callaghan, 1988). A number of chemicals known to injure the central nervous system, including trimethyltin, methylmercury, cadmium, 3-acetylpyridine, and MPTP, have been shown to increase GFAP. In addition, increases in GFAP may be seen at dosages below those necessary to produce cytopathology as determined by Nissl-based stains used in standard neuropathological examinations. Because increases in GFAP may be an early indicator of neuronal injury in the adult, exposure level-dependent increases in GFAP should be viewed with concern.

Chemical-induced alterations in the structure of the nervous system are generally considered neurotoxic effects. To ensure reliable data, it is important that neuropathological studies minimize fixation artifacts and potential differences in the section(s) of the nervous system sampled and control for variability due to the age, sex, and body weight of the subject (WHO, 1986).

4.3.6. Developmental Neurotoxicity

Exposure to chemicals during development can result in effects other than death, gross structural abnormality, or altered growth. There are several instances in which functional alterations have resulted from exposure during the period between conception and sexual maturity (Riley and Vorhees, 1986; Vorhees, 1987). Table 4–6 lists several examples of chemicals known to produce developmental neurotoxicity in experimental animals. Animal models of developmental neurotoxicity have been shown to be sensitive to several environmental chemicals known to produce developmental toxicity in humans, including lead, ethanol, methylmercury, and PCBs (Kimmel et al., 1990).

TABLE 4–6.—PARTIAL LIST OF AGENTS BELIEVED TO HAVE DEVELOPMENTAL NEUROTOXICITY

Alcohols	Methanol, ethanol
Antimitotics	X-radiation, azacytidine
Insecticides	DDT, kepone, organophosphates
Metals	Lead, methylmercury, cadmium
Polyhalogenated hydrocarbons	PCB, PBB
Psychoactive drugs	Cocaine, phenytoin
Solvents	Carbon disulfide, toluene
Vitamins	Vitamin A

Sometimes functional defects are observed at dose levels below those at which other indicators of developmental toxicity are evident (Rodier, 1986). Such effects may be transient or reversible in nature, but generally are considered adverse effects. Data from postnatal studies, when available, are considered useful for further assessment of the relative importance and severity of findings in the fetus and neonate. Often, the long-term consequences of adverse developmental outcomes noted at birth are unknown and further data on postnatal development and function are necessary to determine the full spectrum of potential developmental effects. Useful data also can be derived from well-conducted multigeneration studies, although the dose levels used in these studies may be much lower than those in studies with shorter-term exposure.

Much of the early work in developmental neurotoxicology was related to behavioral evaluations. Recent advances in this area have been reviewed in several publications (Riley and Vorhees, 1986; Kimmel et al., 1990). Several expert groups have focused on the functions that should be included in a behavioral testing battery, including sensory systems, neuromotor development, locomotor activity, learning and memory, reactivity and habituation, and reproductive behavior. No testing battery has fully addressed all of these functions, but it is important to include as many as possible, and several testing batteries have been developed and evaluated for use in testing.

Direct extrapolation of functional developmental effects to humans is limited in the same way as for other endpoints of developmental toxicity, i.e., by the lack of knowledge about underlying toxicological mechanisms and their significance. It can be assumed that functional effects in animal studies indicate the potential for altered development in humans, although the types of developmental effects seen in experimental animal studies will not necessarily be the same as those that may be produced in humans. Thus, when data from functional developmental toxicity studies are encountered for particular agents, they should be considered in the risk assessment process.

Agents that produce developmental neurotoxicity at a dose that is not toxic to the maternal animal are of special concern because the developing organism is affected but toxicity is not apparent in the adult. More commonly, however, adverse developmental effects are produced only at doses that cause minimal maternal toxicity; in these cases, the developmental effects are still considered to represent developmental toxicity and should not be discounted as secondary to maternal toxicity. At doses causing excessive maternal toxicity (that is, significantly greater than the minimal toxic dose), information on developmental effects may be difficult to interpret and of limited value. Current information is inadequate to assume that developmental effects at maternally toxic doses result only from maternal toxicity; it may be that the mother and developing organism are sensitive to that dose level. Moreover, whether developmental effects are secondary to maternal toxicity or not, the maternal effects may be reversible while effects on the offspring may be permanent. These are important considerations for agents to which humans may be exposed at minimally toxic levels either voluntarily or involuntarily, because several agents are known to produce adverse developmental effects at minimally toxic doses in adult humans (e.g., smoking, alcohol).

Although interpretation of functional developmental neurotoxicity data may be limited at present, it is clear that functional effects must be evaluated in light of other toxicity data, including other forms of developmental toxicity (e.g., structural abnormalities, perinatal death, and growth retardation). The level of confidence in an adverse effect may be as important as the type of change seen, and confidence may be increased by such factors as replicability 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 considered an important measure of chemical effect; in the case of functional effects, both monotonic and biphasic dose-response curves are likely, depending on the function being tested.

4.3.7. Physiological and Neuroendocrine Endpoints

One of the key roles played by the nervous system is to orchestrate the general physiological functions of the body to help maintain homeostasis. To this end, the nervous system and many of the peripheral organ systems are integrated and functionally interdependent. For example, specific neuronal processes are intimately involved in maintaining or modulating respiration, cardiovascular function, body temperature, and gastrointestinal function. Because many peripheral organ functions involve neuronal components, changes in such physiological endpoints as blood pressure, heart rate, EKG, body temperature, respiration, lacrimation, or salivation may indirectly reflect possible treatment-related effects on the functional integrity of the nervous system. However, since physiological endpoints also depend on the integrity of the related peripheral organ itself, changes in physiological function also may reflect a systemic toxicity involving that organ. Consequently, the neurotoxicological significance of a physiological change must be interpreted within the context of other signs of toxicity. A variety of general physiological procedures can be applied to neurotoxicological problems. These procedures range in scale from simple measurements, for example, of body temperature, respiration, lacrimation, salivation, urination, and defecation, which may be included in routine functional observational batteries used for chemical screening, to more involved procedures involving measurements of blood pressure, endocrine responses, cardiac function, gastrointestinal function, etc. The latter would be more appropriate for second-level tests to characterize the scope of chemically related toxicity.

The central nervous system also regulates the outflow of the endocrine system, which together with the influence of the autonomic nervous system, can affect immunologic function (WHO, 1986). Hormonal balance results from the integrated action of the hypothalamus, located in the central nervous system, and the pituitary, which regulates activities of endocrine target organs. Each site is susceptible to disruption by neurotoxic agents. Neuroendocrine dysfunction may occur because of a disturbance in the regulation and modulation of the neuroendocrine feedback systems. One major indicator of neuroendocrine function is secretions of hormones from the pituitary. Hormones from the anterior pituitary are important for reproduction (follicle-stimulating hormone, luteinizing hormone), growth (thyroid-stimulating hormone), and response to stress (adrenocorticotropic hormone). Hypothalamic control of anterior pituitary secretions occurs through the release of hypothalamic-hypophysiotropic hormones. Hormones from the posterior hypothalamus (prolactin, melanocyte-stimulating hormone, and growth hormone) are also involved in a number of important bodily functions.

Many types of behaviors (e.g., reproductive behaviors, sexually dimorphic behaviors) are dependent on the integrity of the hypothalamic-pituitary system, which could represent an important site for neurotoxic action. Pituitary secretions arise from a number of different cell types in this gland and neurotoxicants could affect these cells either directly or indirectly. Morphological changes in follicular cells, chromophobe cells, somatotropic cells, prolactin cells, gonadotropic cells, follicle-stimulating hormone secreting cells, luteinizing hormone-containing cells, thyrotropic cells, and cortico cells might be associated with adverse effects on the pituitary, which could ultimately affect behavior and the functioning of the nervous system.

Biochemical changes in the hypothalamus also may be used as indices of potential changes in neuroendocrine function. However, the neuroendocrine significance of changes in hypothalamic neurotransmitters and neuropeptides is usually only inferential and data must be considered on a case-by-case basis.

Most anterior pituitary hormones are subject to negative feedback control by peripheral endocrine glands and, if neurotoxicants modify peripheral secretions, neuroendocrine changes can result from this altered feedback. Modifications in the functioning of these endocrine secretions could occur after toxic exposure; a number of agents have been shown to alter blood levels of glucocorticoids, thyroxine, estrogen, corticosterone, and testosterone. Although such changes are not necessarily due to direct neuroendocrine effects, target organ changes often can be a first indication of neuroendocrine changes.

4.3.8. Other Considerations

4.3.8.1. Structure-activity relationship.

Because of a general lack of epidemiologic or toxicologic data on most chemical substances, attempts have been made in toxicology to predict activities based on chemical structure. The basis for inference from structure-activity relationships (SARs) can be either comparison with structures known to have biologic activity or knowledge of structural requirements of a receptor or macromolecular site of action. However, given the complexity of the nervous system and the lack of information on biologic mechanisms of neurotoxic action, there are relatively few well-characterized SARs in neurotoxicology. Since SARs cannot be used to rule out all neurotoxic activity, it is not acceptable to use them as a basis for excluding potential neurotoxicity. Caution is warranted in interpreting SARs in anything other than the most preliminary analyses. Use of SARs requires detailed knowledge not only of structure, but also of each critical step in the pathogenetic mechanism of neurotoxic injury. Such knowledge is still generally unavailable.

SAR approaches are more successful when the range of possible sites of action or mechanisms of action is narrow. Thus, SARs have had more use in relation to carcinogenicity and mutagenicity than in other kinds of toxicity. The SAR approaches used in the development of novel neuropharmacologic structures deserve consideration in neurotoxicology, but their utility depends on a better understanding of neurotoxic mechanisms.

4.3.8.2. In vitro methods.

In vitro procedures for testing have practical advantages, but studies must be done to correlate the results with responses in whole animals. One advantage of validated in vitro tests is that they minimize the use of live animals. Some of the more developed in vitro tests might be simple and might not have to be conducted by highly trained personnel, but, as with many in vivo tests, the analysis and interpretation of results are likely to require expertise. Experience with the Ames test for mutagenesis confirms the advantages of in vitro procedures, but also illustrates the problems that arise when an assay is used to predict an endpoint that is not exactly what it measures (e.g., carcinogenicity rather than specific aspects of genotoxicity). In vitro changes can be markers for toxicity, even when the structural or functional consequences are not known

or predicted. In addition, in vitro methods can examine the more evolutionarily conserved elements of the nervous system and improve neurotoxicity detection and could also provide suitable systems for studying developmental neurotoxicity.

A broad range of tissue-culture systems are available for assessing the neurologic impact of environmental agents, including cell lines, dissociated cell cultures, reaggregate cultures, explant cultures, and organ cultures (Veronesi, 1991).

Neuronal and glial cell lines are used extensively in neurobiology and have potential for neurotoxicological studies. They consist of populations of continuously dividing cells that, when treated appropriately, stop dividing and exhibit differentiated neuronal or glial properties. Neuronal lines can develop electric excitability, chemosensitivity, axon formation, neurotransmitter synthesis and secretion, and synapse formation. Large quantities of cells can be generated routinely to develop extensive dose-response or other quantitative data.

When neural tissue, typically from fetal animals, is dissociated into a suspension of single cells, and the suspension is inoculated into tissue-culture dishes, the neurons and glia survive, grow, and establish functional neuronal networks. Such preparations have been made from most regions of the CNS and exhibit highly differentiated, site-specific properties that constitute an in vitro model of different portions of the CNS. Most of the neuronal transmitter and receptor phenotypes can be demonstrated, and a variety of synaptic interactions can be studied. Glial cells are also present, and neuroglial interactions are a prominent feature of the cultures. A substantial battery of assays (neurochemical and neurophysiologic) is now available to assess the development of the cultures and to indicate toxic effects of test agents added to the culture medium. Relatively pure populations of different cell types can be isolated and cultured, so that effects on specific cell types can be assessed independently. Pure glial cells or neurons, or even specific neural categories, can be prepared in this way and studied separately, or interaction between neurons and glial cells can be studied at high resolution. The neurobiologic measures used to assess the effect of any agent can be very specific (for example, activity of neurotransmitter-related enzyme or binding of a receptor ligand) or global (for example, neuron survival or concentration of glial fibrillary acidic protein). The two-dimensional character of the preparations makes them particularly suited for morphologic evaluation, and detailed electrophysiologic studies are readily performed. The toxic effects and mechanisms of anticonvulsants, excitatory amino acids, and various metals and divalent cations have been assessed with these preparations. The cerebellar granular cell culture system, for example, has been exploited recently in studies of the mechanism of alkyllead toxicity (Verity et al., 1990).

A related preparation made from single-cell suspensions of neural tissue is the reaggregate culture. Instead of being placed in culture dishes and allowed to settle onto the surface of the dishes, the cells are kept in suspension by agitation; under appropriate conditions, they stick to one another and form aggregates of controllable size and composition. Typically, the cells in an aggregate organize and exhibit intercellular relations that are a function of, and bear some resemblance to, the brain region that was the source of the cells. The cells establish a three-dimensional, often laminated structure. Reaggregate cultures lend themselves to large-scale, quantitative experiments in which neurobiologic variables can be examined, although morphologic and ligand-binding studies are performed less readily than with surface cultures.

Organotypic explant cultures also are closely related to the intact nervous system. Small pieces or slices of neural tissue are placed in culture and can be maintained for long periods with substantial maintenance of structural and cell-cell relations of intact tissue. Specific synaptic relations develop and can be maintained and evaluated, both morphologically and electrophysiologically. Because all regions of the nervous system are amenable to this sort of preparation, it is possible to analyze toxic agents that are active only in specific regions of the central or peripheral nervous system. Explants can be made from relatively thin slices of neural tissue, so detailed morphologic and intracellular electrophysiologic studies are possible. Their anatomic integrity is such that they capture many of the cell-cell interactions characteristic of the intact nervous system while allowing a direct, continuing evaluation of the effects of a potentially neurotoxic compound added to the culture medium. The process of myelination has been studied extensively in explant cultures, and considerable neurotoxicologic information has been gained. A preparation similar to an explant culture is the organ culture, in which an entire organ, such as the inner ear or a ganglion, rather than slices or fragments, is grown in vitro. Obviously, only structures so small that their viability is not compromised can be treated in this way.

In general, the technical ease of maintaining a culture varies inversely with the degree to which it captures a spectrum of in vivo characteristics of nervous system behavior. The problem of biotransformation of potentially neurotoxic compounds is shared by all, although the more complete systems (explant or organ cultures) might alleviate this problem in specific instances. In many culture systems, complex and ill-defined additives—such as fetal calf serum, horse serum, and human placental serum—are used to promote cell survival. A number of thoroughly described synthetic media are now available, however, and such fully defined culture systems can be used where necessary.

5. Neurotoxicology Risk Assessment

5.1. Introduction

Risk assessment is an empirically based process used to estimate the risk that exposure of an individual or population to a chemical, physical, or biological agent will be associated with an adverse effect. Generally, such effects can be quantified and the relative probability of their occurrence can be calculated. The risk assessment process usually involves four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization (NRC, 1983). Risk management is the process that applies information obtained through the risk assessment process to determine whether the assessed risk should be reduced and, if so, to what extent (NRC, 1983). In some cases, risk is the only factor considered in a decision to regulate exposure to a substance. Alternatively, the risk posed by a substance is weighed against social, ethical, and medical benefits and economic and technological factors in formulating a risk management decision. The risk-balancing approach is used by some agencies to consider the benefits as well as the risks associated with unrestricted or partially restricted use of a substance. The purpose of this chapter is to describe the risk assessment process as it has currently evolved in neurotoxicology and present available options for quantitative risk assessment.

5.2. The Risk Assessment Process

5.2.1. Hazard Identification

Agents that adversely affect the neurophysiological, neurochemical, or

structural integrity of the nervous system or the integration of nervous system function expressed as modified behavior may be classified as neurotoxicants (Tilson, 1990b). For hazard identification, the best or most generalizable studies would measure these changes in humans. With the exclusion of therapeutic agents, information on effects in humans is usually derived from case reports of accidental exposures and epidemiological studies. This type of data affords less certainty regarding generalizability as well as less specific exposure information. As discussed in chapter 4, a common alternative method of data generation for hazard identification is the use of animal models. Animal models that measure behavioral, neurophysiological, neurochemical, and structural effects have been developed and validated. Studies that employ these models to evaluate specific potential hazards are used to predict the outcome of exposure to the same hazard in humans.

5.2.1.1. Human studies

Information obtained through the evaluation of human exposure data provides direct identification of neurotoxic hazards. This type of information is generally available from clinical trials required for the approval of therapeutic products for human use. For the purposes of risk assessment of nontherapeutic substances, data on effects of exposure to humans come primarily from two types of studies, case reports and epidemiological (Friedlander and Hearn, 1980) (see chapter 3). Case studies can supply evidence of an agent's toxicity, but are often limited by both the qualitative nature of the signs and symptoms reported and the nature of the exposure data. Epidemiological studies can provide data on the types of neurotoxic effects and the possible susceptibilities of certain populations. Under appropriate considerations, they can generally provide convincing and reliable evidence of potential human neurotoxicity. As with case studies, however, often only qualitative estimates of exposure can be obtained. Controlled laboratory studies have the potential to provide adequate exposure and effects data for accurate hazard identification, but ethical considerations place moral and practical restrictions on such studies except in those instances where direct benefit to the subjects, as in the case of therapeutic agents, may be expected. Excluding instances of therapeutic product development, most studies are limited to measuring the effects of acute, rather than long-term, exposure. This limits their utility in risk assessment because the effect of long-term, low-level exposure to a potentially toxic agent is often the issue of concern.

Methods available to evaluate neurotoxicity in humans include examination of neurophysiological and behavioral parameters. Specific tests to measure neuromuscular strength and coordination, alterations in sensation, deficits in learning and memory, changes in mood and personality, and disruptions of autonomic function are frequently employed (see chapter 3).

5.2.1.2. Animal studies

As discussed in chapter 4, animal models for many endpoints of neurotoxicity are available and widely used for hazard identification. Data from animal studies are frequently extrapolated to humans. For example, if exposure to an agent produces neuropathology in an animal model, damage to a comparable structure in humans is predicted. Similarly, biochemical and physiological effects observed in animals are commonly extrapolated to humans. Agents that produce alterations in the levels of specific enzymes in one animal species generally have the same effect in other species, including humans. Neurophysiological endpoints also tend to be affected by the same manipulations across species. Thus, an agent interfering with nerve conduction in an animal study is often assumed to have the same effect in humans. Behavioral studies in animals are also applied to human hazard identification, although the correspondence between methods employed in animals and humans is sometimes not as obvious. For this reason, behavioral methods developed for neurotoxic hazard identification need to be considered on a case-by-case basis.

5.2.1.3. Special issues

5.2.1.3.1. Animal-to-human extrapolation. The use of animal data to identify hazard to humans is not without controversy. Relative sensitivity across species as well as between sexes is a constant concern. Overly conservative risk assessments, based on the assumption that humans are always more sensitive than a tested animal species, can result in poor risk management decisions. Conversely, an assumption of equivalent sensitivity in a case where humans actually are more sensitive to a given agent can result in underregulation that might have a negative impact on human health.

5.2.1.3.2. Susceptible populations. A related controversy concerns the use of data collected from adult organisms, animal or human, to predict hazards in potentially more sensitive populations, such as the very young and the elderly, or in other groups, such as the chronically ill. In some cases, identification of neurotoxicity hazard does not generally include subjects from either end of the human life span or from other than healthy subjects. Uncertainty factors are used to adjust for more sensitive populations. In addition, single or multigeneration reproductive studies in animals may provide a source of information on neurological disorders, behavioral changes, autonomical dysfunction, neuroanatomical anomalies, and other signs of neurotoxicity in the developing animal (chapter 4).

5.2.1.3.3. Reversibility. For the most part, the basic principles of hazard identification are the same for neurotoxicity as for any adverse effect on health. One notable exception, however, concerns the issue of reversibility and the special consideration that must be given to the inherent redundancy and plasticity of the nervous system.

For many health effects, temporary, as opposed to permanent, effects are repaired during a true recovery. Damage to many organ systems, if not severe, can be spontaneously repaired. For example, damaged liver cells that may result in impaired liver function often can be replaced with new cells that function normally. The resulting restoration of liver function can be viewed as recovery. In the central nervous system, cells generally do not recover from severe damage and new cells do not replace them. When nervous system recovery is observed, it may represent compensation requiring activation of cells that were previously performing some other function, reactive synaptogenesis, or recovery of moderately injured cells. While a damaged liver may recover due to the addition of new cells, severe damage to nervous system cells results in a net loss of cells. This loss of compensatory capacity may not be noticed for many years and, when it does appear, it may be manifest in a way seemingly unrelated to the original neurotoxic event. Lack of ability to recover from a neurotoxic event later in life or premature onset of signs of normal aging may result. It is therefore important to consider the possibility that significant damage to the nervous system may have occurred in experiments where effects appear to be reversible.

5.2.1.3.4. Weight of evidence.

A "weight of evidence" approach to identifying an agent as a neurotoxic

hazard is almost always necessary. With the exception of therapeutic products, a single, complete, controlled study of an agent's effects on the nervous system, conducted in an appropriate representative sample of humans, is rarely, if ever, possible. Rather, those individuals charged with identifying hazard are usually confronted with a collection of imperfect studies, often providing conflicting data (Barnes and Dourson, 1988).

There are several possible approaches, depending on the quality of the evidence. Two examples are the use of data from only the most sensitive species tested and the use of data from only species responding most like the human for any given endpoint. In assessing neurotoxicity of therapeutic products, when human data are available and neurotoxic endpoints detected in animals can be clinically measured, the human findings supersede those of the nonclinical data base. Assuming that all available evidence is to be included, considerations necessary for formulating a conclusion include the relative weights that should be given to positive and negative studies. Sometimes positive studies are given more weight than negative ones, even when the quality of the studies is comparable. Experimental design factors such as the species tested, the number and gender of subjects evaluated, and the duration of the test are given different weights when data from different studies are combined. The route of exposure in a given study and its relevance to expected routes of human exposure are often a weighted factor. The issue of statistical significance is frequently debated. Some argue that an effect occurring at a statistically insignificant level may nevertheless represent a biologically or toxicologically significant event, and should be afforded the same weight as if the finding were statistically significant. In general, however, only statistically significant measures should be considered in hazard identification. The power of various statistical measures is also considered.

5.2.2. Dose-Response Assessment

In the second step of the risk assessment process, the dose-response assessment, the relationship between the extent of damage or toxicity and dose of a toxic substance for various conditions of exposure is determined. Because several different kinds of responses may be elicited by a single agent, more than one dose-response relationship may need to be developed (e.g., neurochemical and morphological parameters).

When quantitative human dose-effect data are not available for a sufficient range of exposures, other methods must be used to estimate exposure levels likely to produce adverse effects in humans. In the absence of human data, the dose-response assessment may be based on tests performed in laboratory animals. Evidence for a dose-response relationship is an important criterion in assessing neurotoxicity, although this may be based on limited data from standard studies that often use only three dose groups and a control group (Barnes and Dourson, 1988).

The most frequently used approach for risk assessment of neurotoxicants and other noncancer endpoints is the uncertainty- or safety-factor approach (Barnes and Dourson, 1988; Kimmel, 1990). For example, within the EPA, this approach involves the determination of reference doses (RfDs) by dividing a no observed adverse effect level (NOAEL) by uncertainty factors that presumably account for interspecies differences in sensitivity (Barnes and Dourson, 1988). Generally, an uncertainty factor of 10 is used to allow for the potentially higher sensitivity in humans than in animals and another uncertainty factor of 10 is used to allow for variability in sensitivity among humans. Hence, the RfD is equal to the NOAEL divided by 100. If the NOAEL cannot be established, it is replaced by the lowest observed adverse effect level (LOAEL) in the RfD calculation and an additional uncertainty factor of 10 is introduced (i.e., the RfD equals the LOAEL divided by 1000).

If more than one effect is observed in the animal bioassays, the effect occurring at the lowest dose in the most sensitive animal species and gender is generally used as the basis for estimating the RfD (OTA, 1990). Sometimes, different RfDs can be calculated, depending on endpoint or species selected. Selection of safety factors may be influenced by several considerations, including data available from humans, weight of evidence, type of toxic insult, and probability of variations in responses among susceptible populations (e.g., very young or very old). Established guidelines have been accepted by several agencies that use the safety-factor approach to account for intraspecies variability, cross-species extrapolation, and exposure duration. In some instances, comparisons between these predicted values and experimental data have been conducted and the results appear comparable for some selected examples (Dourson and Stara, 1983; McMillan, 1987).

The uncertainty-factor approach is based on the assumption that a threshold does exist, that there is a dose below which an effect does not change in incidence or severity. The threshold concept is complicated and controversial. As described by Sette and MacPhail (1992), there are several different ways in which the term threshold is used. Thresholds are defined, in part, by the limit of detection of an assay. As the sensitivity of the analytical method or bioassay is improved, the threshold might be adjusted downward, indicating that the true threshold had not been previously determined.

Another problem inherent with an observation of no discernible effects at low doses is that it is impossible to determine whether the risk is actually zero (i.e., the dose is below a threshold dose) or whether the statistical resolving power of a study is inadequate to detect small risks (Gaylor and Slikker, 1992). Every study has a statistical limit of detection that depends on the number of individuals or animals involved. For example, it would be relatively unusual to conduct an experiment on a neurotoxicant with as many as 100 animals per dose. If no deleterious effects were observed in 100 animals at a particular dose, it might be concluded that this dose level is below the threshold dose. However, we can only be 95 percent confident that the true risk is less than 0.03. That is, if 3 percent of the animals in a population actually develop a toxic effect at this dose, there is a 5 percent chance that a group of 100 animals would not show any effect. The observation of no toxic effects in an extremely large sample of 1,000 animals only indicates with 95 percent confidence that the true risk is less than 0.003, etc. Because thresholds cannot be realistically demonstrated, they are therefore assumed.

The notion of threshold may be useful in explaining mechanisms associated with specific types of toxicity. What little is known about mechanisms of neurotoxicity suggests that both threshold and nonthreshold scenarios are possible (Silbergeld, 1990). However, for one of the most studied neurotoxicants, lead, there has been a steady decline in exposure levels shown to have effects, suggesting to some that no threshold dose is apparent (Bondy, 1985). Sette and MacPhail (1992) also consider the threshold as a mathematical assumption and as a population sensitivity and conclude that "the idea of no threshold seems experimentally untestable. . . ."

The RfD approach relies on single experimental observations (the NOAEL or LOAEL) instead of complete dose-response curve data to calculate risk estimations. Chemical interactions with biological systems are often specific, stereoselective, and saturable. Examples include enzyme-substrate binding leading to substrate metabolism, transport, and receptor-binding, any or all of which may be a requirement of an agent's effect or toxicity. Therefore, a chemical's dose-response curve may not be linear. The certainty of low-dose extrapolation has been determined to be markedly affected by the shape of the dose-response curve (Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation, 1971). Therefore, the appropriate use of dose-response curve data should enhance the certainty of risk estimations when thresholds are not assumed or determined.

Dose-response models have generated considerable interest as more appropriate and quantitative alternatives to the safety-factor approach in risk assessment. Rather than routinely applying a "fixed" safety factor to the NOAEL (based on a single dose) to obtain a "safe" dose, another approach uses data from the entire dose-response curve.

Two fundamentally different approaches in the use of dose-response data to estimate risk have been developed. Dews and coworkers (Dews, 1986; Glowa and Dews, 1987; Glowa et al., 1983) and Crump (1984) demonstrated an approach in which they used information on the shape of the dose-response curve to estimate levels of exposure associated with relatively small effects (i.e., a 1, 5, or 10 percent change in a biological endpoint). Both Dews and Crump fit a mathematical function to the data and provided an estimate of the variability in exposure levels associated with a relatively small effect.

An alternative approach developed by Gaylor and Slikker (1990) first establishes a mathematical relationship between a biological effect and the dose of a given chemical. The second step determines the distribution (variability) of individual measurements of biological effects about the dose-response curve. The third step statistically defines an adverse or "abnormal" level of a biological effect in an untreated population. The fourth step estimates the probability of an adverse or abnormal level as a function of dose utilizing the information from the first three steps. The advantages of these dose-response models are that they encourage the generation and use of data needed to define a complete dose-response curve.

Although more quantitative dose-response assessment models have emerged in recent years, uncertainty remains as to what biological endpoints from which species with what dosing regimen should be analyzed. Within a species, a given agent may produce a variety of effects, including neurochemical, neuropathological, and behavioral effects. In other instances, a chemical may produce alterations of one endpoint but not others (Slikker et al., 1989). Species selection may also dramatically affect the outcome of risk assessments. The Parkinson-like syndrome produced by single doses of MPTP in the human or nonhuman primate is not observed in rats given comparable MPTP doses (Kopin and Markey, 1988). Although endpoint and species selection appear to have a tremendous effect on the outcome of an assessment, only a few studies have systematically investigated the effect on assessment outcome of varying either the species or the endpoint within a species (McMillan, 1987; Hattis and Shapiro, 1990; Gaylor and Slikker, 1992).

5.2.3. Exposure Assessment

This step of the risk assessment process determines the source, route, dose, and duration of human exposure to an agent. The results of the dose-response assessment are combined with an estimate of human exposure to obtain a quantitative estimate of risk. As either the effect of or the exposure to an agent approaches zero, the risk of neurotoxicity approaches zero. It should be recognized that exposures to multiple agents may produce synergistic or additive effects.

Exposure can occur via many routes, including ingestion, inhalation, or contact with skin. Sources of exposure may include soil, food, air, water, or intended vehicle (e.g., drug formulation). The degree of exposure may be strongly influenced by a number of factors, for example, the occupation of the individual involved.

The duration of exposure (i.e., acute or chronic) and interval of exposure (i.e., episodic or continuous) are variables of exposure that are common to all types of risk assessments, including carcinogenicity (OSTP, 1985).

Although not routinely used, biological markers or biomarkers of exposure could theoretically improve the exposure assessment process and, thereby, improve the overall risk assessment of neurotoxicants. Exposure biomarkers may include either the quantitation of exogenous agents or the complex of endogenous substances and exogenous agents within the system (Committee on Biological Markers, 1987). A limited number of examples of biomarkers of exposure have been reviewed by Slikker (1991) and include blood or dentine lead concentrations (Needleman, 1987), cerebrospinal fluid concentrations of dopamine metabolites following MPTP administration (Kopin and Markey, 1988), cerebrospinal fluid concentrations of a serotonin metabolite following MDMA exposure (Ricaurte et al., 1986), and serum esterase concentrations following organophosphate exposure (Levine et al., 1986). The use of muscarinic receptor binding in peripheral plasma lymphocytes has also been described as a potential biomarker of exposure for the organophosphates (Costa et al., 1990). These examples suggest that biomarkers of exposure are available for some agents, but more effort will be required to demonstrate that these biomarkers can routinely be used to improve the exposure assessment process.

5.2.4. Risk Characterization

The final step of the risk assessment process combines the hazard identification, the dose-response assessment, and the exposure assessment to produce the characterization of risk. As previously stated, the current practice is to divide the NOAEL by the appropriate safety factor to obtain the RfD. The magnitudes of the safety factors used to determine RfDs [interspecies extrapolation (10), intraspecies extrapolation (10), and acute vs. chronic exposure (10) = 1000] are based more on conservative estimates than on actual data (Sheehan et al., 1989; McMillan, 1987) and have been questioned for empirical reasons (Gaylor and Slikker, 1990). Uncertainty factors may be decreased as more data become available. Modifying factors are also employed under certain circumstances to account for the completeness of data sets. Along with this RfD numerical value, any uncertainties and assumptions inherent in the risk assessment should also be stated (OTA, 1990). Although the RfD provides a single numerical value, it does not provide information concerning the uncertainty of this number nor does the RfD approach attempt to estimate the potential risk as a function of dose or consider the potential risk at the NOAEL. The risk at the NOAEL generally is greater than zero and has been estimated to be as high as about 5 percent (Crump, 1984; Gaylor, 1989). Concern has been expressed that the application of the

RfD approach to all neurotoxicants is unlikely to be biologically defensible in light of mechanistic data (NRC, 1992). Several other quantitative risk assessment procedures have recently emerged as alternatives to the RfD approach (Kimmel and Gaylor, 1988).

Quantitative risk assessment may be defined as a data-based process that uses dose-response information and measurements of human exposure to arrive at estimates of risk. Assumptions are required to extrapolate results from high to low doses, to extrapolate from animal results to humans, and to extrapolate across different routes and durations of exposure.

In a step toward quantitative risk assessment, Crump (1984) suggested the use of a benchmark dose defined as "a statistical lower confidence limit corresponding to a small increase in effect over the background level." The benchmark dose is determined with a mathematical model and is less affected by the particular shape of the dose-response curve. Although the benchmark approach avoids several problems inherent in the RfD approach (e.g., lack of precision in defining the LOAEL; Kimmel, 1990), the same final step of dividing by arbitrary safety factors is obligatory.

Another approach to quantitative risk assessment is the statistical or curve-fitting approach. If quantal information concerning the proportion of response at a given dose is available but mechanistic information is lacking, statistical models can be used to fit population data (Wyzga, 1990). This approach has been used to fit various models to data of lead toxicity. The data were sufficient to allow discrimination of several models in terms of goodness of fit; the nerve-conduction velocity data from children exposed to environmental lead as a function of blood lead concentration fit a "hockey-stick" type dose-response curve rather than a logistic or quadratic model (Schwartz et al., 1988). These statistical approaches not only provide a method to extrapolate data to lower exposure conditions but also can provide circumstantial evidence to support a proposed mechanism of action.

The development of quantitative risk assessment approaches depends, in part, on the availability of information on the mechanism of action and pharmacokinetics of the agent in question. In the development of a biologically based, dose-response model for MDMA neurotoxicity, Slikker and Gaylor (1990) considered several factors, including the pharmacokinetics of the parent chemical, the target tissue concentrations of the parent chemical or its bioactivated proximate toxicant, the uptake kinetics of the parent chemical or metabolite into the target cell and membrane interactions, and the interaction of the chemical or metabolite with presumed receptor site(s). Because these theoretical factors contain a saturable step due to limited amounts of required enzyme, reuptake, or receptor site(s), a nonlinear, saturable dose-response curve was predicted. In this case of neurochemical effects of MDMA in the rodent, saturation mechanisms were hypothesized and indeed saturation curves provided relatively good fits to the experimental results. The conclusion was that use of dose-response models based on plausible biological mechanisms provide more validity to prediction than purely empirical models. Concomitant with attempts to develop quantitative risk assessment procedures, it is imperative that regulatory policy or risk management procedures also be developed to use appropriately the type of data generated by quantitative risk assessment. However, until alternative risk assessment procedures have been validated, the available RfD approach with its limitations will most likely continue to be used.

5.3. Generic Assumptions and Uncertainty Reduction

The purpose of risk assessment is to determine the risk associated with human exposure to a hazard. The quality of the data from toxicological studies differs. In the case of therapeutic products where human effects information is available, risk assessments rely primarily on the result of controlled clinical trials. Even when clinical trial data are available, however, conducting a risk assessment is complicated by many uncertainties. In the face of these uncertainties, conservative assumptions are usually made at several steps in the risk assessment process. For example, unless adequate clinical data are available, the most sensitive experimental species is frequently used. While conservative assumptions may lead to a risk assessment that adequately protects the human population, this may result in an increased financial burden on the public (e.g., manufacturing costs or loss of jobs); even then it is impossible to be certain that the total population will be protected. Conversely, errors leading to allowable exposure levels that are too high reduce the safety margin for human health and increase health care costs. Thus, there are compelling public health and economic reasons to obtain more precise risk assessments; all assumptions cannot be completely eliminated, but the degree of uncertainty associated with certain specific assumptions can at least be reduced (Sheehan et al., 1989).

Risk assessment for neurotoxicity shares many common features with other noncancer toxicities such as developmental toxicity and immunotoxicity. As such, there are several generic assumptions that apply to all traditional, noncancer endpoint risk assessment procedures (Table 5–1).

TABLE 5–1.—GENERAL ASSUMPTIONS THAT UNDERLIE TRADITIONAL RISK ASSESSMENTS [a,b]

1. A threshold dose exists for noncancer endpoints.
2. NOAEL/LOAEL uncertainty- or safety-factor approaches are reasonable.
3. Variability in the toxic response to the chemical exposure is not due to a heterogeneous population response.
4. Average dose or total dose is a reasonable measure of exposure when doses are not equivalent in time, rate, or route of administration and the average (or total) dose is proportional to adverse effect.
5. Structure-activity correlations can be used to predict human toxicity.
6. The mechanism of action is the same at all doses for all species.

[a] This is not intended to be an exhaustive list.

[b] Modified from Sheehan et al., 1989.

One approach to reducing some of the uncertainties is to critically define and examine the assumptions made in the risk assessment process. Several of the more generic of these assumptions are listed in Table 5–1. Despite their diversity, these assumptions share the attribute of being partially replaceable by factual information. If, for example, the assumption of 100 percent absorption of a toxicant from a contaminated food source is replaced by data demonstrating that 90 percent of the toxicant is not biologically available under human exposure conditions, then a revised risk assessment could allow a 10-fold greater exposure from that source; i.e., the former risk assessment was too conservative by a factor of 10. As another example, many risk assessments employ data from two species.

If experimental animals and humans absorb or metabolize the same fraction of a dose, the potency estimate would not change when extrapolating from animals to humans. Therefore, it is necessary to have information on both human and animal rates before changes in potency estimates are made. If a toxicant acts via a reactive intermediate and humans produce 10-fold more of

the intermediate than either of the test species under similar conditions, then allowable human exposure should be decreased 10-fold (i.e., the allowable exposure levels are 10-fold too high) or an increased danger to human health exists. These findings could then replace the "most sensitive species" principle with facts concerning relevant human exposure and susceptibility. In these examples, the identification of the assumption helps define research needs or knowledge gaps (Sheehan et al., 1989).

In general, the knowledge gaps are many and complex, but some can be filled with practical solutions. The combination of ample dose-response data and a quantitative risk assessment process can eliminate assumptions 1 (existence of a threshold) and 2 (reasonableness of safety factors) of the six generic assumptions (Table 5–1). The uncertainty of assumption 4 (exposure comparisons) could be at least reduced with the proper application of appropriate pharmacokinetic data. Likewise, the uncertainty of generic assumption 3 (variability of heterogeneous populations) can theoretically be reduced with the use of biomarkers of exposure and biomarkers of effect, to more accurately define the relationship between exposure and biological effect in a large population.

Many assumptions remain, however, and uncertainty reduction by filling knowledge gaps will ultimately require greater understanding of biological mechanisms underlying neurotoxicity. A single risk assessment model may not be adequate for all conditions of exposure, for all endpoints, or for all agents. Risk assessment models of the future may well include biomarkers of both effect and exposure as well as biologically based mechanistic considerations derived from both epidemiologic and experimental test system data.

6. General Summary

It is now generally accepted that some chemicals, including industrial agents, pesticides, therapeutic agents, drugs of abuse, food-related chemicals, and cosmetic ingredients, can have adverse effects on the structure and function of the nervous system. It has recently been proposed that exposure to neurotoxicants might also be associated with Parkinsonism and Alzheimer's disease. Several Federal agencies have initiated research programs in neurotoxicology, developed neurotoxicology testing guidelines, and used neurotoxic endpoints to regulate chemicals in the environment and workplace.

The scientific basis for identifying and characterizing chemical-induced neurotoxicity has advanced rapidly during the last several years. The manifestation of neurotoxicity depends on the relationship between exposure (applied dose) and the dose at the site of toxic action (delivered or target dose) and response. Chemical-induced changes in the structure or function of the nervous system at the cellular or molecular level can be observed as alterations in sensory, motor, or cognitive function at the level of the whole organism. Several important features about the nervous system make it particularly vulnerable to chemical insult, including differential susceptibilities at different stages of maturation, the presence of blood brain and nerve barriers that may be the target of toxic action, high metabolic rate, and limited regenerative capability following damage.

Methods devised to detect and quantify agent-induced changes in nervous system function in humans include clinical evaluations and neurotoxicity testing methods such as neurobehavioral, neurophysiological, neurochemical, imaging, and self-reporting procedures. Experimental approaches used in human neurotoxicology include epidemiological studies and, to a limited extent, human laboratory exposure studies. There are several important unresolved issues in human neurotoxicology, including the development of commonly accepted risk assessment criteria and animal-to-human extrapolation.

It is generally assumed that if physical or chemical-induced neurotoxicity is observed in animal models, then neurotoxicity will be produced in humans. Considerable research has been performed to demonstrate the validity of many animal models in an experimental context and to show predictive validity. Methods in animal neurotoxicology are frequently used in a tier-testing framework with simpler, more cost-effective tests to screen or identify neurotoxic potential. In hazard identification, the presence of neurotoxicity at the first tier is used to make decisions about subsequent development of a chemical or about the need to conduct additional experiments to define the level at which neurotoxicity will be observed. A number of methods have been devised for studies in animal neurotoxicology, including neurobehavioral, neurophysiological, neurochemical, and neuroanatomical techniques. It is known that the neuroendocrine system may be affected adversely by neurotoxicants and that there are populations that are differentially vulnerable to neurotoxic agents. Considerable research is in progress to employ structure-activity relationships to predict neurotoxicity and newly developed in vitro procedures are being used to augment or complement currently existing in vivo approaches.

Principles of risk assessment for neurotoxicity are evolving rapidly. At the present time, neurotoxicity risk assessment is generally limited to qualitative hazard identification. Neurotoxicological risk assessments have been generally based on a no observed adverse effect level and uncertainty factors. As with other noncancer endpoints, there is a need to consider more information about the shape of the dose-response curve and mechanisms of effect in quantitative neurotoxicology risk assessment. Research is needed to develop dose-response models that incorporate biologic information and mechanistic hypotheses into quantitative extrapolation of dose-response relationships across species and from high to low dose exposures.

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