Chapter 5

Testing and Monitoring

"Over the last 10 to 15 years, cancer had dominated the discussion of occupational standards and it continues to remain terribly important. At the same time, information on neurotoxins has increased. The notion of chronic and subclinical neurotoxicity has developed. Although these things are progressive and don't occur overnight, you'll see more attention paid to neurotoxicity in the years ahead. "

Philip Landrigan
Occupational Hazards 49:36, 1987

"The reasons for inadequate neurobehavioral testing of chemicals. . relate to economic factors and political decisions, not to inadequacies of the test methods. "

Donald McMillan
Occupational Hazards 49:37, 1987

''We need to know a lot more about how toxicity is expressed in behavior. We need to be able to recommend tests for chemicals before they move into the marketplace. This is why we need more of what NIOSH is doing. As it is, we are still using workers as part of an early-warning system. "

Ronald Wood
Psychology Today, July 1982, p. 30

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INTRODUCTION

People are exposed to chemicals every day in the course of eating, working, and recreation. Some of these chemicals are synthetic; others, whose properties may be unknown, occur naturally in the environment and in food. Modern society could not exist without them. However, the same chemicals that contribute to our high standard of living may also produce unanticipated and undesired effects. Regulatory officials are concerned with weighing the benefits of use against the risks of adverse health effects.

All substances, even water, can be toxic at a high enough level of ingestion. Determining the risk posed to human health by toxic substances requires information about the potential hazard and about the expected level of exposure, resulting in an estimate of the probability that a substance will produce harm under certain conditions (see ch. 6) (105).

There are many approaches to testing for neurotoxicity, and each has both advantages and limitations. Toxic substances can be evaluated through whole animal (in vivo) tests, tissue and cell culture (in vitro) tests, and tests on human subjects. The latter is the best means of predicting the effects of potentially toxic substances on human health. This approach, however, is generally difficult, expensive, and in some circumstances unethical. Consequently, it is usually necessary to rely on animal or in vitro tests.

Most toxicity testing is performed on animals, usually mice and rats. Animals are used for several reasons, one of which is that, biologically, they resemble humans in many ways and can often serve as adequate models for toxicity studies. On the other hand, it can be difficult to extrapolate the results of animal studies to humans. It is also important to keep in mind that the biochemical and physiological processes underlying human neurological and psychiatric problems are highly complex and often cannot be modeled in any single system.

In vitro tests can be used to complement animal tests and reduce the number of animals used in routine toxicity testing. In vitro testing may also be less expensive and less time-consuming. By understanding the structure or function affected by a toxic substance in vitro, it is sometimes possible to predict adverse effects in the whole animal. Like all testing strategies, in vitro tests have limitations, including the inability to analyze behavioral effects such as loss of memory or irritability.

Some human toxicological data are derived from accidental exposures to industrial chemicals and some from epidemiological studies. Prescription drugs are tested on humans to determine safety and efficacy.

This chapter briefly describes some methods of neurotoxicity testing and the advantages and limitations of each. The first section addresses animal toxicity tests, including the types of neurotoxicity tests currently proposed for regulatory use by the U.S. Environmental Protection Agency (EPA). The second section describes alternatives to animal tests, including in vitro approaches, and the third section describes human testing. Finally, approaches to monitoring of toxic substances are briefly discussed.

ANIMAL TOXICITY TESTS

In designing animal tests and evaluating data, appropriate weight is given to the following factors on a case-by-case basis, taking into account the seriousness of the hazard and the assumptions needed to estimate human health risks (105):

- the relationship between dose and response;
- the effects at the molecular, cellular, organ, organ system, and whole organism levels;
- the reproducibility of the study results and possible explanations for lack of reproducibility;
- the effects of structurally similar substances on humans or animals;
- any known metabolic differences between humans and the test species that could affect response;
- statistical uncertainties and difficulties in extrapolating to a low dose; and
- other factors, such as sex, species differences, and route of administration.

An Office of Technology Assessment (OTA) report, *Alternatives to Animal Use in Research, Testing, and Education*, contains a detailed discussion of the use of animals in research and associated ethical

concerns (105). The issues raised there will not be readdressed in this report.

Toxicity testing should aim to obtain all the data needed for accurate risk assessment at the lowest possible cost. Factors that influence cost include the number of appropriate test species, the nature of the parameters studied, the choice of test subjects, the controls required, and the skilled staff necessary to perform the studies. In addition, toxicity testing requires a substantial investment in labor. Aside from the maintenance needs of the animals used, many observations are necessary. Acute studies often involve observations of behavior and appearance as well as histopathological observations. Subchronic and chronic studies require more detailed pathological studies as well as weekly clinical examinations of all the animals used in the studies (92). Testing costs will be discussed in more detail in chapter 8.

Designing Useful Tests

Animal tests are used to determine the functional, structural, and biochemical effects of toxic substances. Experimental animal models have limitations, however, and the accuracy and reliability of a quantitative prediction of human toxicity depend on a number of conditions, such as choice of species, choice of tests, similarity of human and animal metabolism, design of the experiment, and method of extrapolation of animal data.

When designing animal toxicity tests, therefore, it is essential that the examiners clearly define the objective of their study and understand how the resulting data will be used. Several questions should be answered in advance: Will the data obtained from the animal tests be meaningful? Will the data be useful in the risk assessment process? Can the data be extrapolated from animals to humans?

The World Health Organization (WHO) recently suggested several general objectives of neurotoxicology testing (123):

- identify whether the nervous system is altered by the toxic substance,
- characterize the nervous system alterations associated with exposure,
- ascertain whether the nervous system is the primary target for the chemical, and
- determine dose- and time-effect relationships to establish no observed adverse effect levels (NOAELs).

The initial goal is to determine whether or not the nervous system is affected by a substance for which no toxicological data exist. This often involves screening for neurotoxicity using tests that predict the potential of a substance to produce adverse effects. To be most effective, the tests should be simple, rapid, and economical to administer. Once a chemical is known to produce a neurotoxic effect, further studies can be performed in order to characterize the nature and mechanism of the alterations. Screens are generally designed to explore the consequences of exposure and to indicate whether or not the nervous system is adversely affected.

Chemicals are unlikely to affect all major components of the nervous system at the doses tested; therefore, it is important to use a variety of tests that measure different functional, morphological, or chemical alterations in order to maximize the probability of detecting neurotoxicity. The methods used may differ with the objective of the study, the age of the animal, and the species examined (123).

Potential neurotoxic risks are difficult to assess because of the complexity of the nervous system. Some of the problems in assessment are associated with the wide variations in response that can occur. Other problems are related to the examiner's incomplete understanding of what is being measured by a given test. Therefore, no single test can be used to examine the total functional capacity of the nervous system (123).

Animal Choice

In preliminary screening of known or suspected toxic substances, numerous economic factors influence the design of the evaluation. It is useful if there exist adequate anatomical, physiological, and toxicological databases on the species chosen for study to allow meaningful interpretations of effects and appropriate hypotheses about mechanisms and sites of action (123).

Most routine toxicity testing is carried out with only one or two species. For example, cancer bioassays frequently involve the use of rats and mice, and the monkey may be used for identifying the effects of MPTP, a byproduct in the illicit synthesis of a meperidine analog. Hens have been used to evaluate the neurotoxic potential of organo-

phosphorous pesticides. Most other neurotoxicity screening studies use laboratory rats. Ideally, more than one animal species should be tested—if only a single species is tested, it is possible to conclude that human exposure is acceptable when in fact it is not. However, routine multispecies testing is a costly and demanding enterprise. The facilities and services needed for animal husbandry and the equipment and technical expertise needed to carry out the research make multispecies testing economically impractical in many instances (59).

There are other variables besides species that should be considered. For example, the sex of the test animal may influence results of the study. Some toxic substances may have a greater adverse effect on females than males or vice versa. Consequently, EPA testing guidelines require both male and female rats for neurotoxicity testing.

Another important factor is the age of the animal. The effects of a toxic substance may vary dramatically, depending on the stage of maturation of the animal. For example, cell loss in the nervous system due to natural aging processes may predispose an animal to the adverse effects of toxic substances. Most preliminary assessments are designed to provide information on the population with the greatest potential for exposure, namely, adults. However, aged populations or those undergoing rapid maturation are often especially vulnerable to environmental exposures; thus, tests to assess the neurobehavioral functioning of these populations are necessary for a complete evaluation.

The ideal tests are those that permit longitudinal assessment of animals of both sexes at any stage of development (i.e., at young childhood, prepuberty, and adulthood) (67). Whenever possible, the choice of animal model should take into account such factors as the differences in metabolism of substances between species, genetic composition of the species, and the sensitivity of the test animals to the toxic effects of the substances (50 FR 39458).

Dosing Regimen

Some compounds produce one kind of toxic effect following a single exposure and other effects following prolonged or repeated exposure. In environmental toxicology, a major objective is the detection of cumulative toxicity following continued (or intermittent) exposure. Thus, a multiple-dosing regimen is most commonly used. This is particularly important in neurobehavioral testing, since both quantitative and qualitative changes in the response to environmental factors can occur with repeated exposure, or at some later time following a single exposure (67,123). Normally, assessments are made for a period of time following termination of the dosing regimen, both to determine the reversibility of any observed effects and to see if any new effects appear (123).

Substances are administered in varying doses, the dose being a function of the concentration of the substance and the duration and frequency of exposure. Significant differences in response may occur when the same quantity of toxic material is administered over different exposure periods. Acute exposure to substances may produce both immediate and delayed toxic effects (such is the case for some organophosphorous pesticides). These effects may differ from the effects following long-term exposure. Repeated exposure to certain solvents may produce immediate effects after each dosing as well as delayed adverse effects from long-term exposure (47).

Acute toxic responses result when an animal is subjected to high concentrations of a substance over a short period of time. The acute response may be sudden and severe, and usually lasts for a brief period of time; in some cases, however, it is permanent. If the dose is sufficiently high, death may result. Lower doses (lower concentrations over longer periods of time) may not immediately cause death. As the dose decreases, the response is generally less severe and may take longer to develop. In chronic exposures, clinically adverse effects may take years to develop (47).

Route of Exposure

The most common routes by which toxic substances enter the body are, in descending order, inhalation (through the lungs), **oral** (through ingestion), and dermal (through the skin). Although substances generally produce the greatest effect and most rapid response when given intravenously, this is an unlikely route of entry except in the case of drug therapies or drug abuse. The manner in which a potentially toxic agent enters the body can influence the time of onset, intensity, and duration of the toxic effects. The route of exposure may also influence the degree of toxicity and the organs most severely affected.

Exposure to toxic chemicals in the atmosphere is unavoidable unless devices are used to remove the contaminants from the air before they enter the respiratory tract. In order for any contaminant to reach the alveoli of the lungs (where gas exchange takes place), it must be either a gas or of a certain particulate size (less than 10 microns in diameter) so that it is not removed in the airway to the lungs. The actual and potential' hazards associated with exposure to toxic agents via inhalation are evident in industrial workplaces and in urban areas with polluted atmospheres (55,1 17).

Most episodes of acute toxicity result from intentional or accidental ingestion of a chemical. For instance, a person may deliberately take an overdose of a psychoactive drug. Poisonous mushrooms may be accidentally ingested. Sufficiently large particles of inhaled toxic matter may collect in the throat and be swallowed.

The simplest route of exposure for humans and animals is accidental or intentional contact of the chemical with the skin. The skin is the most readily accessible organ to all forms of foreign chemicals, yet it is also an efficient barrier to many toxic substances. Many substances can be absorbed through the skin, including substances in fragrances (AETT), antidandruff shampoos (zinc pyridinethionine), and solvents (methyl n-butyl ketone) that have proven to be neurotoxic in humans or animals, or both (3,44,47). The degree of absorption is influenced by the type of compound(s) involved and the condition of the skin. For example, cuts or abrasions on the skin's surface will allow the agent to bypass the epidermis, the outer, protective layer of the skin. Once through the epidermis, the substance can easily pass into the circulatory system. Depending on the concentration and duration of the exposure, some substances, solvents, for example, can easily pass through the epidermis.

Extent and Duration of Exposure

The exposure of animals to chemicals is often divided into four categories: acute, subacute, subchronic, and chronic. **Acute is** defined as exposure to a chemical for less than 24 hours. The purpose of an acute test is to observe the evidence of toxicity after administration of the compound and the degree of lethality (55). While acute exposure usually refers to a single administration, repeated or continuous doses may be given within a 24-hour period for some substances with limited acute toxicity. An example is acute exposure by inhalation, which refers to continuous exposure for less than 24 hours. Repeated exposures are divided into subacute, subchronic, and chronic categories. Subacute exposure refers to repeated exposure to a chemical for 1 month or less, subchronic exposure occurs typically from 1 to 3 months, and chronic exposures occur for more than 3 months (47).

As mentioned earlier, the toxic effects following a single exposure to a substance may be quite different from those produced by repeated exposure. This may occur because of compensatory changes elicited by repeated administration or because of cumulative effects of mechanisms different from those causing acute toxicity. For example, the primary acute toxic effect of carbon disulfide is depression of central nervous system activity; however, repeated exposures can result in peripheral neuropathy or parkinsonism. Acute exposure to rapidly absorbed substances is likely to produce immediate toxic effects, but acute exposure can also produce prolonged toxicity that may or may not be similar to the toxic effects of chronic exposure. Likewise, chronic exposures may produce some immediate effects after each administration in addition to the chronic effects (47).

The extent of exposure is another important factor in the characterization of exposure parameters. Generally, but not always, fractionation of the dose reduces the effect. A single dose of a compound that produces an immediate, severe effect might produce less than half the effect when given in two equal doses and no effect when given in 10 doses over a period of several hours or days. Chronic toxic effects occur if the compound accumulates in the organism's system, if it produces irreversible toxic effects, or if there is insufficient time for the system to recover from the toxic damage (47).

Other Considerations

Several additional factors are considered in designing neurotoxicological tests. One condition that may affect toxicity is the nutritional state of the animal. Changes attributed to exposure to toxicants might be due to relatively nonspecific effects related to inhibition of growth or decreases in food or water consumption.

Another factor is the housing conditions of the experimental animals. Sometimes animals are housed individually in cages during toxicological

studies, an arrangement that may alter their responsiveness to the test compounds. For example, a chemical that causes depletion of the neurotransmitters norepinephrine and dopamine produces less depression of motor activity in isolated rats than in grouped rats (125).

Temperature of the environment is another important factor. Normally, the response of an animal to a toxic compound decreases as the environmental temperature is lowered, but the duration of the overall response may be delayed. Also, some drugs are more toxic in certain environmental temperatures than in others. For example, compounds affecting the neurotransmitter acetylcholine may produce significantly greater toxicity in a warm environment than in a colder one. Some substances inhibit sweating. Eventually, the body temperature becomes elevated because the absence of perspiration prevents cooling (38). In such a case, toxic effects may result from hyperthermia, not directly from the effect of the substance on the nervous system.

Validation

Validation is a critical component of the test development process because it ensures that data generated as a result of testing will be useful in evaluating the health risk posed by a particular substance. The value of any toxicity test lies in its ability to measure the endpoint it is designed to detect. For neurotoxicity, the endpoints are adverse changes in the structure or function of the nervous system. General acceptance of a new toxicity test usually requires demonstration that the test is reliable, sensitive, and specific. For validation studies, chemicals with known neurotoxic potential and those known not to be neurotoxic are studied to determine the ability of the test to distinguish between them. Because toxic substances can have many different effects on the nervous system, known neurotoxic substances with different effects on the nervous system are chosen for validation studies. Before test guidelines are proposed for national or international use, validation studies commonly include a multilaboratory phase to test the reproducibility of the testing paradigm in different laboratories (58,81).

Evaluating Chemicals for Neurotoxicity

It is impossible to thoroughly examine the neurotoxicity of each of the chemicals in commerce.

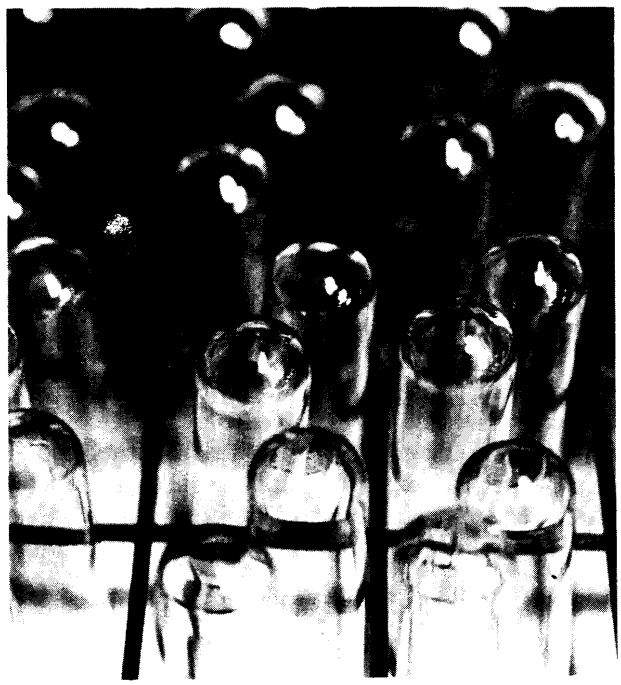


Photo credit: U.S. Environmental Protection Agency

However, it may be possible through a well-developed screening program to flag the substances either currently in use or recently introduced that have neurotoxic potential. Screening is conducted to provide an initial evaluation of the effects of various substances on the nervous system. The results of screening may be used to reduce the number or quantity of hazardous substances in commerce or to aid in determining which additional studies should be undertaken to further characterize their toxicological properties (67). An efficient screen should evaluate a variety of neurological effects rather than just one. Screens should also be sensitive, reproducible, and capable of being administered rapidly (32,33),

Testing strategies often involve a tiered approach. Tiered testing involves a stepwise progression of more specific and sophisticated tests, beginning with a general screen to determine if further testing is necessary. In the initial screen of the tiered testing approach, the outcomes of acute studies are interpreted. If acute effects are identified, then experiments involving repeated exposures are performed in the second tier. The third tier is composed of detailed studies of subtle effects or mechanisms of toxicity. At each stage the examiner builds on the data collected from the previous tier.

Typically, 5 to 10 animals of the same species and strain are used in the tests. It is important to select the proper animal model initially because it is desirable to use the same model in subsequent tiers. Using the same animal is more efficient, costs less, and allows consistent analysis of data. Some toxicity tests only require the acute dosing regimen, and it is not necessary to conduct repeated dosages. Box 5-A illustrates one example of a tiered testing approach. Other investigators have proposed slightly different schemes (32-34,62).

As in vitro tests become available, tiered testing schemes may be modified to take advantage of both whole animal and tissue and cell culture testing approaches. For example, a future scheme might call for in vitro tests as a screen, followed by in vivo tests (32,37). In vitro tests will be described later in this chapter.

Types of Animal Tests

The EPA has taken the lead in devising neurotoxicity tests for use in regulatory programs. In 1985, the Agency devised a final rule on general toxicity testing guidelines under the Toxic Substances Control Act (50 FR 39398-39418). The guidelines are categorized into three subparts: subpart B describes the procedures for general toxicity testing (i.e., acute dermal, inhalation, and oral exposure); subpart C includes testing procedures for subchronic dermal, inhalation, and oral exposure; and subpart D describes testing procedures for chronic exposure.

General toxicological tests evaluate a broad spectrum of potential toxicological effects, including some effects on the nervous system; however, these tests are not designed to examine comprehensively the possible neurotoxic properties of chemicals. In 1985, EPA proposed specific guidelines for neurotoxicity testing (50 FR 39458-39470). EPA has proposed guidelines for the functional observational battery (FOB) and specific tests to analyze motor activity, schedule-controlled operant behavior (SCOB), developmental neurotoxicity, neuropathology, and the effects of organophosphorous pesticides (1 12). When specific neurotoxicity testing is necessary, EPA currently plans to require the FOB, together with motor activity and neuropathology tests. At the present time, these three tests are referred to by EPA as the core test battery. EPA's Office of Toxic Substances and Office of Pesticides Programs are currently considering a requirement to use the core tests routinely in evaluating new and old chemicals and pesticide products. When appropriate, other tests may also be required.

Box 5-A—Tiered Animal Testing To Identify Adverse Neurobehavioral Effects of Substances

Tiered testing is an efficient and cost-effective approach to evaluate the toxicity of chemicals. In the first tier of an experiment, the recommended strategy is to identify acute hazards of substances. The second tier is designed to characterize the toxicity in repeated exposure, and the third is used to undertake detailed studies of special impairments or of mechanisms of chemical injury. Each tier provides useful information for subsequent tiers.

First tier—Animals are exposed to the substance being evaluated. The exposure period is short and covers a wide range of concentrations. The investigator seeks to identify any evidence of mortality, morbidity, or morphological changes. The experimenter also observes behavior. The first tier helps establish the parameters of exposure that are appropriate for the second tier. It may also suggest mechanisms by which the effect is produced, which may assist in the design of more sensitive experiments in the third tier.

Second tier—Animals are repeatedly or continuously exposed to substances being evaluated. This tier provides an opportunity to characterize delayed toxicity, to observe the development of tolerance, and to characterize the reversibility of adverse effects.

Third tier—At this stage, highly focused studies are performed to fully characterize toxicity, using methods dictated by the nature of the system. This tier can identify subtle sensory or perceptual impairments, affective disorders, or cognitive and intellectual dysfunction. A detailed hazard characterization not only can facilitate the identification of the most sensitive situation, but also may clarify the mechanism of action of the substance.

The above schemes may be modified in the future as in vitro tests become available.

SOURCES: A.M. Goldberg and J.M. Frazier, "Alternatives to Animals in Toxicity Testing," Scientific *American* 261(2):24-30, *1989;* R.W. Wood, American Psychological Association, testimony before the Neurotoxicity Subpanel of the FIFRA Science Advisory Panel, U.S. Environmental Protection Agency, Washington, DC, Oct. 15, 1987.

In August 1989, EPA sponsored a meeting of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel to examine various issues related to proposed guidelines for neurotoxicity and mutagenicity testing under the Act and to review the classification of several selected compounds (54 FR 35387).

Unless otherwise specified, it is assumed that both acute and subchronic testing will be conducted for both FOB and motor activity. Although some experts have recommended that neuropathological examinations be conducted following acute exposures, at the present time EPA anticipates requiring such analysis only after repeated exposures. These neurotoxicity tests represent an initial approach to identifying hazardous chemicals and are not specifically designed to develop the data necessary for full-scale risk assessments (101). (See ch. 6.)

The EPA core battery does not represent a complete screening assessment of the nervous system. For example, it does not adequately assess cognitive function, neurophysiology, or neurochemistry. Some neurotoxicologists have challenged the usefulness of the core battery, saying that it does not go far enough. Nevertheless, EPA plans to require just the core battery, with the option of using more comprehensive tests for selected compounds. Additional tests that EPA might require in conjunction with or in place of the core battery include SCOB, developmental neurotoxicity, and neurotoxic esterase assay (101).

Which tests are most appropriate for routine use in screening for neurotoxicity is the subject of disagreement in the scientific community. Some scientists believe that developmental and SCOB should be part of the EPA core test battery because they measure different aspects of neurotoxicity than do the FOB, motor activity, and neuropathology tests. Others believe that the motor activity and SCOB tests should not be used as part of an initial screen, because they may not be direct measures of neurotoxicity. EPA believes that the initial screen should include FOB, motor activity, and neuropathology assessments because these tests provide adequate initial measures of neurotoxicity and enable investigators to judge whether or not additional (second tier) testing is necessary. Descriptions of various neurotoxicity tests follow.

Functional Observational Battery

An FOB is a collection of noninvasive tests to evaluate sensory, motor, and autonomic dysfunction in either animals exposed to substances or animals having endured direct damage to the nervous system (57). FOBS are generally used as screens to determine which substances require additional testing.

EPA published a test guideline for an FOB in 1985. The EPA guideline incorporates aspects of tests developed and used in industry and academia (32-34,42,79,80). The battery is designed to be used in conjunction with general toxicity tests or neuropathological examinations, or both (50 FR 39458-39460). It serves as a screening tool (thus, it is considered a first tier test), indicating which substances should be further characterized using second tier methods. It is not intended to provide an overall evaluation of neurotoxicity. EPA is currently refining and validating its FOB.

The EPA test battery is administered to female and male rats, usually 10 per dose group per sex. Three doses of the test substances are used, with doses chosen so that the highest dose produces obvious signs of toxicity. The doses are selected on the basis of values from previous literature and experiments in order to ensure the detection of neurobehavioral effects (69,70). The observer is not aware of the dose identification. The observer records each response subjectively, using established rating scores. After all data are collected, they are entered into a computer, summarized, and analyzed using statistical methods (17,68-70). Box 5-B summarizes the procedures for conducting the EPA FOB.

The FOB is advantageous because it can be easily administered and can provide some notion of the possible functional changes produced by exposure to neurotoxic substances. It also allows evaluation of the dose-response and time course characteristics of the neurological and behavioral changes produced by exposure to a substance. Furthermore, the equipment used is relatively inexpensive, and the total time to complete an entire evaluation is short (68,69). Potential problems include difficulty in defining certain measures, a tendency toward subjective biases in assessing behavior (123), and the need for trained observers.

Box 5-B-Conducting the EPA Functional Observational Battery

In conducting the EPA functional observational battery (FOB), the technician first observes and describes the rat's posture in the home cage, then closure of the rat's eyelid and any convulsions or tremors that may be present. Next, the animal is picked up and rated for ease of handling and removal from the cage. The rat is observed and rated for signs, such as lacrimation and salivation, that the autonomic nervous system has been adversely affected. The rat is then placed on a cart top for 3 minutes, during which time the number of rears are counted and the gait, mobility, and level of arousal are rated. At the end of the 3 minutes, fecal and urine output are recorded.

Next, the technician rates the rat's responses to several stimuli, such as the approach of a pencil, snap of a metal clicker, touch of the pencil on the rat's rump, and pinch of the tail with forceps. Using a pen flashlight, the observer tests the rat for pupil constriction in response to light. The righting reflex is then measured by the ability of the rat to flip over in midair and land on its feet. Using strain gauges, the rat's forelimb and hindlimb grip strength are measured. The rat's hind feet are painted, and the technician then holds the rat a few inches above the cart top and drops it in order to measure landing foot splay. Finally, the rat's weight and rectal temperature are recorded. The entire procedure takes approximately 6 to 8 minutes per animal.

SOURCES: V. Moser, Director, NSJ Technology Services Corp., Research Triangle Park, NC, personal communication, Nov. 16, 1988; V. Moser, J. McCormick, J.P. Creason, et al., "Comparison of Chlordimeform and Carbaryl Using a Functional Observational Battery," ***Fundamental and Applied Toxicology*** 11:189-206, 1988.

Photo credit: John O'Donoghue

One component of the functional observational battery (FOB) evaluates a rat's response to an auditory stimulus.

Motor Activity

Motor activity is generally defined as any movement of the experimental animal, and it is most often evaluated after acute and subchronic exposures. The acute motor activity test is used to examine changes in animal movement following the administration of a range of acute doses. This test can also be used to determine the potential of a substance for producing acute neurotoxicity, and it may be used as a screen to evaluate certain classes of substances for neurotoxicity. The subchronic motor activity test is used to determine whether repeated dosing with suspected chemicals results in changes in activity, This test may be used to determine a substance's potential for producing subchronic neurotoxicity (50 FR 39460) (60). There is disagreement as to whether motor activity is a primary indicator of neurotoxicity. For example, the primary action of a toxicant may be at some site other than the nervous system; the changes in motor activity maybe secondary, that is, a result of the primary effect.

Proposed EPA guidelines require that the test substance be administered in different amounts to groups of animals. Levels of exposure that result in significant changes in motor activity are compared to levels that produce toxic effects not originating in the central nervous system (50 FR 39460). Observation measurements may be either quantitative or qualitative. The quantitative approach measures the frequency, duration, and sequencing of various motor components of behavior. The qualitative approach is used to gather data on the presence or absence of certain components of activity (90).

The use of observational methods to detect subtle changes in behavior has limitations. Many man-hours are required to obtain and evaluate the data. Some studies also require more than one observer. Because of possible subjective influences on data collection, a great deal of technical knowledge is required to ensure reliability. Finally, subject-observer interaction is an important consideration.

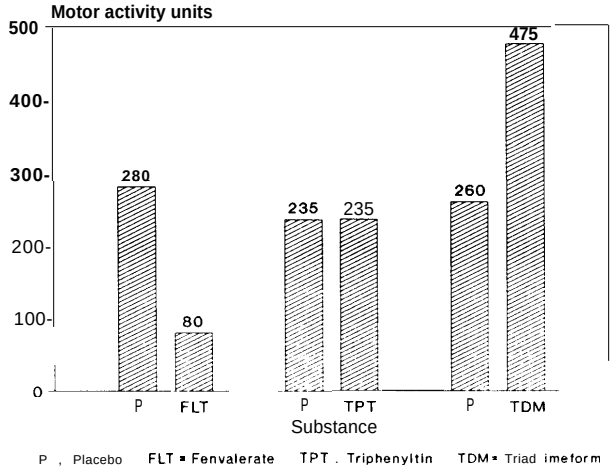
For example, the presence of the observer may modify the animal's behavior (90).

The techniques of observational analysis have included videotape recordings and computerized pattern recognition. In most cases, videotaping has minimized the problem of subject-observer interaction and has provided a permanent record of behavior which can be used for standardizing observations. The computer techniques have alleviated the problems of subjectivity (subject-observer interaction and subjective bias) and laborious data-collection procedures (90).

Some of the automated techniques that have been developed for motor activity testing include photocell devices, mechanical devices, field detectors, and touch plates. Photocell devices provide direct measures of motor activity in which beams of light traverse a cage and collide with photoreceptors. This technique involves placing the rat in a figure-8 maze and recording any movement of the experimental animal that interrupts the beam of light. The number of beam interruptions is counted and recorded by a computer for a l-hour time period (60,68). The figure-8 maze is only one of a variety of chambers used for motor activity examinations. For example, another device commonly employed for assessing motor activity is the Motron Electronic Mobility Meter, which differs from the figure-8 maze because of its rectangular shape and the density and arrangements of the photodetectors that are used to record motor activity (60). Automated motor activity measures may be used to generate dose-response data. This is typically done by placing rats in a plexiglass box, Two video cameras monitor the animal's behavior, and the video signals are transferred to computers in order to identify common patterns in movement and behavioral classification of the data (71).

Toxic substances may have a variety of effects on motor activity. To generate the data illustrated in figure 5-1, motor activity was measured for 1 hour in a group of rats in a figure-8 maze after administration of a toxic substance or placebo (P). The numbers represent motor activity units for the entire hour. Group FLT received the pesticide fenvalerate, which depressed activity. Group TPT received the pesticide triphenyltin, which had no effect on activity. Group TDM received the pesticide triadimeform, which stimulated activity, Experiments are ordinarily conducted with many doses of a toxic substance to determine how motor activity changes with level of exposure (59).

Figure 5-l—The Effects of Toxic Substances on Motor Activity



SOURCES: K.M. Crofton and L.W. Reiter, "The Effects of Type I and II Pyrethroids on Motor Activity and the Acoustic Startle Response in the Rat," *Fundamental and Applied Toxicology* 10:624-634, *1988;* K.M. Crofton, V.M. Boncek, and R.C. MacPhail, "Evidence for Monoaminergic Involvement in Triadimefon-induced Hyperactivity ," *Psychopharmacology* 97:326-*330, 1989;* S. Padilla, R.C. MacPhail, and L.W. Reiter, "Neurotoxic Potential of Pesticides: Age-related Effects of Pesticides to Youth in Agriculture," U.S. Environmental Protection Agency report, Health Effects Research Laboratory, 1985.

Motor activities recorded with mechanical devices involve a vertical or horizontal displacement of the chamber in response to the animal's motions. Some of the mechanical devices used include stabilimeters and running wheels. Stabilimeters record the movement of the animal when it causes the chamber floor to be displaced from its resting position. Running wheels are designed so that the wheel is positioned on a horizontal axle and the animal's running causes the device to rotate. Running wheels have been used in behavioral toxicology for over three-quarters of a century to study the effects of food deprivation, water deprivation, estrus, lesions of the central nervous system, and locomotor activity (90).

Field detectors are used to record the disturbances that an animal creates in moving within a test cage. Touch plates measure motor activity by recording contacts of the animal with sections of the chamber floor (90).

There are many advantages of motor activity tests. These include the availability of automated test equipment, ease of testing, and objectivity of data (60). Additional factors include obtaining reproducible data that are sensitive to the effects of acute exposure to various toxic substances. These methods do not require any special training or surgical preparations prior to testing.

Several organizations, including the National Academy of Sciences, the World Health Organization, and the Federation of American Societies for Experimental Biology, have recommended that motor activity testing be included in evaluating the toxicity of potential and known neurotoxic substances (30,64,74,123). However, further testing is usually needed to provide more specific information on the adverse health effects of the test substance. Furthermore, the data collected may not provide information on the origin of the problem or indicate what subsequent tests should be administered (64). There is general agreement within the scientific community that questions remain concerning the specificity of motor activity measures. For example, sickness resulting from chemical exposure is not always associated with changes in motor activity (60).

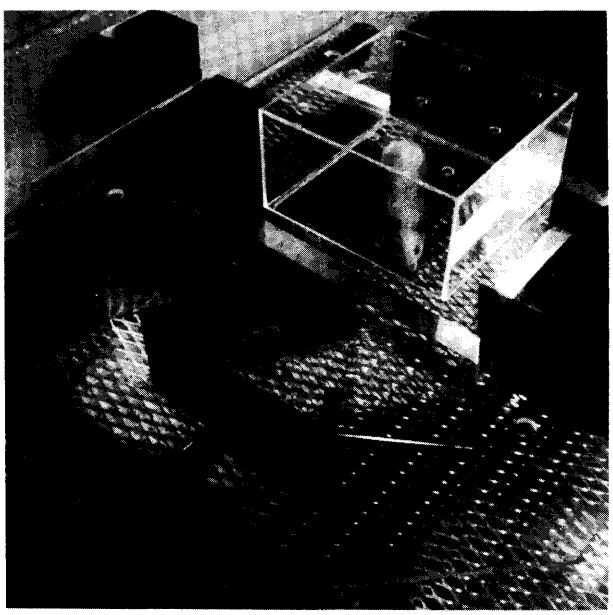


Photo credit: V Moser and R.C. MacPhail

The figure-8 maze is used to evaluate changes in motor activity after exposure to neurotoxic substances.

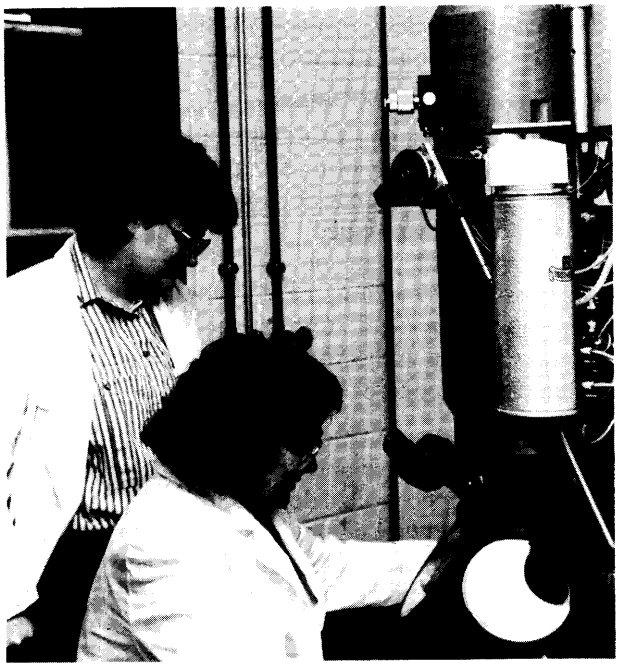


Photo credit: Julia Davis, NSI Technology Services Corp., Research Triangle Park, NC

The electron microscope is a useful tool in examining nerve tissue damaged by toxic substances.

Neuropathology

Neuropathology is the third component of the EPA core test battery (50 FR 39461). The neuropathological examination is designed to develop data on structural and functional changes in the nervous system as a result of exposure to toxic substances. EPA's guidelines recommend procedures to detect pathological alterations produced by neurotoxic substances. Morphological examination of animals exposed to neurotoxic substances helps to distinguish between pharmacological and structural types of adverse effects, describes the relative frequency and severity of the lesions, establishes the location of structural changes in the central nervous system, serves as a basis for relating particular classes of compounds to particular kinds of damage, and reveals the cellular components that have been damaged. Additional neuropathological techniques are currently in use to determine NOAELs and to examine the effects of toxic substances on the nervous system (48,100).

There is general agreement that neuropathological studies should be conducted in parallel with other neurotoxicity tests. Neuropathological evaluations may be performed following acute, subchronic, and

chronic exposures to toxic substances (50 FR 39461).

Developmental Neurotoxicology

Developmental neurotoxicology (behavioral teratology), an emerging discipline within the toxicological sciences, is concerned with behavioral and related effects in the offspring of parents exposed to neurotoxic substances prior to conception, during gestation, during lactation, or any combination of these times (45). Research efforts are under way to understand the basic principles of behavioral neurotoxicity, the biological mechanisms involved, and the appropriate methods for testing and obtaining data to be used by regulatory agencies in setting standards (45). In recent years, major advances have been made in methods for detecting the adverse behavioral effects of toxic substances on the developing organism. In 1979, the National Center for Toxicological Research (NCTR) developed a battery of tests to be used for the Collaborative Behavioral Teratology Study. NCTR served as the pilot test facility for conducting the study, and five other laboratories were involved in evaluating a standard protocol. The study was designed to assess the reliability of the test methods used and to detect the sensitivity of each (1,14,45,114,115).

Regulatory efforts in behavioral teratology began in 1975, when Great Britain and Japan produced guidelines for testing pharmaceutical substances. In 1983, the European Economic Community developed similar guidelines. WHO proposed draft testing guidelines for drugs and other substances in 1986 (45). That same year, EPA proposed testing guidelines for several glycol ethers (51 FR 17883; 51 FR 27880). A final test rule for diethylene glycol butyl ethers (53 FR 5932) was set in 1988 and for triethylene glycol monomethyl ethers (54 FR 13472) in 1989. These were the first testing guidelines directly related to developmental neurotoxicity to be promulgated by a U.S. regulatory agency.

Developmental neurotoxicity tests are used to characterize various aspects of damage to the developing nervous system, including adverse structural and functional changes. This information serves as a basis for relating particular classes of compounds to particular kinds of damage; it can then be used to predict what classes of compounds may be neurotoxic. Developmental neurotoxicity tests are also used in determining the magnitude of damage resulting from particular exposure levels, and they aid in establishing NOAELs (51 FR 17890). The guidelines for glycol ethers consist of evaluations of morbidity and mortality, growth and physical development, neurological and physical abnormalities, auditory startle habituation, learning and memory, developmental locomotor activity, and neuropathology. Recently, a consent order for the testing of 1,1,1 -trichloroethane was published (54 FR 34991); it includes developmental neurotoxicity testing.

In 1987, FIFRA's Science Advisory Panel approved the development of a generic testing guideline for developmental neurotoxicity testing (along with a guideline for adult neurotoxicity testing). Generic guidelines have also recently been proposed for developmental and adult neurotoxicity testing of pesticides. These tests are designed to determine the effects of maternal exposure to pesticides on the nervous systems of offspring. The proposed generic test guidelines require administration of the test substance to several groups of pregnant animals during gestation and lactation. Selected offspring are then tested for neurotoxicity. This evaluation is designed to detect any effects on growth and development, gross neurological effects, or behavioral abnormalities. These guidelines will be required for the testing of pesticides on a case-by-case basis. Testing may be required for substances that cause central nervous system malformations, substances already known to be neurotoxic in adults, hormonally active substances, and substances that are structurally related to known neurotoxicants (46).

In April 1989, a workshop on the comparability of human and animal developmental neurotoxicity was sponsored by EPA and the National Institute on Drug Abuse to evaluate and compare the effects of known neurotoxic substances on the developing nervous system. The workshop focused initially on several agents known to adversely effect humans, including selected abused substances (primarily methadone and cocaine), alcohol, lead, polychlorinated biphenyls, diphenylhydantoin, methyl mercury, and X-irradiation. It is possible to make qualitative comparisons of effects across species, especially when major categories of function are compared. Making quantitative comparisons in data is more difficult (46).

Based on this information, work groups then focused on the underlying basis for comparability of

effects across species, the appropriateness of current testing approaches, alternative approaches to risk assessment, and the considerations (triggers) that should be used in determining when to require testing. Participants agreed that the support for cross-species comparability was great enough that a reliable effect (including permanent and transient effects) should be considered a potentially adverse effect in humans. Also, developmental effects, in the presence or absence of maternal toxicity, should be considered adverse. Since no single category of function was found to be routinely the most sensitive, it was agreed that a battery of functions should be included in any developmental neurotoxicity testing screen. Although limitations were identified, workshop participants felt that a reference dose should be established to identify a level below which no increase in developmental neurotoxicity is expected, An abbreviated test battery was proposed for screening purposes. Whether to use this abbreviated battery or a full-scale testing protocol may depend on the type of information already available. For example, a substance that causes central nervous system malformations should be thoroughly evaluated for developmental neurotoxicity, whereas a substance that is structurally related to known neurotoxic substances might be tested first using the abbreviated battery (46).

EPA has published risk assessment guidelines for developmental toxicity (51 FR 34028) and has recently proposed amendments to these guidelines (54 FR 9386). Developmental neurotoxicity data may aid in evaluating the long-term consequences of adverse effects discovered at the time of birth and the relationship of the behaviorally effective dose to the toxic dose. These data may also aid in identifying effects that should be monitored in exposed populations (45). EPA is currently developing guidelines for the use of data on adult and developmental neurotoxicity in risk assessments.

Schedule-Controlled Operant Behavior

Changes in behavior are a useful indicator of exposure to neurotoxic substances because behavior involves the integration of motor, sensory, and higher order nervous system activities (102). Regulatory officials increasingly recognize behavioral change as an important endpoint of neurotoxicity. Several organizations, including the National Academy of Sciences and WHO, have recommended that operant behavior testing be included in evaluations

Photo credit: D. Cory-Slechta

Schedule-controlled operant behavior (SCOB) tests are used to evaluate a rat's learned behavior in scheduled intervals.

of potential and known neurotoxic substances (74,75, 123). Operant behavior refers to "behavior that is maintained by its own consequences' (50). Schedule-controlled operant behavior refers to reinforcing an animal's response to stimuli according to an explicit schedule, thereby producing orderly patterns of behavior (50).

There are several reasons why operant behavior tests may be useful. Operant behavior is critical for adaptation and long-term survival of animals. Tests of this kind allow reliable and quantitative examination of the effects of substances on behavior, and the extensive literature on operant behavior provides a conceptual framework for analysis of effects. Finally, operant conditioning allows the researcher to tailor the behavior to the needs of the experiment (98). Disadvantages of using this type of test include the cost of equipment and of data acquisition and analysis systems, the time involved in training animals to certain schedules, and the difficulties in interpreting the toxicological significance of some of the subtle endpoints used as indices of operant performance.

In 1985, EPA established guidelines for evaluating the effects of toxic substances on simple learning processes using SCOB tests. SCOB evaluates the effects of acute and chronic exposures on the rate

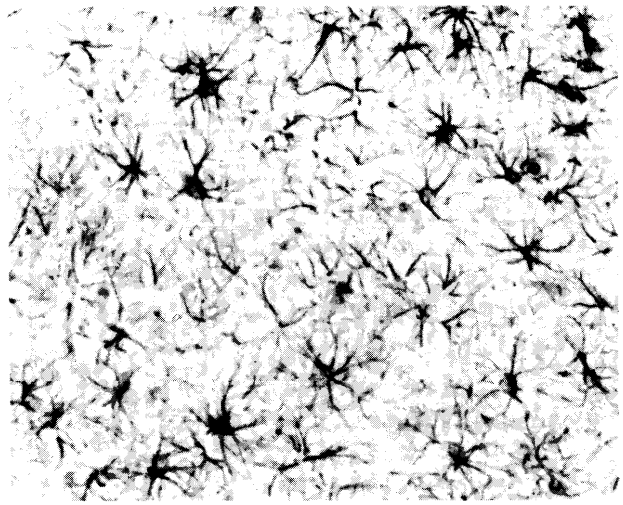
and pattern of responses under schedules of reinforcement (50 FR 39465). Following testing for behavioral effects, additional tests may be necessary. Operant behavior studies may be used in conjunction with neuropathological examinations.

EPA's approach to operant behavior testing involves placing the animal in an apparatus containing a lever and a device to deliver a reinforcer, such as milk. One method is to train the animal under a fixed-ratio reinforcement schedule, in which a fixed number of presses on the lever is followed by a reward of milk. For example, if one rewards an animal for exactly each third lever press that it makes, the ratio between responses (lever presses) and reward is fixed (50,68). Animals may also be trained under variable-ratio reinforcement schedules. In other words, the technician varies the schedules so that sometimes the third response yields milk, sometimes the seventh, and sometimes the hundredth. The animal never knows when the next reward is coming (50). These schedules of reinforcement may be used to generate moderate response rates that may increase or decrease as a function of exposure to toxic substances (50 FR 39466). Several kinds of SCOB tests are currently used in industry (49,50,89,102).

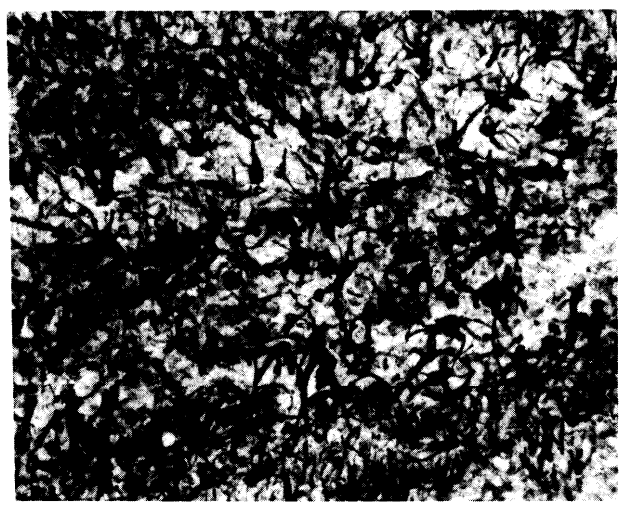
A variety of other testing schemes are commonly used to examine behavior. These include tests to determine the effects of neurotoxic substances on motor coordination, tremor, sensory processes, reflexes, and learning and memory (23,27,29,49,66,102). There is some disagreement in the scientific community as to the optimal approach for evaluating operant behavior.

Biochemical Markers

Various biochemical markers have been used to assess the effects of toxic substances on adult and developing nervous systems. EPA recently developed a proposed guideline for the assessment of developmental neurotoxicity using a glial fibrillary acidic protein (GFAP) radioimmunoassay (77). GFAPs are proteins located in the glia, the non-neuron satellite cells of the central nervous system. When glial cells are damaged by toxic substances, they substantially increase production of GFAP. The proposed test is designed to develop data on changes in the amount of GFAP in the developing nervous system after postnatal exposure to a toxic substance. Such an assay is a useful adjunct to developmental neuropathological examinations (76,77), Assays of proteins in neurons and glia can be used to detect and characterize specific responses and alterations in brain development due to toxic substances. While not designed to uncover basic mechanisms underlying specific neurotoxic effects, this approach can aid in defining neurochemical mechanisms underlying altered brain development (78).



A. Control



B. Rat exposed to trimethyltin.

Photo credit: J.P. O'Callaghan, U.S. Environmental Protection Agency

Trimethyltin increases levels of glial fibrillary acidic protein (GFAP) in astrocytes of the rat brain, a sign of nervous system damage.

Specialized Tests for Organophosphorous Pesticides

Exposure to some organophosphorous pesticides produces delayed effects, including weakness of limbs and improper function of certain motor

neurons. Evidence of toxicity first appears approximately 2 to 3 weeks after initial exposure. In 1985, EPA established guidelines for neurotoxic esterase assay for organophosphates (50 FR 39463). These guidelines describe the procedure for measuring the inhibition of an enzyme known as neurotoxic esterase (NTE) in the brain or spinal cord of hens exposed to organophosphorous substances (50 FR 39463). This assay is intended to serve as an adjunct to behavioral and pathological examinations of hens and is not intended to replace in vivo tests.

EPA also established guidelines in 1985 for a test of acute delayed neurotoxicity of organophosphorous substances (50 FR 39466-39467). This test involves administering a single dose of these substances orally to adult hens and observing them for symptoms such as gait changes, lack of coordination, and paralysis. The animals are observed daily for approximately 3 weeks until effects are determined. All signs of toxicity are recorded, as well as the duration and extent of exposure. In addition, the hens are evaluated for motor ability at least twice a week, with various tests. If neurotoxic effects are not seen immediately, the dosage may be repeated and the observation period extended (50 FR 39466-39467). Later, pathological examinations are also conducted on the animals.

Subchronic delayed neurotoxicity refers to a prolonged lack of coordination resulting from repeated exposure to a toxic substance over a limited period of time. In 1985, EPA established guidelines for a test of subchronic delayed neurotoxicity of organophosphorous substances (50 FR 39467). This test involves administering these substances orally to hens for approximately 3 months. It is usually conducted after obtaining information from acute tests. Evaluators observe the hens daily for such indicators as gait changes, lack of coordination, and paralysis. Following the observation period, pathological tests of selected neural tissues are conducted using perfusion techniques and microscopic evaluations. In addition to providing information on the possible health effects of repeated exposures to organophosphorous substances, this test may provide information on dose-response, thus aiding in determining an estimate of a no-effect level.

Neurophysiology Techniques

Neurophysiological tests for assessing the health effects of potential and known neurotoxic substances are usually adopted by neurotoxicologists from testing techniques used in the basic neurosciences. These tests are designed to provide specific types of information, and the technique or set of techniques chosen for a given application will depend on the nature of the scientific issues under investigation (9).

In general, neurophysiological testing techniques depend on the electrical properties of nerve cell membranes. The firing of a single neuron involves the movement of electrically charged ions across the membrane. This movement of charged particles creates electrical potentials which can be measured. The measured potentials, in turn, reflect the functioning of the neuron or neurons that generated them. Neuronal potentials are usually measured by placing electrodes on or near the neural tissue of interest. In many cases where the neural tissue is not directly available, such as the human brain, the electrodes can be placed at remote sites for detection of electrical activity which is conducted through the cranial tissues. The electrical signals recorded from the electrodes are typically amplified, filtered, and passed on to a data acquisition device such as a computer (9).

It is convenient to categorize electrophysiological testing techniques by the size of the recording electrodes used. These range from a few microns to several millimeters. The former, termed "microelec - trodes," can be used to penetrate cell membranes and measure the function of single neural cells or parts of cells, such as membrane ion channels or synaptic endings. Moving up in size, “multiunit electrodes” can be placed in the vicinity of several cells and can measure the activity of each neuron in a cluster of neurons simultaneously. Still larger ‘‘macroelectrodes’ can measure the summed activity of many neurons, possibly thousands of cells. With macroelectrodes, the activity of individual cells is no longer detectable; instead, the activity of neural systems can be monitored (9). Neurophysiological tests may be used to study neural function either in vitro or in vivo, and they can measure spontaneously emitted neural responses or those evoked in response to some type of stimulation (9).

For neurotoxicological applications, microelectrode techniques and in vitro procedures are useful for investigating mechanisms of action of known neurotoxic substances because of the specificity of the techniques. For investigating the potential neurotoxicity of compounds with unknown properties,

in vivo macroelectrode procedures are more useful because of their generality. One set of macroelectrode techniques, sensory evoked potentials (EPs), is being developed by EPA for potential use in neurotoxicology testing paradigms. This approach has been endorsed by several industrial organizations (9).

Sensory evoked potentials can be used to identify which of the sensory systems in the nervous system are affected by neurotoxic substances and to provide information about the nature of these changes. In addition, sensory systems are model systems for studying 'generic' dysfunctions, since they include all the components of other systems but can be studied relatively noninvasively. Evoked potentials are essentially electrical signals that are generated by the nervous system in response to a stimulus. Using neurophysiological techniques, these signals can be measured and recorded. Various types of evoked potential techniques are currently in use, including brainstem auditory evoked responses, flash evoked potentials, pattern reversal evoked potentials, and somatosensory evoked potentials (25,56,61).

The electroencephalograph (EEG) records spontaneous, ongoing electrical activity in the brain (activity that, unlike EPs, is not associated with presentation of a stimulus). Electrodes are surgically implanted in a rat's skull or pasted onto a human's scalp. The electric potential differences between the electrodes are measured and the changes in the potential difference are recorded. EEGs can provide a detailed record of electrical activity at several brain sites, allowing investigators to identify general regions of the brain that may be adversely affected by acute or long-term exposure to known or potential neurotoxic substances. However, EEG data can be difficult to interpret, and the technique provides limited information on the mechanisms of action of toxic substances (4,43,97). The limitations of EEGs spurred the innovation of methods for measuring evoked potentials.

Brainstem auditory evoked responses (BAERs) can be used to detect specific losses in the auditory system and thus to determine specific regions of the rat's nervous system that have been damaged (25). This approach has been used to assess the effects on hearing of various solvents, such as toluene (56,61, 82,83,88).

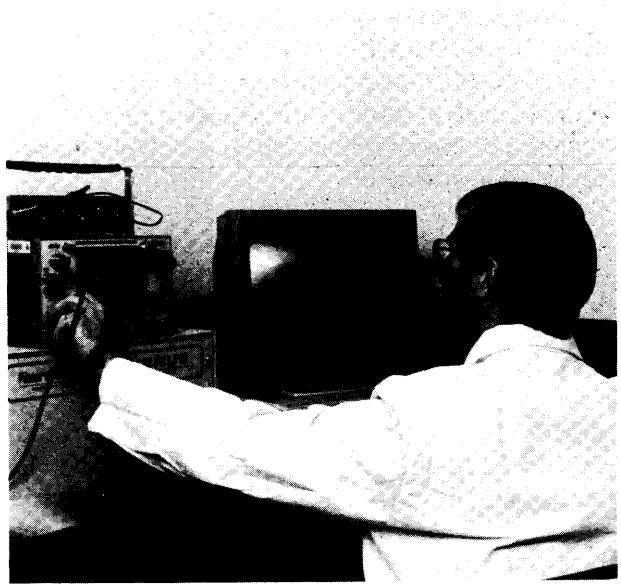


Photo credit: Julia Davis, NSI Technology Services Corp., Research Triangle Park, NC

Experimental neurophysiologist examines a visual evoked potential recorded from a subject watching the checkerboard stimulus seen at right.

Visual evoked potentials, which include flash evoked potentials (FEPs) and pattern reversal evoked potentials (PREPs), are used to evaluate the effects of toxic substances on those components of the nervous system responsible for vision (25,61). The visual system is vulnerable to neurotoxic substances, and acute and chronic exposure to such substances can lead to damage of the retina and the nerve cells in various areas of the brain that process the information received from the retina. Visual evoked potentials have been used to assess the effects of various heavy metals, pesticides, and solvents on visual function in rats. Potentials can be generated using stimuli ranging from diffuse light flashes to complex patterns of shapes and colors (25,61,83).

FEPs in rats are altered by exposure to many heavy metals, pesticides, and solvents. One technique for using FEPs in neurotoxicological studies involves flashing a strobe light of high intensity (turning on and off an intense stimulus) at the test species followed by observing and analyzing the effects on the visual system. One common technique involves placing the rat in a chamber surrounded with mirrors on three walls and on the fourth wall a strobe light which flashes at various intensity levels. Stimulus intensity, pupil diameter, and level of light

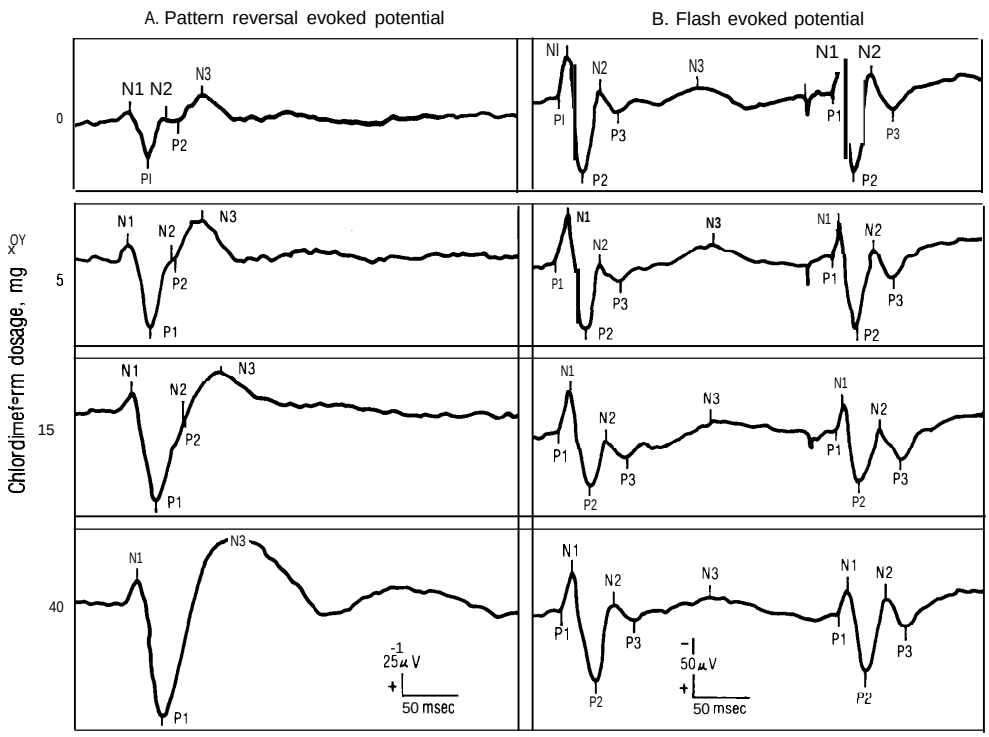
adaptation are the major parameters of concern in recording FEPs (4,25,56,61,82,97). Following FEP examinations, a neuropathological examination may be conducted to identify any retinal or brain lesions (damage or loss of retinal cells) caused by exposure to the toxic substance.

PREPs are used in the diagnosis of optic neuritis, multiple sclerosis, and other illnesses that affect the visual system in humans. Visual evoked potentials can be created by changing a pattern of bright and dark areas on a screen in front of an animal without altering the overall level of illumination. Patterns for PREP testing are generated by reversing the checks on a checkerboard display (black for white and vice versa) or the bars in a horizontal or vertical arrangement. One drawback of this technique is that it is difficult to ensure that animals focus on the patterns, especially without training (4,25,56,61 ,82, 83,97). On the other hand, PREPs can be recorded in awake rats without concern for the focal point. When the stimulus is in the rat's visual field, the eyes will be in focus (10).

Figure 5-2 indicates the results of testing the chemical chlordimeform on the rat visual system. As the dosage of the toxic substance is increased (from O to 40 micrograms per kilogram), the amplitude (size) of the PREPs increases (note, e.g., the distance from points N1 to Pi), but the amplitude of the FEPs is unchanged. The chlordimeform enhances the response to high-contrast, but not to low-contrast, stimuli (12).

Somatosensory evoked potentials (SEPs) are commonly used to determine the effects of both potential and known toxic substances on the nervous system. The somatosensory nerves are the longest cells in the body, extending from the limbs to the head. In testing, an electric current is applied to the sensory nerve of particular interest and the SEPs are measured. Responses can be examined at many points along the nerve. This approach has been used to study the effects of acrylamide (4,25,26,56,61 ,82) and sulfuryl fluoride on the rat's somatosensory system (63).

Figure 5-2-Pattern Reversal Evoked Potential (PREP) and Flash Evoked Potential (FEP) After Treatment With Chlordimeform



SOURCE W Boyes and R S Dyer, "Chlordimeform Produces Profound, Selective, and Transient Changes in Visual Evoked Potentials of Hooded Rats," *Experimenta/ Neurology 86434-447,* 1984

SEPs have been used extensively in neurotoxicological studies because they provide rapid, effective, and quantifiable methods for testing sensory functions (including the visual, auditory, and somatosensory systems). Another advantage is ease in surveying the entire sensory pathway to the brain. However, the equipment associated with this technique is expensive, and special training is often required to operate it. Another limitation is that, due to the large variability among rats, many must be tested to obtain statistically reliable results.

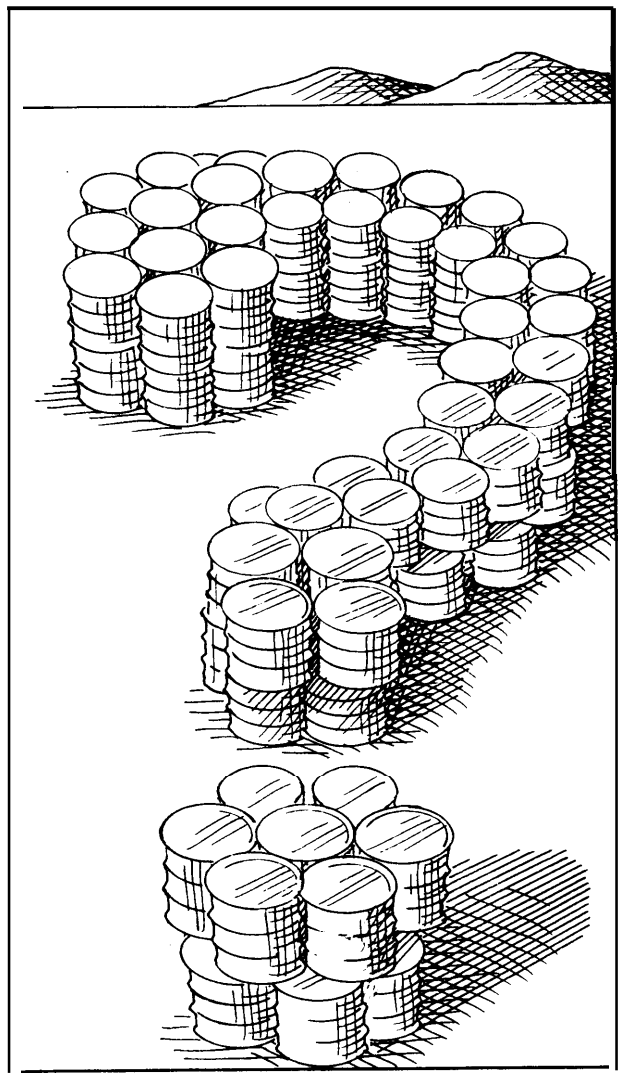
Animal Testing Issues

How Well Are Animal Test Results Extrapolated to Humans?

An important goal of toxicology is to increase the capability of predicting human responses from animal toxicity tests and to understand the causes of interspecies differences in susceptibility to toxic substances. The greatest difficulty in extrapolating animal data to humans is the difference in responses between humans and animals to toxic substances. Humans may be more sensitive to certain substances than animals and vice versa. In addition, since the human population is more heterogeneous than any animal species, the range of doses producing an effect on humans maybe larger than that for animals (122).

Sex, age, health, nutritional state, and genetic makeup may affect an animal's response to toxic substances and must be considered when selecting an animal model. Also, similarity between animal and human metabolism is an important consideration because it may influence the final determination of whether a chemical will be therapeutic or toxic, will be stored or excreted, or will cause acute or chronic effects in humans (65).

When the risks of toxic substances are being assessed, the potential exposure of humans is a critical consideration. Toxicological data on experimental animals should be applied to the situations and routes of exposure that are likely to occur for humans. For example, data collected from the oral administration of a substance to animals have less relevance to a situation in which humans are exposed by inhalation. In addition, an evaluator should be cautious when applying data obtained on young, healthy animals to a human population that is diseased, malnourished, or diverse in its genetic makeup. The data that are to be evaluated to determine a potential risk should be obtained from animal models that are as similar to humans as possible (65). When assessing functional effects, the measures taken in animals should relate to the functions that are at risk in humans. Thus, if human complaints are confusion, memory loss, or irritability, the animal data should be addressed, to the extent possible, to changes in these functions.



Illustrated by: Ray Driver

ALTERNATIVES TO ANIMAL TESTS

Some individuals argue that more animals are used for testing than are needed and that changes in experimental design or improved methods of data analysis could reduce the number of animals used. Alternatives to animal tests, such as in vitro tests,

serve the same fundamental purpose as whole animal tests: to establish the toxicological properties of a chemical in order to protect and improve human health and the environment. In vitro approaches use animal, human, or plant cells, tissues, or explants maintained in a nutritive medium for use as a model system in toxicity testing.

Concern about the use of animals in testing seems to be accelerating at the same time as concern about product and drug safety. However, the need for more experimental animals is an incentive for the development of new techniques, especially faster and less expensive ones (105). While Federal regulatory agencies currently rely on animal tests to predict human toxicity, in vitro alternatives are likely to play an increasingly important role in future toxicological evaluations.

In vitro tests are often used to complement animal tests and reduce the number of animals being used for routine toxicity testing. Methods for integrating in vitro tests into routine toxicity testing are necessary to enhance understanding of the neurotoxic potential of toxic substances (37).

Toxicologists have identified three major reasons for developing in vitro techniques: scientific-**academic, economic,** and humane. There are many scientific-academic reasons for developing in vitro methods. There are more than 60,000 chemicals in EPA's inventory of toxic substances and thousands more chemical formulations, many of which have not been tested for toxicity. Current testing methods are time-consuming; for example, it might take from 3 months to 2 years to complete a battery of chronic studies. With the enormous number of substances that have not been tested and with new substances continually entering commerce, rapid, inexpensive methods are needed for screening.

In vitro testing is already of critical importance in academic scientific research. This approach is often employed to determine the mechanism of action of toxic agents because in vitro systems are less complicated and can be manipulated easily. Tissue culture methodologies have advanced rapidly, and new equipment and facilities will ensure continued progress (36). It has been estimated that more than $70 million has been spent in the United States over the past decade to develop in vitro testing (37). There are numerous opportunities to apply the knowledge that has been gained in basic research to the development of methods of toxicity testing.

The cost of in vivo research and testing is increasing. In vitro approaches are generally more economical, being both less expensive and less time-consuming. In addition, they are also more humane because they reduce animal use and minimize animal suffering (36).

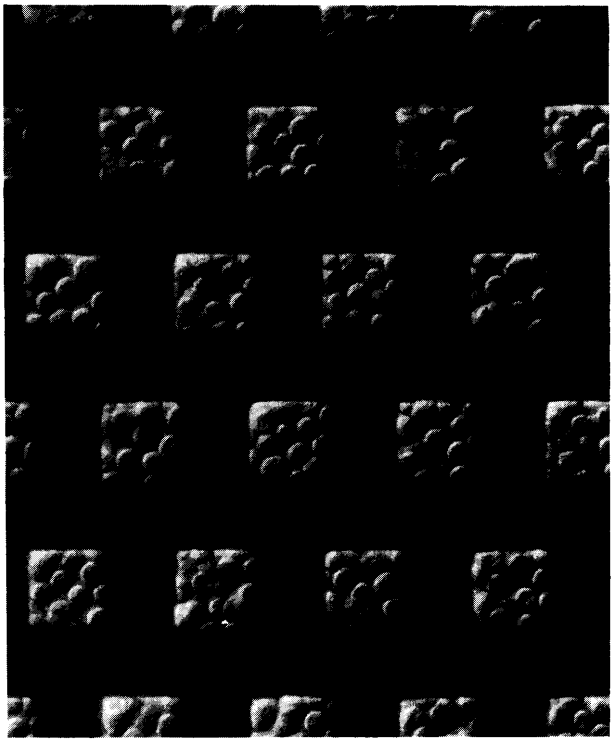
In Vitro Neurotoxicity Test Development

Interest in using in vitro testing approaches to assess neurotoxicity has increased considerably in recent years. In 1980, a symposium on the use of tissue culture in toxicology, held in Sosterberg, Holland, focused on the potential application of in vitro approaches to the study of neurotoxic substances. Participants emphasized the need for improved methodologies and increased awareness in the regulatory community of the utility of in vitro techniques. Since that time, efforts to develop in vitro tests have advanced rapidly (36,37,103).

In vitro tests do have some limitations. They cannot mimic the complex biochemical and physiological interactions that take place in vivo. Also, the supply of normal human cells available for toxicological testing is currently limited. In order for human cells to be used routinely for toxicity testing, some method of making them more readily available must be devised. In addition, not all human cell types can be cultured (103).

A number of companies in the United States are currently developing in vitro toxicological tests. For competitive reasons, industry initiatives are generally not made public. Consequently, they will not be addressed in this report.

The Food and Drug Administration (FDA), the National Institutes of Health (NIH), the Consumer Product Safety Commission (CPSC), and EPA are examining potential in vitro testing approaches (116). In particular, the National Toxicology Program of the Department of Health and Human Services is evaluating in vitro systems and has asked for proposals on alternative test development (1 16). The CPSC is attempting to make greater use of existing chemical, biological, and human data in order to avoid animal tests, to reduce the number of animals used in tests, and to modify existing methods so as to reduce pain and suffering (95). EPA has also taken action to reduce the use of animals in toxicity research and testing.



Photograph by J.C. Owicki and K.M. Kereso

Photomicrograph of living cells in the wells of chambers that allow monitoring of changes in cellular metabolism following exposure to toxic substances.

Numerous in vitro techniques are currently in use. Tissue culture involves maintaining or growing organs, tissues, or cells in vitro for more than 24 hours. Tissue culture can be further subdivided into cell culture and organ culture (22,105).

Tissue Culture

Many tissues from humans and animals can be successfully maintained and studied in culture. Roux originally used tissue culture in 1885 to maintain chick embryos outside the egg (99). Nervous tissue was among the first tissues to be cultured. In 1907, R.G. Harrison developed a method for maintaining frog neural tissues in vitro for weeks (40). In the 1930s, advances were made in defining the media required for maintaining cells and tissues in culture, and by the 1950s, tissues could be cultured in entirely synthetic media. At the same time, scientists became aware of the importance of adding antibiotics to culture systems. Before antibiotics, bacterial growth interfered with the developing cells, and all work had to be done in aseptic conditions. It is now standard procedure to inhibit bacterial growth with antibiotics (99,105).

Pure cultures of cells and mixtures of cells have different properties. These differences may be used to study various aspects of cell activity, such as differentiation. In this process, one can distinguish between cells that have a capacity to form other cells (undifferentiated cells), and cells that have reached their final stage of development and will not undergo any further change (differentiated cells) (21,99).

In cell cultures, the colony consists of a mass of differentiated or undifferentiated cells, and individual cell types are not easily identified. However, where a number of different kinds of cells are growing together, such as in organ cultures, the cells retain their normal function and differentiated form; thus, the different types of cells are easy to identify (99). Tissues can be kept alive outside the living animal for months or years in cell cultures; however, whole organs can be sustained in cultures for only a few days to a few weeks.

Assessing toxicity using tissue culture approaches generally involves adding a test substance to the culture, observing the viability of the cells, and identifying any structural or functional changes.

Applications of In Vitro Techniques to Neurotoxicity Testing

Various types of in vitro techniques are being developed to evaluate the effects of potential and known neurotoxic substances. These approaches can be grouped into three general categories: primary cultures, cell lines, and cloned cells.

Primary Cultures

Primary culture refers to the removal and maintenance of cells, tissues, and organs in vitro. Embryo culture, for example, has proven to be very useful in neurotoxicological studies. Recently, the Chemical Industry Institute of Toxicology (CIIT) in Research Triangle Park, North Carolina, developed a rodent fetal cell culture system for in vitro testing. This approach involves removing certain regions of the brain from mouse embryos and culturing them in a chemically defined environment. After the culture is exposed to various known and potential neurotoxic substances, the tissues and cells can be examined for morphological and biochemical changes (20). This technique is useful because neuronal tissues undergo normal or near-normal development, and cellular and tissue interactions can be analyzed.

CIIT scientists are using a class of substances known as monohalomethanes to validate this test system. Animal and human exposure to monohalomethanes may result in a variety of neurological symptoms, such as tremors, lack of coordination, epileptic seizures, and coma. The results from in vitro studies using monohalomethanes are compared with documented animal studies to determine correlations between in vitro and in vivo methods. Development of a database to compare results from in vitro and whole animal studies, human studies, and epidemiological studies may aid in validating this system (20). A similar embryo culture approach was used successfully by others to demonstrate that ethyl alcohol can retard the growth and differentiation of fetal tissues (13).

Retinal neurons may also be employed to evaluate the effects of toxic substances on the nervous system. This approach involves dispersion and culture of retinal cells removed from chick embryos. Culture methods have recently been improved, allowing growth of low-density, clump, and flat cell-free cultures of chick embryo neurons. These cultures can be used to analyze the effects of toxic substances on cell differentiation using time-lapse video recordings. In addition, various biological techniques may be used to define and characterize observed effects (2).

Techniques for culturing neonatal mouse retinal neurons and photoreceptors have also been developed recently. Cells from the retinas of 2-day-old mice can be cultured in serum-free, completely chemically defined environments. They serve as useful models for evaluating the survival and differentiation of photoreceptor cells, which are critical to visual processes (87).

A "monolayer" culture system has been developed to allow the survival and differentiation of chick embryo retinal neurons and photoreceptors without contamination. Photoreceptor cells can be purified with kainic acid and B-bungarotoxin, which, when added to the culture medium, destroy many retinal neurons without affecting the photoreceptors (86). The technique of selectively destroying cells is a recognized means of cell separation in tissue culture. Once purified photoreceptors are available, the effects of various toxic substances can be determined without the complicating factors introduced by multiple cell types.

Muscle cells can also be cultured, allowing investigators to analyze the effects of toxic substances on the neuromuscular system. Cultured muscle cells from rats and chicks have been used in electrophysiological studies to examine the sensitivity of acetylcholine receptors. Toxic substances have also been used to aid in characterizing the structure and function of acetylcholine receptors (91). This type of system could be adapted to assess the effects of toxic substances on the neuromuscular junction.

Another useful testing method involves organotypic cultures, cultures that preserve the connections and spatial relationships between neurons and glia (126). One such culture used in neurotoxicity studies is of the ganglion (a collection of nerve cells external to the brain or spinal cord) (96). In addition, the mouse embryo spinal cord has been used to study the effects of various neurotoxic substances, including organophosphorous pesticides **(35).** Organotypic cultures have also been used to examine the mechanisms of action of a wide range of neurotoxic substances, including such metals as mercury and thallium and such organic compounds as chloroquine (a drug used to treat rheumatic fever) and 2,5-hexanediol, a metabolize of n-hexane (126).

Explant cultures are also useful in evaluating neurotoxicity. They involve placing a small piece of nerve tissue in a culture medium and maintaining it for several weeks or months at a time. Explants have been used to evaluate the effects of chemicals on the myelin sheath surrounding nerve cells and on the synaptic connections between these cells (96).

Cell Lines

Cell lines take advantage of the immortal properties of certain types of malignant nervous system cells. For example, the neuroblastoma C-1300 and the rat glioma C-6 cell lines have been used in neurochemical and morphological studies for evaluating the effects of a variety of neurotoxic substances (22,35). One group of investigators recently fused rat retinal cells with mouse neuroblastoma cells to create a hybrid cell line that proved to be very useful in evaluating the neurotoxic effects of the amino acid glutamate and related compounds (73). Cell lines are especially useful because a large quantity of single cell types are available for biochemical analysis, the cells can be easily examined microscopically, and electrophysiological evaluations may be undertaken (96).

Advantages and Limitations of In Vitro Testing

In vitro tests are advantageous for several reasons. They involve simpler procedures and consequently take less time to complete than animal tests. For example, technicians can conduct morphological, biochemical, and physiological studies on the same preparation (93). Furthermore, cultures can be transferred from one region of the country to another, allowing evaluation of the same culture in various laboratories specializing in particular tests. Cultures can be made of human cells, hence the difficulty of species variation and of extrapolation of data is minimized. Substances may be studied in isolation, and responses by selected cell populations can be examined. Also, the cellular environment can be controlled through modification of the concentration and nature of specific nutrients, which is difficult using animals (21,99,20).

On the other hand, in vitro tests normally do not account for the route of exposure to a substance, its distribution throughout the body, or its complete metabolism. Also, because in vitro systems generally do not duplicate the neural circuitry of the entire animal, toxic endpoints (e.g., behavioral changes, motor disorders, sensory and perceptual disorders, and lack of coordination) may be difficult to define (93). Other concerns are that substances added to the culture to keep it viable (e.g., antibiotics) might interact with the tested substance, that cell lines of cancerous cells may respond to toxic substances differently than normal cells, and that it may not be possible to perform chronic toxicity studies due to the relatively short lifespan of many cultures (cell lines using immortal cells are a possible exception). Nevertheless, all test systems have limitations, and there is general agreement that the many advantages of in vitro testing present a strong incentive for continued development and increased utilization (21,99,20).

HUMAN TESTING

Millions of U.S. workers are exposed full- or part-time to general toxic or neurotoxic substances (3). Nearly 400,000 cases of occupational diseases are recognized annually (111). Preventing the adverse health effects of chemicals is largely dependent on understanding the toxicological properties of new and existing chemicals. Various standardized human tests are available to assess the adverse effects of toxic substances on the nervous system; however, because of the ethical issues inherent in performing some human tests and the difficulty of obtaining trained staff and expensive equipment, there have been relatively few human studies conducted (24).

Overview of Human Tests

Human testing may occur in response to occupational, environmental, or laboratory exposures. The methods used to assess the toxicity of substances vary from one setting to another, since some approaches are appropriate in one situation but not in others. For example, when determining early symptoms of chronic exposure, subjects exposed occupationally are better test groups than groups exposed environmentally. On the other hand, in certain epidemiological studies, subjects exposed environmentally may be helpful because of the large diversity of individuals and wide range of ages (74).

In the occupational setting, workers are often exposed unintentionally to toxic substances. In the general environment, exposure groups may include individuals and families living near sources of industrial pollution, people living in large industrial cities where they are exposed to vehicle exhaust and fuel additives, and farmers and agricultural workers exposed to pesticides in the field (74). Epidemiological studies of these individuals are required to determine the extent to which neurotoxic substances are affecting human health.

Neurobehavioral Tests

Neurobehavioral tests can provide objective evaluations of nervous system and neurobehavioral functions. Test methods have been utilized both in evaluation of groups of workers exposed to substances and in laboratory examinations of individuals suspected of having occupational illnesses. In the evaluation of a group of workers, neurobehavioral tests are used to assess exposure-effect relationships and, in some cases, to serve as guides for establishing standards for workplace exposures. In the laboratory setting, neurobehavioral methods are useful in quantifying the degree of functional disability and in making a diagnosis (44).

Several considerations are involved in the selection of testing techniques to determine the effects of neurotoxic substances on workers' health. It is very important to consider the purpose of the examina-

tion. For example, the study may be designed to identify effects on individual workers who are exposed or on a population of workers exposed as a group. Furthermore, the frequency and duration of exposure must be determined: a study of acute effects may require tests measuring different functions and properties than a study of chronic effects. Finally, in some tests a certain time period must elapse before effects become apparent (44,67). Researched most commonly select tests that are known to measure functions affected by several neurotoxic substances; provide a complete analysis of nervous system effects, ranging from reflexes to complex behaviors; are known to measure one or more well-defined functions, whether psychological or neurophysiological; and are cost-effective in terms of the information they provide (44,67).

Neurobehavioral test results are influenced by many factors. These can be divided into three general classes: subject, examiner, and environmental. Subject factors include the individual's age, sex, education, socioeconomic status, health and drug history, and motivation. Table 5-1 summarizes the subject factors influencing neurobehavioral test results. Examiner factors are another important consideration. In order to ensure the cooperation of subjects and to maximize the reliability of the data, it is important to establish a good working relationship between examiner and participants. It is also important that a well-trained examiner speak and interact with all subjects in a consistent and standardized manner (44). Environmental factors that influence neurobehavioral studies include the test surroundings, subject-experimenter interaction, and season of the year.

Table S-l-Subject Factors Influencing Neurobehavioral Test Results

Age: The performance on neurobehavioral tests varies with age. When comparing exposed groups, subjects should be matched by age as closely as possible.
Sex: There are biological and social differences that must be considered when designing tests that include male and female workers.
Years of school **education:** Amount of education also influences the performance on neurobehavioral tests.
Socioeconomic status: Socioeconomic status includes a combination of educational, cultural, and occupational factors that may affect test results. This factor takes into account the years of school education, regular income, and special occupational training.
Health and drug history: Any disease that affects neurological functions will affect neurobehavioral studies. Some of these diseases include epilepsy, diabetes, and arthritis. If an individual has any of these health problems, the evaluator may want to exclude the individual from the study. Drugs must also be considered. Psychoactive drugs, in particular, can alter performance on the study. In addition, certain consumed foods and beverages may alter the individual's alertness and performance. These include coffee, colas, and chocolate, all of which contain caffeine.
Motivation: The attitude of the participants must also be taken into account.

SOURCE: B.L. Johnson (cd.), *Prevention of Neurotoxic Illness in Working Populations (New York, NY:* John Wiley& Sons, 1987).

Finland's Institute of Occupational Health Approach

During the 1950s, the first neurotoxicity test battery for occupational exposure was developed at Finland's Institute of Occupational Health (FIOH). The battery was designed to study the effects of various substances, especially solvents, on workers. The 14 neurobehavioral tests listed in table 5-2 are typical methods used at FIOH to evaluate effects on intelligence, short- and long-term memory, learning ability, perception, motor performance, and personality. The battery is now used routinely in Finland (39).

Psychological testing is usually conducted at the Institute, although sometimes it is conducted at an industrial facility. The tests are usually performed on an individual basis. Before the tests are administered, the patient is interviewed. The tests are presented in a fixed order, as indicated in table 5-2. The examination takes 1 to 3 hours, depending on the tests used and the time available for interviews (39).

World Health Organization's Recommended Approach

During a meeting cosponsored by WHO and the National Institute for Occupational Safety and Health in 1983, neurotoxicologists recommended a core set of tests, known as the Neurobehavioral Core Test Battery, that could be used in screening for neurotoxic effects. This test battery is particularly useful in developing countries or in places where there are limitations in the setting or the literacy of the test population (3).

Table 5-3 lists the tests used in this battery. They were chosen to allow development of uniform, consistent data from a variety of occupations and neurotoxic exposure situations (3). Most of the core tests require the use of paper and pencil in order to minimize the need for mechanical instruments (a concern for developing countries). These tests generally require minimal training to administer; how-

Table 5-2-Behavioral Test Battery for Toxicopsychological Studies Used at the Institute of Occupational Health in Helsinki

Test method+Test description
Wechsler Adult Intelligence: -determining similarities between items; measures verbal ability -determining synonyms of words; measures general intelligence and verbal ability —reproducing patterns of design using blocks; measures visual ability -determining the missing parts of pictures; measures perception —associating symbols and digits; measures memory and speed —recalling digits in series; measures verbal memory
Wechsler Memory Scale: –logical memory, visual reproduction, and associative learning
Benton Visual Retention Test: —recalling and reproducing figures; tests memory and visual retention ability
Kuhnburg Figure Matching Test: —recalling various figures on cards; measures speed and memory
Bourdon *Wiersma Vigilance Test:* —strike over all groups of 4 dots as printed on the test sheet (50 rows); each row contains 25 groups of 3,4, or 5 dots; performed as accurately and quickly as possible; measures speed and perception
Figure Identification: —identifying figures; measures speed and perception
Symmetry ***Drawing Test:*** -drawing the other symmetric half of figures; measures perception and motor speed
Santa Ana Dexterity Test: —test for manual dexterity; hand-eye coordination; measures the ability to perform skillful movements with hands and arms
Flnger Tapping Test: —taps a counter with thumb rapidly; measures motor speed
Reaction Time: —reactions of hands or feet from visual and auditory signals; measures simple reaction time to respond to stimulus
MIra Test: -draw simple, straight, and broken lines without seeing the paper and pencil; measures psychomotor behavior and psychomotor ability
Rorschach Inkblot Test: —variables: adaptability, emotionality, spontaneity v. inhibition, rational self-control, originality of the perception; measures personality, nonintellectual personality disturbances, changes in mood, readiness for affective reactions
Eysenck Personality Inventory: —measures two dimensions of personality: neuroticism and extroversion-introversion
Questionnaire: —measures changes in mood, emotionality, and subjective well-being; two forms used: 1) measures sleep disturbances, fatigue, neurotoxic behavior; and 2) measures disturbances in control of mood, emotions, attention, fatigue

SOURCE: H. Hanninen and K. Lindstrom, ***Behavioral Test Battery for Toxicopsychological Studies Used*** at ***the Institute of Occupational Health in Helsinki*** **(Helsinki: Institute of Occupational Health, 1979), pp. 1-58.**

Table 5-3—WHO Neurobehavioral Core Test Battery

Functional domain	Core test
Motor speed, motor steadiness	**Aiming (Pursuit Aiming ii): assess** the control and precision of hand movements; individual is required to follow a pattern of small circles, placing a dot in each circle around the pattern; subject's score is the number of taps in the circle within 1 minute
Attention, response speed	Simple reaction time; see table 5-2 for description
Perceptual-motor speed	**Wechsler Adult Inteiiigence** Scale: a sheet contains a list of numbers that are associated with certain simple symbols and a list of random digits with blank spaces below them; subject asked to write correct symbols in blank spaces as fast as possible
Manual dexterity	**Santa Aria: see** table 5-2 for description
Visual perception, memory	**Benton Visual Retention:** see table 5-2 for description
Auditory memory	**Wechsler Adult intelligence Scale:** recall digits in series forwards and backwards immediately after hearing them

SOURCE: B.L. **Johnson, (cd.),** ***Prevention of Neurotoxic Illness in Working Populations (New York, NY:*** John Wiley & -Sons, 1987).

ever, the reaction time test requires the use of an electrical instrument that necessitates some training. The total amount of time necessary to complete the core test battery is approximately 45 minutes (44).

Computer-Based Testing

Computer-based neurobehavioral tests have recently been developed in response to the need for standardized testing methods that objectively and efficiently collect data on various neurotoxic effects seen in exposed workers. Computer testing has been used to study acute exposures of workers in laboratory (experimental) studies and to study chronic effects on workers in epidemiological studies. Some computer-based tests are reliable for conducting comparative studies of workers, but methods appropriate for clinical studies have not been developed (52).

The most extensively used computer-based test battery is the Neurobehavioral Evaluation System. The tests selected analyze a broad range of central nervous system functions, including psychomotor performance, memory, perceptual ability, vocabulary ability, and mood (53,7).

Various computer-based tests have been developed for epidemiological applications, including the

MicroTox System (27); Swedish Performance Evaluation System (41); Milan Automated Neurobehavioral System, a computer implementation of many of the tests in the WHO Neurobehavioral Core Test Battery (15); and the Cognitive Scanner, developed in Denmark (51).

Computerized techniques have several advantages and limitations. Some of the primary advantages are reproducibility of testing conditions, ease of scoring, immediate reporting of results to the subjects, and storage of data in the computer's memory for future use. In addition, highly trained staff are not required (52,7). The limitations of these techniques center on the cost and availability of equipment. In addition, computer techniques usually emphasize speed of response; thus, other behavioral responses may not be adequately measured.

Neurophysiological Techniques

As is the case for animal testing, a variety of neurophysiological techniques can be used to assess the health effects of potential and known neurotoxic substances on humans. Many of the same techniques used in animal studies can be employed for evaluating worker exposure to various neurotoxic substances. These include the sensory evoked potentials, electromyograph, and electroneurograph. Sensory evoked potentials include brainstem auditory evoked responses, flash evoked potentials, pattern reversal evoked potentials, and somatosensory evoked potentials. Most of these techniques have been summarized earlier in this chapter; they will not be readdressed here. (See the section on neurophysiological techniques of animal testing.) EPA summarized several situations in which analysis of sensory evoked potentials would be useful (82), including determining the sensory effects of injured workers who are unconscious, immobile, or unable to respond verbally; sensory testing of workers claiming compensation when malingering is suspected; sensory testing of workers whose complaints do not correspond to clinically significant deficits in routine clinical examination; distinguishing peripheral from central nervous system damage in sensory pathways; and monitoring of workers chronically exposed to chemicals known to be neurotoxic.

Electromyography (EMG) and electroneurography (ENG) are established testing techniques well-suited to studies of various neuromuscular disorders. They are also often used in clinical examinations in neurology, orthopedics, and neurosurgery. EMG records electrical activities using a needle electrode inserted into the muscle. Researchers note several characteristics, including electrical activity in the muscle when the needle is inserted, electrical activity of the resting muscle, and electrical activity of motor conduction velocity during voluntary muscle contraction (43). ENG measures the electrical signals generated by the nerves. The electromyograph has not been used extensively for evaluating the health effects of neurotoxic substances on test animals, because few toxicologists are trained in EMG procedures. Interpretation of the results requires special training, and it can be difficult to control the degree of muscle contraction in test animals (97,43).

Human Exposure Studies

Information collected in human neurotoxicity studies may have several important uses, including:

- providing indications of toxic effects that can serve as early warnings of chronic disease processes;
- testing the adequacy of existing or proposed exposure limits;
- identifying human performance capacities that may be impaired by short-term exposure to toxic chemicals; and
- providing data on the neurotoxic effects of exposure to more than one chemical or other workplace conditions (e.g., physical agents, work level, drugs) that may interact to modify the neurotoxicity of single substances (44).

Fundamental components of this type of study are controlled exposure to the substances being studied, methods for estimating the body burden of the substances, appropriate tests and experimental design to reflect the neurobehavioral response of the subjects to the substance, and control groups or control conditions. However, human exposure studies are among the most difficult and expensive controlled laboratory experiments to conduct. Because humans have complex personalities, each individual brings to the experiment several attributes that may be difficult for an investigator to control. Such variables include age, sex, education, motivation, and work history (24).

Human studies typically require more examiner-subject interaction than other types of tests. A certain amount of controlled and consistent interaction is necessary to reduce the anxiety caused by the test

situation. Several factors may affect the interaction, including the presence of more than one examiner, and the personality, experience, and sex of the examiner. Interaction effects occur when subjects are tested in groups in large exposure chambers. The results of a study may change if the subjects are tested in groups of two or more rather than singly, in groups of both sexes rather than one sex, or in groups in which the subjects are friends rather than strangers (24).

Selection of Study Populations

The success of any human toxicity test depends on a well-designed study that has a clearly defined purpose. Two major reasons for conducting a study in the industrial setting are: 1) an awareness that a group of people collectively has similar health complaints and that a potential occupational health problem exists, or 2) a potential hazard has been identified and more information is needed to define the extent of the hazard. When undertaking human studies, it is important to select a well-defined group. If the purpose of the study is a potential health problem, the study population may have been identified by a formal complaint from an individual or company to a Federal agency. Usually, the source of the complaint appears to be limited to a work site or a plant. In this circumstance, a preliminary screening questionnaire may be conducted to determine the study group (125).

Steps in Conducting Workplace Research

There are several fundamental steps in conducting a workplace research study, and there are several significant dangers to be avoided. The identification of a suitable work group is the first, difficult step. The evaluator should consider the willingness of a company to allow worker participation. Prior to beginning the test, the evaluator must seek out the companies involved and convince them of the value of the test in order to ensure participation. Most employees will cooperate as long as they are convinced that data on them will be kept confidential (3).

Testing conditions are determined by the industry involved and past experience with the test selection. Testing sites are usually clinics, hospitals, laboratories, and conference centers. It is standard practice to describe the purpose and the benefit of the study to test subjects, what unpleasant tests they will encounter, who is responsible for the study, and whom to contact if they have questions or experience difficulties. They should also be informed that they may withdraw from the study at any time if they feel that it is unsatisfactory in any way (3).

Records should be kept on file for each research project. They should contain information on the day-to-day decisions regarding the study and any unusual events that take place. In addition, there should be a comprehensive report containing information on worker characteristics such as age, sex, race, and education; the number of years that the worker has been at his or her profession; the measurements or pattern of exposure over the years; the methods used to obtain the measurements; complete descriptions of all tests; descriptions of statistical tests used; and any adverse effects and diseases that were determined (3).

Epidemiological Studies

Epidemiological studies play a very important role in evaluating the effects of neurotoxic substances on workers and in developing strategies for the prevention of occupational diseases affecting the nervous system (44). The advantage of such studies over animal testing is that they provide direct evidence of effects on human health. However, human studies are difficult to conduct and evaluate. One limitation is that if the exposure results only in acute effects, epidemiological studies must be performed shortly after exposure occurred.

Another limitation is the complex relationship that exists between toxic exposures and human disease. Humans vary greatly not only in their exposure to substances, but also in their physiological response to exposure. Despite these difficulties, extensive techniques for evaluating data from human studies have been developed. Epidemiology has proved to be a reliable means of evaluating qualitative and quantitative relationships between exposure to toxic substances and human disease (16). Because epidemiological studies generally identify correlations between exposures and effects, it is often necessary to undertake animal studies to identify cause and effect relationships.

Occupational epidemiology is the study of the distribution of a disease among a working population and the factors that influence this distribution. This field attempts to identify relationships between diseases and occupational exposures to chemicals. The value of such epidemiological studies is in-

creased when they are used with toxicological studies on humans or animals. They are important in identifying possible associations that can be tested in laboratory environments. Furthermore, they can be used to evaluate human health risks suggested by laboratory exposures (16).

Legal and Ethical Considerations in Neurotoxicity Testing and Monitoring

Deliberate exposure of humans to neurotoxic substances in the course of research calls for all of the basic protections afforded research subjects under existing Federal law. Department of Health and Human Services regulations require institutions performing research on human subjects to create and use Institutional Review Boards to check proposed projects for compliance with regulations if those projects are funded by the Department or its constituent agencies (45 CFR 46.103(b)). Although these regulations are legally binding only on institutions receiving Federal funds, they are usually considered minimum standards for other institutions and research situations as well.

After there has been an appropriate evaluation of the value, scientific merit, probability of generating knowledge, and risk-benefit ratio of a proposed study, subjects can be selected and their consent solicited. Federal law requires that specific information be disclosed before valid consent can be obtained. Under Federal regulations (45 CFR 46.116) and some State statutes, all reasonably foreseeable risks and discomforts that subjects might experience must be disclosed.

Risk information is not the only type of information that requires greater elaboration in the research setting. Federal law also mandates disclosure regarding the nature and purpose of the research; anticipated length of the subject's participation in the study; procedures to be followed; identification of experimental procedures; benefits that may reasonably be expected to accrue to the subject or others from the study; steps to be taken, if any, to maintain confidentiality of records identifying participants; whether compensation and treatment are available for injury arising from a study where more than minimal risk is involved; and who should be contacted if subjects have questions regarding the research or their rights, as well as the contact person in the event of research-related injury (45 CFR 46.1 16(a)).

Workplace exposures to neurotoxic substances may be accidental or nonaccidental. The primary ethical obligation in the case of an accidental exposure to a neurotoxic substance is prevention. Box 5-C illustrates the important ethical issues that arise from chronic workplace exposure to neurotoxic substances such as mercury, A continuing issue in both types of workplace exposure is whether it is appropriate to notify workers about past exposures to hazardous substances, including neurotoxic substances. Many persons believe that groups of workers who have been exposed to hazardous substances in the past should be informed of this whenever possible. However, the possibility that some workers will be mistakenly identified and informed has to be weighed against the value of a retrospective notice procedure.

Prevention of Human Exposure to Neurotoxic Substances

Some of the disorders caused by neurotoxic substances can require extensive therapy and medical care. In addition, a significant number of these may be irreversible if exposure levels are high. The severity of these effects is an excellent reason for implementing methods of preventing exposure to neurotoxic substances.

Several approaches are used. One method is to increase awareness of the effects of neurotoxic substances through educational programs (6). These programs are designed to educate supervisors and workers about the signs and symptoms associated with exposure to certain toxic substances in the workplace. Managers may reduce risk of exposure to substances by substituting a less hazardous substance for the substance of concern, using adequate engineering controls, developing improved working conditions, and providing proper protective equipment, such as respirators, gloves, eye shields, and boots (6,125).

All occupational safety and health programs should be directed toward recognizing and preventing problems early. This includes communication among Federal agencies, manufacturers, and users of potentially neurotoxic substances.

Medical controls are another important aspect of an exposure prevention program. The extent of the controls will depend on the hazards and seriousness of the risks involved. Preemployment physical examinations, including detailed histories of previ-

Box 5-C—Ethical Issues Associated With Chronic Exposure to a Neurotoxic Agent

One example of an occupational exposure to a neurotoxic agent is the case of workers assigned the task of recovering mercury from old or broken thermometers.

On October 16, 1986, two executives and a supervisor of the Pymm Thermometer Company were indicted on charges of assault for allegedly endangering the lives of workers by knowingly and continually exposing them to mercury, conspiracy for hiding the existence of a cellar workshop from the Occupational Safety and Health Administration (OSHA) inspectors, and falsifying records in an attempt to conceal the cellar operation. According to the brief filed on behalf of the workers:

> Already aware of the dangerous conditions on their main manufacturing floor, defendants created and maintained even worse conditions in a cellar mercury-reclamation operation. In order to salvage some of the valuable mercury that was being wasted in its main manufacturing process, Pymm constructed a crushing machine that ground up broken and defective thermometers, spewing mercury-laden dust into the face of the machine operator. The machine was housed in a windowless, underventilated cellar, where defendants stored boxes leaking mercury from the broken and faulty thermometers to be processed (85).

One worker who was employed in this area for approximately 11 months suffered permanent brain damage from mercury poisoning (85). Exposure to mercury can cause tremors, headaches, and nausea, and more severe cases of mercury poisoning have been linked to brain damage, kidney disease, loss of vision and hearing, and motor impairment. Humans can absorb mercury by inhaling the vapors in the air. Mercury passes from the lungs into the bloodstream, which transports and deposits it first in the brain and then in other parts of the body, including the spinal cord and peripheral nervous system. Once in the body, mercury binds to proteins in the central nervous system. As long as mercury circulates and remains in the body's soft tissue, some of it can be returned to blood and plasma, to be extracted and excreted through the kidneys and intestines. In this way, the body rids itself of about half of one day's intake over a period of 40 to 70 days. When, however, a person takes in mercury faster than it be can excreted, the body begins to store mercury in bones and teeth (47). OSHA's limit for exposure during an 8-hour day is 0.1 milligram per cubic meter of air.

Chronic exposure of workers to a known neurotoxic agent like mercury raises ethical arguments about the duties of employers not to knowingly inflict harm on workers, the use of coercion in exposure to neurotoxic agents, the right of an employee to know that he or she is working in a harmful area, and the right of the employee to experience the full benefit of Federal efforts to ensure a safe workplace through OSHA inspectors and accurate record keeping. The employers in a case such as this could make an ethical argument that the greatest good for the greatest number entails recovery of mercury, but they are not ethically or legally free to pursue this objective when it clearly inflicts a known hazard on workers. The ethical dilemma in a case such as this would be an arguable ethical right of the worker to assume the risks of exposure to a known neurotoxic agent, such as mercury, in order to pursue some other value, such as increased pay. In order to explore whether the worker would have such a right it would be necessary to ensure that the worker was freely and knowingly opting to take such a risk. In addition, it would be important that the individual not impose unnecessary risk on others, for example, by exposing family members to mercury by bringing it home on work clothes. In the Pymm case, it is alleged that when the workers asked about any possible dangers of working with mercury, the employer lied and provided no training, protective clothing, or other safety equipment (85). Although the company officers were convicted by the jury on the assault charges, the trial court judge overturned the verdict. The State appealed to the appellate division of the Supreme Court of the State of New York. The case is continuing.

SOURCES: C.D. Klaassen, M.O. Amdur, and J. Doull (eds.), ***Casarett and Doull's Toxicology (New*** York, NY: Macmillan, 1986); ***People of the State of New York v. William Pymm, Edward A. Pymm, Pym Thermometer, Inc., and Pak Glass Machinery Corp.,*** Brief for the Appellant, Mar. 21, 1988.

ous exposures to substances and relevant preexisting conditions, are often very useful. Such examinations can identify persons who are likely to be susceptible to specific toxic substances. In addition, they allow the occupational physician to take necessary steps to limit employee exposure to certain hazards. Routine medical examinations also aid in monitoring the effectiveness of worker safety programs and verify the effectiveness of engineering controls. Symptoms of a high level of exposure to a substance in a-group of workers may indicate a failure that must be corrected. Consequently, more stringent engineering controls may be implemented to improve the working environment. A variety of engineering controls may be used to minimize exposure to neurotoxic substances. Because OTA described these in detail

in a previous report (104), they will not be addressed here.

MONITORING OF TOXIC SUBSTANCES

Numerous methods are currently being used to monitor exposure to and adverse health effects of toxic substances, including substances that may affect the nervous system. These methods include specimen banking (long-term storage of biological specimens for toxicological analysis), monitoring of animal tissues (e.g., marine mammal tissues and mussel tissues), and biological monitoring. Monitoring studies are used to develop baseline data, to determine whether and to what extent humans and other organisms are exposed, and to assess exposure trends. The following discussion summarizes some of the current domestic and international monitoring programs.

Specimen Banking

Domestic and International Programs To Monitor Toxic Substances

The purpose of specimen banking programs is to track the concentrations of contaminants in tissues over time. Data from programs of this kind are very useful to public health and regulatory officials, who must ensure that human exposure to toxic substances is limited. These data are also critical to epidemiological and other scientific investigations designed to link adverse health effects with particular toxic substances. Human tissue monitoring was first undertaken in the Federal Republic of Germany and the United States. Other countries now have plans to collect and store human tissues, including Canada, Japan, and Sweden. In 1980 and 1981, the West German Specimen Banking Program began collecting and storing human specimens at the University of Munster and at the central bank at the Atomic Research Center in Julich (54). Three types of human material were collected: whole blood, adipose tissue (fat tissue), and liver tissue. Biological specimens from terrestrial, freshwater, and marine environments were also collected (54).

In 1973, EPA, in collaboration with the National Bureau of Standards (NBS),[1] proposed the establishment of a National Environmental Specimen Bank, a systematic approach to specimen banking and monitoring for effects of toxic substances. Since 1975, EPA and NBS have been involved in research-related programs for specimen sampling, analysis, and storage (118,120,1 19). Furthermore, in 1975, the Federal Republic of Germany and EPA agreed to cooperate in general activities of specimen banking (120). A workshop was sponsored by EPA and NBS in 1976 to design a pilot National Environmental Specimen Bank program and to evaluate the long-term storage of samples. The primary goals of this program are the collection, processing, storage, and analysis of specimens (120).

In addition, EPA has established two monitoring programs to assess exposure to pesticides and to identify changes in exposure levels. The first program analyzes pesticides in urine and blood serum; the second monitors and stores adipose tissue (54).

From 1976 to 1980, the National Center for Health Statistics (NCHS) sponsored the National Health and Nutritional Examination Survey II (NHANES II) to establish base-line data on public exposure to various classes of pesticides, including the organophosphate, carbamate, chlorophenoxy, and organochlorine classes (54,72). Researchers set out to obtain health and nutritional information by conducting direct physical examinations and tests (including blood, serum, and urine specimens) for pesticide exposure in the general population in various regions of the United States. The program has provided estimates of the total prevalence of selected illnesses, impairments of health and nutritional status, and the distribution of many conditions in the population by sex, age, income levels, race, and region (72). Technicians have developed systematic methods of collecting, analyzing, and interpreting the data for the studies in order to detect potentially toxic substances. In addition, from 1982 to 1984, the Hispanic Health and Nutrition Examination Survey (HHANES) was conducted by NCHS to provide data on the health and nutritional status of the Hispanic population of the United States (31).

In 1985, NCHS began planning NHANES III (a survey to be conducted between 1988 to 1994) to assess nutrition status, osteoporosis (abnormal decrease in density and loss of calcium in the bone), arthritis, lung disease, heart disease, diabetes, AIDS, kidney disease, growth and development of children, and health and disability of older citizens (54,109).

[1] In 1988, the National Bureau of Standards became a component of the National Institute of Standards and Technology.

Currently, all data are collected by computerized methods in mobile examination centers, which increases the quality and availability of the data for analysis.

The current goals of NHANES III include examining the national prevalence of various diseases and risk factors, documenting and investigating reasons for trends, understanding disease etiology, and investigating the natural history of selected diseases (109),

Another type of program was established by EPA some years ago to monitor toxic substances in human adipose tissue. In 1970, the Agency initiated and sponsored a National Human Adipose Tissue Survey to determine incidence, levels, and other indicators of exposure to pesticides in the general population of the United States (54). This program monitors the levels of various pesticides in adipose tissue collected from cadavers during autopsies (54).

WHO is conducting a multinational specimen banking program for human tissues. Specimens from the heart, brachial artery, aorta, and diaphragm of cadavers are being evaluated. This program is designed to compare exposure to trace metals with the development of cardiovascular diseases (54). Additional human monitoring programs include a serum program conducted by the Centers for Disease Control and collection of preserved human tissues in formaldehyde at the EPA Pesticide Research Laboratory (54)$_0$

Monitoring of Nonhuman Tissues

In 1987, the Alaskan Marine Mammal Tissue Archival Project was established by the Minerals Management Service to collect and store Alaskan marine mammal tissues in order to monitor toxic substances. To reach this goal, three objectives were set: to collect marine mammal tissues that are suitable for determining levels of organic and inorganic substances; to transport and archive tissues in a condition that is ideal for long-term storage and analysis; and to determine the most appropriate collection protocols for long-term storage of marine mammal tissues (8,1 11).

In 1984, the National Oceanic and Atmospheric Administration within the U.S. Department of Commerce conducted studies through its National Status and Trends Program for Marine Environment Quality to determine the environmental quality of the coastal and estuarine regions of the United States. The objectives of this program are to determine concentrations of substances in biological tissues and sediments and to examine and record changes in these concentrations. Since 1984 and 1986, respectively, samples have been collected at approximately 50 benthic surveillance sites and 150 Mussel Watch sites. Benthic (bottom-dwelling) fishes are collected at the Benthic Surveillance sites and their livers are removed and stored for further chemical evaluation. At the Mussel Watch sites, molluscs are collected for chemical analysis. Commonly assayed substances include polyaromatic hydrocarbons, polychlorinated biphenyls, pesticides, and the elements arsenic, cadmium, chromium, lead, mercury, silver, and tin (106,107,108).

Biological Monitoring

Monitoring programs are designed to observe, measure, and judge on a continuous basis the potential health effects of substances and make proper decisions on the adequacy of control measures. Monitoring is more than just sampling the air where workers are being exposed or conducting medical examinations of workers. It is an entire series of activities that are undertaken to make proper judgments on the protective controls needed or the adequacy of the control measures in place, or both. One approach commonly used in occupational health is biological monitoring. This makes it possible to determine both the occurrence of exposure and the presence of particular substance(s) in body fluids (i.e., blood or urine) or organs in order to evaluate health risk (5).

Biological monitoring programs are designed to detect the presence in the body of substances from all routes of exposure. The appropriate frequency of monitoring may be influenced by several factors, including intensity and duration of exposure and toxicity of the substances. Monitoring is generally done more often when the toxic substances being evaluated are expected to produce irreversible changes.

One limitation of biological monitoring is that it is sometimes difficult to establish whether exposure to toxic substances is responsible for observed changes in the biological parameters. Individuals are often exposed to several substances simultaneously, and one must consider whether a different substance or a combination of substances caused the observed toxic effects. Variability in individual responses may be another limitation to monitoring. Multiple

Photo credit: U.S. Environmental Protection Agency

factors may cause variability in response among workers exposed to the same substance. Thus, it may be difficult to determine the normal response for a given individual (5).

Internationally, the Global Environment Monitoring System created a biological monitoring system to evaluate the health risks from exposure to lead, cadmium, and pesticides. The study of lead exposures was conducted between 1979 and 1981 and involved 10 countries. In 1984, a follow-up study was conducted in four countries. Blood samples from volunteers were taken and analyzed for lead and cadmium content. In 1981, a study of selected organochlorine pesticides, including DDT and PCBs in human milk, was conducted in 10 countries to assess the population's exposure to these substances (124).

Other Monitoring Programs

As part of the Federal Emergency Planning and Community Right-to-Know Act of 1986, EPA was required to generate a database on toxic substances released into the environment from industrial sites throughout the country. Commonly known as the Toxics Release Inventory (TRI), the database contains information on approximately 328 toxic substances (see box 5-D). Results of the inventory indicate that in 1987, approximately 18 billion pounds of toxic substances were released directly into the air, surface waters, land, or underground injection wells in the United States. In addition, 4.6 billion pounds were transported offsite for disposal or treatment. TRI will enable regulatory and public health officials, researchers, and the public to monitor what quantities of particular chemicals are being released from sites around the country. The first data were published in 1989, and the inventory will be updated annually. The database pertains only to manufacturing industries; Federal facilities are not accounted for (94,1 13). Figure 5-3 illustrates the neurotoxic substances among the TRI's top 25 chemicals emitted into the air.

Box 5-D-Neurotoxicants Released Into the Environment by Industry: The Toxics Release Inventory Supplies New Evidence

Until recently, regulators had no comprehensive answer to a basic question underlying toxic substances regulation: What amounts of toxic substances are we actually dealing within the United States? Despite dozens of databases devoted to toxic chemical regulation, such as data on air pollution permits, surface water discharges controlled under Federal water pollution control regulations, and hazardous wastes regulated under the Resource Conservation and Recovery Act, no single compendium contained estimates of the overall amounts of chemicals released into the environment. The Toxics Release Inventory, which grew out of reporting requirements mandated in the 1986 Superfund amendments (Superfund Amendments and Reauthorization Act sec. 313), provides a preliminary answer—at least for the 327 chemicals covered by the statute that are discharged into air or water or dumped on land by manufacturers in 20 specified industries.

Inventory data show, for example, that manufacturing facilities emitted significant amounts of neurotoxicants to the air in 1987. Overall, facilities released 2.6 billion pounds of the 327 toxic chemicals on the Inventory list. A brief review of the scientific literature reveals that 17 of **the top 25 chemicals, accounting** for 1.8 **billion pounds (77 percent) of the total for the top 25, have documented neurotoxic effects ranging from narcotic effects (drowsiness or fatigue) to more permanent and debilitating effects, such as hearing impairment and blindness. Of these 17 neurotoxicants, only benzene, which is a known human carcinogen, has been regulated as a hazardous pollutant under the Clean Air Act.** The neurotoxic effects of two additional chemicals-l,l,l - trichloroethane and glycol ethers, which account for another 189 million pounds (8 percent) of the top 25-are being investigated under the Toxic Substances Control Act section 4 test rules. In sum, manufacturers released a total of nearly 2 billion pounds of potential or known neurotoxicants (85 percent of the top 25) in 1987. Figures on 1988 releases, which will become available in 1990, should give some indication as to whether emissions of these neurotoxicants are increasing or decreasing.

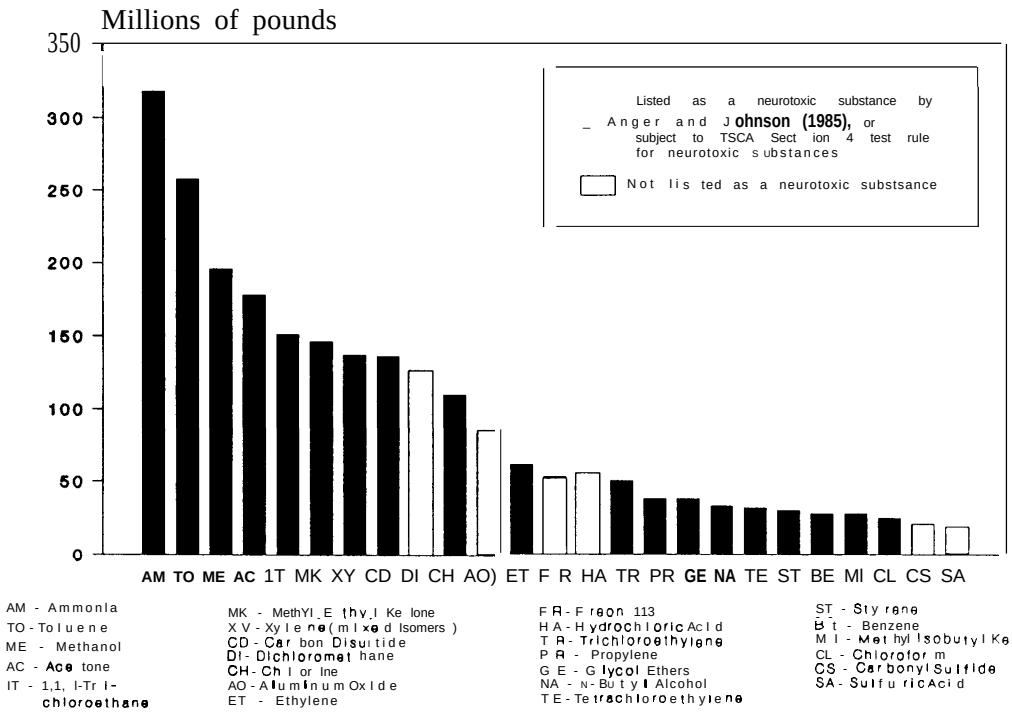
The Inventory data do not cover many sources of toxic chemicals in the environment, notably consumer products and agricultural chemicals, nor do they address the chemical releases and exposures in the occupational setting. Furthermore, the data do not reveal the amounts to which people are actually exposed (chemicals may break down or be transported rapidly through the environment after being released, or they may accumulate in the environment) or the probable risks from exposure. The Inventory data do, however, suggest that significant amounts of identified neurotoxicants are finding their way out of factories and into the environment; these releases are plausible candidates for further study or control.

SOURCES: U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, ***The Toxics Release Inventory: A National Perspective, 1987,*** EPA 560/4-89-006 (Washington, DC: 1989); W.K. Anger and B.L. Johnson, "Chemicals Affecting Behavior," ***Neurotoxicity of Industrial and Commercial Chemicals,*** vol. 1, J.L. O'Donoghue (cd.) (Boca Raton, FL: CRC Press, 1985), pp. 51-148.

A wide variety of additional monitoring programs has been undertaken by several Federal agencies. For example, in 1978, the U.S. Department of Agriculture (USDA) and the Human Nutrition Information Service devised a survey called the Nationwide Food Consumption Survey to measure the food and nutrient content of the U.S. diet, the dollar value of food used in the average U.S. household, and food and nutrient intakes of individuals at home and away from home. In addition, since 1965, FDA has conducted a survey known as the Total Diet Study to collect and analyze diet samples from retail markets to assess concentrations of metals, pesticide residues, and other substances commonly found in the diet. In 1987, FDA analyzed 936 food samples in the diets of U.S. consumers and found that the levels of intake of the pesticides assayed for were less than 1 percent of acceptable levels set by WHO and the United Nation's Food and Agriculture Organization (110). Also, the National Residue Program is conducted by USDA to evaluate pesticide residue levels and other potentially hazardous substances present in meat and poultry. In 1984, EPA's Office of Pesticide Programs developed a Tolerance Assessment System in order to estimate potential human exposure to pesticides in the diet and analyze the risks that could result from exposure (31).

The Agency for Toxic Substances and Disease Registry of the Department of Health and Human Services recently set up a registry of persons exposed to toxic substances at hazardous waste sites and at emergency chemical spills. The registry will

Figure 5-3-Neurotoxic Substances Are Prominent Among the Toxics Release Inventory's Top 25 Chemicals Emitted Into the Air in 1987



SOURCES: Data obtained from W.K. Anger and B.L, Johnson, "Chemicals Affecting Behavior," ***Neurotoxicity of industrial and*** Commercial ***Chemicals,*** vol. 1, **J.L. O'Donoghue (ad.) (Boca Raton, FL: CRC Press, 1985), tables 1 and 2, pp. 70-141; TSCA sec.4,52FR31445; TSCA sec. 4,53 FR 5932; 54 FR 13470; 54 FR 13473; U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances,** ***The*** Toxics ***Release Inventory: A National Perspective, 1987,*** **EPA** ***560/4-89-006*** (Washington, DC: 1989).

provide information needed by researchers to assess the long-term health effects of both low-level chronic exposures and high-level acute exposures (108).

SUMMARY AND CONCLUSIONS

The adverse effects of toxic substances on the nervous system may be evaluated through three categories of toxicological tests: whole animal, tissue and cell culture, and human subjects. Each approach has both advantages and limitations, and in practice combinations of these tests may be used in a complete toxicological evaluation. The best means of predicting human health effects is to evaluate the effects of potentially toxic substances directly on human subjects. However, this approach is difficult and frequently presents ethical dilemmas. Consequently, it is often necessary to rely on animal tests in making predictions of human health effects. In some cases, in vitro tests can be used to detect the neurotoxic potential of toxic substances. As more in vitro testing techniques become available and are validated, they will be useful in initial screening, as complements to various animal tests, or both.

Several industrial and Federal organizations have developed animal tests to evaluate the effects of known and potential neurotoxic substances. In industry, various testing approaches are currently in use and protocols are continually being revised and improved. In the Federal arena, EPA has developed guidelines under the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act specifically for determining neurotoxic properties of toxic substances. The guidelines are composed of a core set of tests consisting of the functional observational battery (a series of tests designed to screen rapidly for neurotoxic potential), tests of motor activity, and neuropathological examinations. For regulatory purposes, EPA plans to utilize the core tests and supplement them with additional neurotoxicity tests when appropriate. These may include schedule-controlled operant

behavior, neurotoxic esterase assay for organophosphorous substances, acute and subchronic delayed neurotoxicity of organophosphorous substances, and developmental examinations. Neurophysiological evaluations are also used in identifying neurotoxic substances and in evaluating their adverse effects; however, EPA currently has not developed guidelines for using these tests in regulatory activities.

Several human tests are in use to determine the neurotoxic potential of suspected and known toxic substances. These include neurobehavioral evaluations and various neurophysiological tests. In addition, computerized techniques are rapidly advancing to aid in studies of neurotoxicity.

Monitoring of toxic substances is critical because it enables investigators to systematically trace toxic pollutants and their sources that are contaminating the air, land, and water. Monitoring programs include human and animal specimen banking, biological monitoring, and related efforts. Toxicity monitoring programs now under way in Federal agencies address neurotoxicological concerns in varying degrees. However, much more could be done in this area.

Until recently, Federal agencies have devoted little attention to neurotoxicity testing. EPA is the leader in developing test guidelines to evaluate neurotoxicity. The regulatory programs of other agencies would benefit from joint test development, and more active involvement of industry and academia in test development and validation programs would help ensure the optimal design of neurotoxicity tests for general use in regulatory programs,

EPA is continuing to examine the testing guidelines already produced to determine whether a wider range of tests is needed to evaluate the neurotoxic properties of toxic substances, For example, the schedule-controlled operant behavior and developmental tests provide additional information about certain effects that cannot be determined by the FOB, motor activity, and neuropathology examinations.

The Federal Government is encouraging the development of in vitro neurotoxicological tests. As these tests become available, testing schemes may be modified to take advantage of both in vivo and in vitro approaches. Finally, monitoring programs under way at various organizations and Federal agencies would benefit by giving greater attention to substances with neurotoxic potential and by incorporating a wider range of neurological and behavioral effects into monitoring schemes.

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