Comprehensive Neurotoxicity Assessment

Beverly M. Kulig
Department of Neurotoxicology and Reproduction Toxicology, TNO Nutrition and Food Research Institute, Rijswijk, The Netherlands

Significant progress has been made in recent years in terms of both the conceptualization of neurotoxicity assessment strategies as well as in the development of behavioral techniques for evaluating neurotoxic exposures. A tiered approach, for example, has been advocated as an assessment strategy in which testing would proceed in a stepwise fashion from general screening using simple behavioral methods and neuropathology (tier 1) to the characterization of effects (tier 2) using more specific testing techniques. With respect to tier-1 testing, behavioral observational methods have been recommended for screening purposes, and these technically simple techniques, together with automated methods for motor activity assessment, are being increasingly incorporated into chemical and drug safety evaluations for regulatory purposes. With respect to tier-2 testing, more technically sophisticated techniques and behavioral paradigms are available for characterizing the behavioral effects of chemical exposures on motor, sensory, and cognitive processes. Paradigms involving learned and unlearned behavior, for example, have been described for quantifying a variety of clinical signs of motor impairment including paretic gait disorders, tremor, and coordination deficits. Likewise, robust noninvasive behavioral methods capable of tracking changes in visual, auditory, and somatosensory thresholds during the course of exposure are also available. With respect to cognitive testing, numerous maze and operant techniques and paradigms measuring different aspects of performance, learning, and memory have been elaborated. This paper presents an overview of behavioral techniques currently used to assess neurotoxicity in adult laboratory animals and discusses their application to hazard identification and other areas of risk assessment. — Environ Health Perspect 104(Suppl 2):317–322 (1996)

Key words: neurotoxicity, functional observational battery, motor impairment, sensory function, cognitive behavior

Introduction

Neurotoxic effects of chemicals, as reflected by behavioral changes, have been known through exposure of humans and lower animals since antiquity. The neurotoxic properties of lead, for example, were identified as early as 200 B.C., and the first recorded regulatory measures in industrial hygiene ever taken were aimed at protecting workers from the neurological effects of mercury exposure in the Idrian mines in 1665 (1).

Over the last 100 years, industrial activities have introduced a vast array of new chemicals into the home and environment and have greatly increased the possibility of toxic exposures in the general population. At present, more than 65,000 chemicals are commercially used by industry, including some 3,350 pesticides, 8,627 food additives, 1,815 pharmaceuticals, and 3,410 cosmetic ingredients (2,3). In addition, more than 1,000 chemicals are introduced into the market each year.

In reviewing the neurotoxicology literature, Anger and Johnson (4) identified approximately 850 chemicals for which there is some evidence for an adverse effect on nervous system functioning and behavior. Further, at the regulatory level, maximum workplace exposure levels have been established for 588 chemicals; of these, 167 have been regulated, at least in part, on the basis of their effects on the nervous system. In the United States alone, an estimated 14 million persons are occupationally exposed to these chemicals (5).

Despite the magnitude of neurotoxicant exposures in working populations and evidence that considerable amounts of these chemicals are finding their way into the environment, relatively few chemicals have been even marginally evaluated for their effects on nervous system function and behavior (3). Although historically morphologic and classical toxicologic methods have been used to provide evidence of neurotoxicity, there has been increasing acceptance of the use of behavioral methods in evaluating neurotoxicity (3,6). At the regulatory level, the U.S. Environmental Protection Agency (U.S. EPA) has published generic neurotoxicity testing guidelines for the testing of chemicals and pesticides (3), and the U.S. Food and Drug Administration (FDA) is currently revising its guidelines to include behavioral measures of neurotoxicity assessment (7). Further, on an international level, the use of behavioral methods for neurotoxicity assessment has also been proposed by the Organization for Economic Cooperation and Development (OECD) (8).

Neurotoxicity Assessment Strategies

Because of the heterogeneous nature of the nervous system, different neurotoxic chemicals can affect neurologic and behavioral functioning in different, specific ways. Thus, to evaluate the neurotoxicity of a chemical, measures of different types of functions are necessary. Further, for the purpose of regulatory testing, a number of panels and committees have recommended a tiered approach to neurotoxicity assessment (3,9,10). Such a tiered approach typically involves a stepwise progression from general screening using simple behavioral assessment methods (functional observational battery) and neuropathology (tier 1) to the characterization of effects (tier 2) using more specific behavioral, electrophysiologic, neurochemical, and neuropathological techniques (6).

A functional observational battery (FOB) consists of a collection of noninvasive tests to evaluate neurologic and behavioral signs in
exposed animals (11,12). Usually, observations of the animal, which in most studies is the rat, are carried out both while the rat is in its home cage and while it is moving freely about in a test arena for a specified period of time. Animals are observed for changes in arousal, reactivity to handling, presence and type of convulsions, alterations of gait and mobility, and autonomic signs. Several simple tests are also performed to evaluate sensory functions (e.g., reactions to noise, tactile stimulation, pain) as well as motor functions (e.g., grip strength measurements, landing foot splay). In regulatory testing, automated motor activity assessment is also included in tier-1 neurotoxicity screening.

Despite the apparent simplicity of these tests, they have been shown to be quite sensitive in detecting the neurotoxic effects of a number of compounds (11) and, indeed, sometimes surpassing automated techniques (13). Several issues that have been repeatedly raised with respect to the use of tier-1 testing are the sensitivity of tests for assessing sensory impairments as well as the lack of inclusion of any tests at the screening level for measuring cognitive behavior or learned performance.

In developing a test strategy in our own laboratory, we have used simple tier-1 tests, as well as more sophisticated techniques, to examine the neurotoxic effects of a number of compounds. In many cases, we often used both simple and more specific behavioral test methods in the same study rather than applying a tiered-testing approach. This has been especially true in chronic studies of inhaled organic solvents (14), where the costs of exposing animals are high relative to those associated with behavioral testing. Further, despite the simplicity of tier-1 test methods, some techniques such as grip-strength measurements show quite acceptable levels of interanimal variability and are quite stable in the same animal over time. Thus, some of the techniques appropriate for tier-1 screening in high-dose studies may also be sufficiently sensitive for use in long-term time-course studies at lower doses. In addition, we also worked on the development of automated tests of sensory function. The rationale for developing auditory threshold measurements was based on our failure to detect auditory dysfunction with simple tests of stimulus reactivity for compounds that are known ototoxic agents. Finally, given the reports in the human literature on solvent effects and cognitive functioning, our testing battery also includes measures of learned performance.

In this paper, an overview of these techniques as well as those used by other investigators is presented.

**Tests for Quantifying Clinical Signs of Motor Impairment**

The potential of industrial compounds to affect motor function in humans is well documented (4,5), and many of the quantitative neurobehavioral techniques thus far developed have concentrated on this functional domain. Quantitative measurements of grip strength, walking patterns, tremor, coordinated movement, and motor activity have all been successfully applied to evaluating the effects of chemical exposures on different aspects of motor function (15).

One method for measuring neuromuscular function, which has become widely used since its original description by Meyer et al. (16), is the measurement of fore- and hindlimb grip strength. Although included in the U.S. EPA and proposed OECD neurotoxicity testing guidelines as a tier-1 test, grip-strength measurements are quite sensitive in detecting neuromuscular impairments, even at relatively low exposure levels (15,17).

In our laboratory, the effects of long-term exposure to a number of chemicals including acrylamide, n-hexane, methylmercury, and carbon disulfide have been examined in studies lasting from 12 to 36 weeks (14,15). Examination of the stability of baseline responding in control animals and low variability of the data demonstrates the sensitivity of grip-strength measurements in detecting and quantifying the effects of chemical agents that are toxic to peripheral nerve, as well as the utility of this method in extended exposure studies. These qualities, together with low cost and ease of administration, make grip-strength measurements suitable both for screening studies as well as studies designed to evaluate the time course of effects or mechanisms of action.

In addition to measures of neuromuscular function, a number of techniques have been described for detecting and quantifying disturbances of gait and motor coordination. A simple and direct approach to quantifying ataxic and paretic gait disturbances is the analysis of records of successive footprints made during locomotion (18).

To obtain a record of footprints while the animal is in motion, the hindpaws are either grease or inked and the animal is allowed to walk on a nonmoving, paper-covered surface. To facilitate consistent walking in one direction, testing is usually conducted by placing the animal at one end of a narrow walkway covered with paper and allowing it to traverse the length of the walkway to a darkened enclosure at the other end. The analysis of gait topography has been applied to evaluate a number of different types of experimental treatments including drugs (19), models of peripheral nerve damage and multiple sclerosis (20), and neurotoxic agents. Recently, for example, Pryor (21) used this type of gait analysis in demonstrating that high-level exposure to toluene produces a persistent motor syndrome marked by a shortened stride and a wide-based gait analogous to that seen in heavy toluene abusers.

Analysis of successive footfalls indicates that stride length, stride width, and measures of gait symmetry for normal adult rats are highly consistent (22). There has been, however, little standardization among laboratories with respect to the exact procedures used to collect footfall patterns. Further, it is not yet known to what degree reduced body size or weight may influence gait topography. Thus, an approach similar to that used by Pryor (21) for evaluating the contribution of body size should probably be adopted. One of the drawbacks of gait analysis is the large amount of time necessary to evaluate the hard-copy records. However, the possibility of using computer-supported analysis techniques has been reported (23).

In addition to examining changes in gait topography while the animal is walking on a stationary surface, a number of simple and complex techniques have been developed to examine coordinated movement in other situations. The test of landing foot splay, for example, has been shown to be a relatively stable and sensitive test of motor impairment (24) and has been incorporated into tier-1 testing methodologies. Other tests of coordinated movement such as negative geotaxis (17) and rotorod performance (25) appear to be less reliable screening methods.

In our own laboratory, automated techniques using a video-microprocessor system have been developed for quantifying coordination deficits in small animals and overcome many of the problems seen with rotorod testing. In initial studies using these techniques, impaired motor function could be demonstrated for a number of different types of motor neurotoxicants including short-term administration of acrylamide, ethanol, tremorine, and 2,5-hexanediol, as well as long-term inhalation exposure to organic solvents (14,26).

Table 1 presents a comparison of the effects of a 12-week exposure to acrylamide...
Table 1. A summary of the effects of 12 weeks of exposure to acrylamide on different measures of motor impairment.

| Tests and measures | Significant effects, p< | First detected |
|--------------------|-------------------------|----------------|
| Motor activity      |                         |                |
| Ambulation          | 0.005                   | Week 9         |
| Rearing             | 0.002                   | Week 8         |
| Grip strength       |                         |                |
| Forelimb            | 0.001                   | Week 9         |
| Hindlimb            | 0.001                   | Week 9         |
| Coordination        |                         |                |
| Composite score     | 0.001                   | Week 6         |
| Misteps             | 0.001                   | Week 6         |
| Walking failure     | NS                      |                |
| Nerve conduction    |                         |                |
| Peak latency        | 0.001                   | Week 12        |
| Peak amplitude      | 0.05                    | Week 12        |
| Body weight         | NS                      |                |

NS, not significant.

on different types of motor performance measured in our laboratory. From these data, it is obvious that any of the end points measured, with the exception of body weight and walking failure, would have been adequate for identifying acrylamide as a neurotoxicant. However, there are obvious differences in the time at which effects could be detected. Although one may be tempted to conclude that the measurement of rearing and coordination by automated methods is more sensitive, it may also be that different effects develop with different time courses.

A further novel approach to measuring coordinated movement employs an operant paradigm involving wheel running for food reward (27). In this study, changes in complex motor behavior produced by methanol were reflected in dose-related increases in interresponse times of wheel rotations, indicating decreasing velocity with increasing doses at levels equal to 10% of the LD₅₀.

In addition to changes in gait and coordination, tremor is also an important clinical sign of neurotoxicant exposure. Tremor refers to rhythmic, involuntary oscillations of the whole body or a particular body part and, as a clinical sign, is seen in various neurological disease states, such as Parkinson's disease and multiple sclerosis, and as a consequence of exposure to particular therapeutic drugs or chemicals (28,29). In addition, tremor can also result from environmental overexposure to a variety of compounds including metals, pesticides, and organic solvents (4), and some of the most dramatic instances of human neurotoxic disease have involved occupational exposure to tremogens. The only possibility for detecting tremor using nonautomated techniques is by visual observation. Several methods for detecting and measuring whole-body tremor using different types of transducers have been described (30–32).

In addition to methods based on whole-body tremor, operant techniques have been used for examining the spectral profile of tremorgenic agents (33,34). One ingenious technique employing operant procedures to examine forelimb tremor in the rat uses an operant chamber equipped with an isometric force transducer as the operandum. The force transducer is located in a recessed aperture in such a way that the rat must exert a predefined force on the transducer to gain access to the reinforcement dipper. During response execution, oscillations of the rat's right forelimb detected by the force transducer are recorded and analyzed to obtain spectral density functions of tremor. Using this technique, Fowler and his colleagues (34) reported frequency-dependent changes in force oscillations in haloperidol-treated rats. A similar use of operant technology has also been described for examining motor effects in primates; this method appears to be quite sensitive in detecting the onset of manganese-induced motor impairment (33).

Motor Activity Assessment

Of all the tests of motor function, measures of spontaneous activity have become the behavioral parameters most extensively used to examine the effects of neurotoxicant exposures. Although some of the recent focus on motor activity may, in part, be due to its proposed use as a regulatory end point for neurotoxicity screening, efforts to study motor activity date back almost a century. There are a number of features that make motor activity an attractive behavioral end point for examining the effects of chemical exposures. Motility is an inherent feature of all animals, and motor activity occurs spontaneously without the need for deprivation or pretraining of the animals. Further, measures of motor activity have been shown to be sensitive to treatments known to affect central nervous function, including brain damage and drugs. Finally, the quantification of motor activity lends itself relatively easily to automation, which is an important consideration in a field of study in which the number of compounds that are potentially worthy candidates for evaluation can be expected to be substantial.

Motor activity assessment has been extensively used in neurotoxicity assessment; a number of surveys of methods and critical reviews have been published over the last 10 years examining different types of automated motor activity assessment techniques (14,35). Currently, the most common methods use photocell detection methods or videomaging techniques, which can be used to track the animal. Motor activity assessment is an apical test for detecting neurotoxicant effects rather than for documenting a specific clinical motor effect. Although automated motor activity assessment has been incorporated into tier-1 regulatory testing, it has also been used together with other techniques for characterizing behavioral changes during long-term exposure in relation to blood and brain levels of organic solvents (36).

Evaluating Sensory Functions

Although a considerable amount of importance has been placed on the motor effects of toxic exposures, possible effects on sensory function are also of growing concern. Surveys of the literature have indicated that approximately 44% of chemicals that possess neurotoxicant effects have an impact on sensory function (37). The measures designed to evaluate sensory function in the context of tier-1 neurotoxicity testing include simple tests of reactivity to visual, auditory, and somatosensory stimuli. Using this approach, we have been unable to obtain convincing data of sensory impairments for compounds that have been described in the literature as toxic to the visual or auditory nervous system. It may be that this lack of sensitivity is due to differences in dose or exposure duration and that only a direct empirical comparison could answer this question. However, if one considers the specific sensory effects described in the literature, it is difficult to imagine these observational methods being capable of detecting specific hearing and visual impairments (38).

Basically, two different types of behavioral paradigms have been described for evaluating sensory effects—those based on operant conditioning and those using reflex-modification techniques. Instrumental techniques have included the use of both active avoidance paradigms as well as psychophysical operant discrimination methodologies in both rodents and primates.

One instrumental technique that has been successfully used to uncover auditory deficits in rats is the multisensory conditioned avoidance paradigm (17,39). Using this technique, Pryor and his colleagues (39) were able to uncover a neurotoxic effect not previously noted with other methods, namely, the ability of toluene, xylene, and
styrene to produce irreversible frequency-specific hearing loss. Psychophysical operant discrimination techniques provide a very elegant approach to the evaluation of neurotoxicant-induced specific sensory deficits. These techniques have been successfully used to examine toxicant effects on a variety of sensory processes including visual, auditory, and somatosensory functions, as well as the irritant properties of vapors. A number of reviews of this technology are available (40,41). Some of the most elegant studies using operant discrimination techniques in primates demonstrate the selective effects of neurotoxic agents such as acrylamide (42) and developmental methylmercury exposure (43) on different aspects of visual functioning.

One of the principal drawbacks usually cited in discussions of operant sensory testing paradigms is the relatively long periods of time required to achieve stable baseline levels of responding. Because of this limitation, other authors have investigated the use of reflex-modification techniques as a possible tool both in terms of screening and characterization of sensory deficits, particularly with respect to the auditory system (37).

The application of reflex-modification procedures in rodents uses the whole-body startle response, which reflexively occurs in response to a sudden loud auditory stimulus. The technique is based on the fact that perceived stimuli presented shortly before the startle stimulus will reduce the amplitude of the startle response, which can be measured as changes in downward force with force transducers or other suitable devices (44). By adapting the technique using the eye-blink response rather than the whole-body startle response, the technique can also be applied in human studies (45). Reflex audiometry has been applied to evaluating the ototoxic properties of a number of drugs and chemicals including ototoxic antibiotics (46), trimethyltin (47), and trichloroethylene (48). One of the disadvantages of reflex audiometry is the relatively long testing sessions that are required to obtain a full audiometric function across a wide range of frequencies. However, it is also possible to limit the number of frequencies tested in such a way that reflex modification can also be used as a screening method.

**Evaluating Changes in Learned Performance and Cognitive Behaviors**

Behavioral impairments indicative of cognitive changes have been associated with exposure to a number of chemicals. Developmental exposure to lead (49), methylmercury (50), and PCBs (51), for example, have been causally related to delayed development and intellectual impairments in children. Further, chronic exposure to organic solvents has been associated with the development of toxic encephalopathy and deficits in behavioral performance at occupational exposure levels (52). Given the fact that these and other neurotoxicants are ubiquitous in the environment, it is not surprising that concern has been raised regarding the lack of measures of learning, memory, and behavioral performance from tier-1 testing in the regulatory sphere.

There have been many models developed to evaluate different aspects of learning and other higher-order functions in animals. Some studies have concentrated on the effects of chemical exposures on the performance of learned behaviors, using, for example, free operant techniques (53), while others have attempted to develop models to study acquisition and memory (54). Further, a variety of techniques (shuttle-box learning, maze techniques, and operant techniques) have been used with different behavioral paradigms including active and passive avoidance learning, reversal learning, repeated acquisition, and delay tasks.

With respect to changes in the performance of learned behavior, schedule-controlled operant behavior (SCOB) has been extensively applied to the study of different classes of chemicals (55), and schedule-controlled operant techniques for neurotoxicity evaluation have been included in neurotoxicity testing guidelines. Because the aim of many of the studies employing SCOB has been to demonstrate the usefulness of these methods in neurotoxicity screening, experimental protocols used, for example, to study inhaled organic solvents have typically employed high-level, short-duration exposure schedules (56,57). Although such studies can provide highly reliable quantitative information by which to judge the relative potency of different compounds to affect behavior in a given test system, they also give the impression that very high concentrations of solvents are necessary to affect learned behavior in rodents.

In our own laboratory, we have been concerned with the effects of organic solvents on psychomotor slowing in the performance of learned behavior. One of the most consistent effects reported in the human occupational literature is a slowing in the performance on reaction-time tasks in workers exposed to organic solvents. Since most threshold limit values (TLVs) for organic solvents have been chosen to avoid acute behavioral effects of this nature (14), it seemed worthwhile to determine whether rats were also affected at occupationally relevant levels. Thus, we developed an animal model of a two-choice discrete-trial operant task in the rat and examined the levels at which effects on learned performance began to occur. One of the interesting findings in these studies is the sensitivity of latency measures of this method to the acute effects of low-level exposure and the change in acute effects in the context of repeated exposures (14).

Figure 1 shows the effects of inhalatory exposure to perchloroethylene for 3 days on the number of short-latency (<2 sec) two-choice responses and the number of long-latency responses. On day 1 of exposure, performance was significantly affected only in the high-concentration groups. However, with repeated daily exposure, effects also became apparent in the low-dose groups as well. To what degree changes in the acute effects of solvents are related to changes in body burdens with repeated exposure is currently being investigated for compounds that produce different effects in the short-term repeated exposure situation.

In addition to the study of chemical effects on the performance of free-operant...
or discrete-trial tasks, a number of investigators have used different behavioral paradigms to examine the effects of chemicals on learning and memory processes. Active and passive avoidance tasks have been extensively used in pharmacology for many years to examine drug effects on acquisition learning and memory, and several advantages and disadvantages of these approaches have been discussed previously (54, 58). One of the principal drawbacks with the use of these techniques is that they do not allow for the repeated evaluation of changes in memory or learning ability during the course of long-term exposure.

One possible approach to studying learning ability is the use of repeated acquisition paradigms using either maze tasks or operant chambers. Several repeated acquisition paradigms have been described in the literature using rodents including reversal learning (59), repeated acquisition of response chains (60), and repeated acquisition in mazes (61). Analogous approaches for primates have also been described (62). One of the primary advantages of using repeated acquisition paradigms is the possibility of incorporating control measures into the design of the task to distinguish changes in general performance measures from those related to acquisition (60). A similar approach can also be employed in delayed alternation and delayed matching tasks for studying chemical effects on working memory (54, 63). The application of memory and learning paradigms such as delayed alternation, reversal learning, repeated acquisition, and delayed-matching have been applied for many years to study the effects of drugs and brain lesions on learning and memory processes. To what degree these techniques can be applied to neurotoxicology assessment is yet to be determined. However, the general approach holds considerable promise for evaluating neurotoxicant-induced effects on learning and memory.

**Concluding Remarks**

To develop a comprehensive approach to neurotoxicity assessment, the necessary tools for evaluating the effects in question are obviously of utmost importance. Over the last 10 years, significant advances have been made in developing behavioral toxicology assessment techniques, both simple and complex. Methods for quantifying toxicant-induced motor deficits that are sufficiently sensitive and easy to perform have been developed and can be used on a routine basis for neurotoxicity screening. Although detecting sensory changes is more complex, the utility of both operant and reflex methodologies have been demonstrated in detecting sensory toxicants. With respect to cognitive functions, one can expect continued refinements in techniques, which will add incremental improvements to the existing methodologies outlined above.

The availability of methodologies for hazard identification, however, is only one aspect of a comprehensive assessment strategy for evaluating the neurobehavioral effects of chemical exposures. Study designs that address the role of different factors such as toxicokinetics, concentration × time relationships, sex and age differences, and co-exposures, which contribute to the expression of neurotoxicity, are also of considerable importance. In many cases, the application of techniques currently available to these issues would be of great value in assessing the risk to human health posed by chemical exposures.

---

**REFERENCES**

1. Hunter D. The Diseases of Occupations. 5th ed. London:English Universities Press, 1974:240–297.
2. Lave LB, Upton AC. Toxic Chemicals, Health, and the Environment. Baltimore:Johns Hopkins University Press, 1987.
3. U.S. Congress. Neurotoxicity: Identifying and Controlling Poisons of the Nervous System. OTA-BA-436. Washington:U.S. Government Printing Office, 1990:361.
4. Anger WK, Johnson BL. Chemicals affecting behavior. In: Neurotoxicity of Industrial and Commercial Chemicals. Vol I (O'Donagheue JL, ed). Boca Raton, FL:CRC Press, 1985:51–148.
5. Anger WK. Neurobehavioral testing of chemicals: impact on recommended standards. Neurobehav Toxicol Teratol 6:147–153 (1984).
6. WHO. Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals. Environmental Health Criteria Document 60. Geneva:World Health Organization, 1986.
7. Tilson HA, MacPhail RC. Interpretation of neurobehavioral data in toxicologic studies. In: Neurobehavioral Toxicology: Analysis and Interpretation (Weiss B, O'Donagheue JL, eds). New York:Raven Press, 1994:345–357.
8. Organization for Economic Cooperation and Development. OECD Guideline for the Testing of Chemicals. Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. Revised Draft Document, January 1994.
9. National Research Council. Environmental Neurotoxicology. Washington:National Academy Press, 1992:53–93.
10. ECETOC. Evaluation of the Neurotoxic Potential of Chemicals. Monograph No 18. Brussels:European Centre for Ecotoxicology and Toxicology of Chemicals, 1992:30–38.
11. Moser VC. Screening approaches to neurotoxicity: a functional observational battery. J Am Coll Toxicol 8:85–93 (1989).
12. O’Donagheue JL. Screening for neurotoxicity using a neurologically based examination and neuropathology. J Am Coll Toxicol 8:97–115 (1989).
13. Moser VC, MacPhail RC. Comparative sensitivity of neurobehavioral tests for chemical screening. Neurotoxicology 11:335–344 (1990).
14. Kulig BM. Methods and issues in evaluating the effects of organic solvents. In: Behavioral Measures of Neurotoxicity (Russell RW, Flattau PE, Pope AM, eds). Washington:National Academy of Sciences, 1991:159–183.
15. Kulig BM, Lammers JHCM. Assessment of neurotoxicant–induced effects on motor function. In: Neurotoxicology. Target Organ Series in Toxicology (Tilson HA, Mitchell C, eds). New York:Raven Press, 1992:147–179.
16. Meyer OA, Tilson HA, Byrd WC, Riley MT. A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav Toxicol 1:233–236 (1979).
17. Pryor GT, Uyeno ET, Tilson HA, Mitchell CL. Assessment of chemicals using a battery of neurobehavioral tests: a comparative study. Neurobehav Toxicol Teratol 5:91–117 (1983).
18. Jolicoeur FB, Rondeau DB, Hamel E, Butterworth RF, Barbeau A. Measurement of ataxia and related neurological signs in the laboratory rat. Can J Neurol Sci 6:209–215 (1979).
19. Becker A, Palissa A, Grimm M. Gait analysis—a useful method for quantitatively measuring ataxia in mice. Z Versuchstierkld 31:89–94 (1988).
20. Wietzohler H, Eckert S, Stevens A. Measurement of ataxic and paretic gait in neuropathies of rats based on analysis of walking tracks. J Neurosci Meth 32:35–95 (1990).
21. Pryor GT. A toluene-induced motor syndrome in rats resembling that seen in some human solvent abusers. Neurotoxicol Teratol 13:387–400 (1991).
22. Parker AJ, Clarke KA. Gait topography in rat locomotion. Physiol Behav 48:41–47 (1990).
23. Steinberg H, Sykes EA, McBride A, Terry P, Robinson K, Tillotson H. Computer analysis, using a digitizer, of ataxic mouse gait due to drugs. J Pharmacol Meth 21:103–113 (1989).
24. Edwards PM, Parker VH. A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats. Toxicol Appl Pharmacol 40:589–591 (1977).
25. Dunham NW, Miyia TS. A note on a simple apparatus for detecting neurological deficits in rats and mice. J Am Pharmacol Assoc 46:208–209 (1957).
26. Kulig BM, Vanwersch RAP, Wolthus OL. The automated analysis of coordinated movement in rats during acute and prolonged exposure to toxic agents. Toxicol Appl Pharmacol 80:1–10 (1985).
27. Youssef AF, Weiss B, Cox C. Neurobehavioral toxicity of methanol reflected by operant running. Neurotoxicol Teratol 15:223–227 (1993).
28. Martellini P. Tremor: a clinical and pharmacological survey. J Neural Transm 22(Suppl):141–148 (1986).
29. Stein RB, Lee RG. Tremor and clonus. In: Handbook of Physiology, Section I. The Nervous System. Vol 2: Motor Control, Part 1 (Brooks VB, ed). Bethesda, MD:American Physiological Society, 1981:325–343.
30. Gerhart JM, Higby J-S, Uphouse LL, Tillson HA. Chlorodecone-induced tremor: quantification and pharmacological analysis. Toxicol Appl Pharmacol 66:234–243 (1982).
31. Johnson JD, Meisheimer TL, Isom GE. A new method for quantification of tremors in mice. J Pharmacol Meth 16:329–337 (1986).
32. Lehtinen MS, Gotthoni PR. A system for measuring tremor intensity in rats. IEEE Trans Biomed Eng 8:RME–32 (1985).
33. Newland MC, Weiss B. Persistent effects of manganese on effortful responding and their relationship to manganese accumulation in the primate globus pallidus. Toxicol Appl Pharmacol 113:87–97 (1992).
34. Fowler SC, Liao R-M, Sjoldager P. A new rodent model for neuroleptic-induced pseudo-parkinsonism: low doses of haloperidol increase forelimb tremor in the rat. Behav Neurosci 104:449–456 (1990).
35. Reiter LW, MacPhail RC. Motor activity: a survey of methods with potential use in toxicity testing. Neurobehav Toxicol 1(Suppl):53–66 (1979).
36. Kulig BM, Lammers JHCM, Jaspers RMA. Acute and persistent neurotoxic effects of perchloroethylene in the rat. Toxicologist 12(1):276 (1992).
37. Crofton KM, Sheets LP. Evaluation of sensory system function using reflex modification of the startle response. J Am Coll Toxicol 8:199–211 (1989).
38. Stebbins WC. Concerning the need for more sophisticated animal models in sensory behavioral toxicology. Environ Health Perspect 44:77–85 (1982).
39. Pryor GT, Howd RA, Rebert CS. Hearing loss in rats caused by inhalation of mixed xylene and styrene. J Appl Toxicol 7:55–61 (1987).
40. Maurissen JP. Quantitative sensory assessment in toxicology and occupational medicine: applications, theory and critical appraisals. Toxicol Lett 43:321–343 (1988).
41. Rice DC. Testing effects of toxicants on sensory system function by operant methodology. In: Neurobehavioral Toxicity: Analysis and Interpretation (Weiss B, O’Donague JL, eds). New York: Raven Press, 1994:299–318.
42. Merigan WH, Barkdell E, Maurissen JP, Eskin TA, Lapham LW. Acrylamide effects in the macaque visual system. I: Psychophysics and electrophysiology. Invest Ophthalmol Vis Res 26:309–316 (1985).
43. Rice DC, Gilbert SG. Early chronic low-level methylmercury in monkeys impairs spatial vision. Science 216:759–761 (1982).
44. Ison JR. Reflex modification as an objective test of sensory processing following toxicant exposure. Neurotoxicol Teratol 6:437–445 (1984).
45. Reiter LA, Ison JR. Reflex modification and loudness recruitment. J Aud Res 19:201–207 (1973).
46. Young JS, Fechter LD. Reflex inhibition for animal audiometry: a technique for assessing ototoxicity. J Acoust Soc Am 73:1686–1693 (1983).
47. Crofton KM, Dean KF, Menache MG, Janssen R. Trimethyltin effects on auditory function and cochlear pathology. Toxicol Appl Pharmacol 105:123–132 (1990).
48. Jaspers RMA, Mujsjer H, Lammers JHCM, Kulig BM. Mid-frequency hearing loss and reduction of acoustic startle responding in rats following trichloroethylene exposure. Neurotoxicol Teratol 15:407–412 (1993).
49. Needleman HE, Guenoe C, Leviton A, Reed R, Maher H, Barret P. Deficits in psychologic and classroom performance of children with elevated dentine lead levels. N Engl J Med 300:689–695 (1979).
50. Marsh DO. Dose-response relationships in humans: methyl mercury epidemics in Japan and Iraq. In: The Toxicity of Methyl Mercury (Eccles CU, Annau Z, eds). Baltimore: Johns Hopkins University Press, 1987:45–54.
51. Tilson HA, Jacobson JL, Rogen WJ. Polychlorinated biphenyls and the developing nervous system: cross-species comparisons. Neurotoxicol Teratol 12:239–249 (1990).
52. WHO and the Nordic Council of Ministers. Chronic Effects of Organic Solvents on the Central Nervous System and Diagnostic Criteria. WHO Environmental Health Series Copenhagen: 1985.
53. Glowa JR. Behavioral effects of volatile organic solvents. In: Behavioral Pharmacology: The Current Status (Seiden LS, Balster RL, eds). New York: Alan R. Liss, 1985:537–552.
54. Eckerman DA, Bushnell PJ. The neurotoxicology of cognition: attention, learning and memory. In: Neurotoxicology (Tilson H, Mitchell C, eds). New York: Raven Press, 1992:213–270.
55. Cory-Slechta DA. Schedule-controlled behavior in neurotoxicology. In: Neurotoxicology. Target Organ Series in Toxicology (Tilson HA, Mitchell C, eds). New York: Raven Press, 1992:271–294.
56. Glowa JR, Dews PB. Behavioral toxicology of organic solvents. II: Comparison of results on tone by flow-through and closed chamber procedures. J Am Coll Toxicol 2:319–323 (1983).
57. Moser VC, Coggeshall EM, Balster RL. Effects of xylene isomers on operant responding and motor performance in mice. Toxicol Appl Pharmacol 80:293–298 (1985).
58. Bignami G, Alleva E, Amorico L, De Aceta L, Giardini V. Bidirectional avoidance by mice as a function of CS, US and apparatus variable. Anim Learn Behav 13:439–450 (1985).
59. Bushnell PJ. Delay-dependent impairment of reversal learning in rats treated with trimethyltin. Behav Neural Biol 54:75–79 (1990).
60. Cohn J, Cox C, Cory-Slechta DA. The effects of lead exposure on learning in a multiple repeated acquisition and performance schedule. Neurotoxicology 14:329–346 (1993).
61. Bushnell PJ, Angell KE. Effects of trimethyltin on repeated acquisition (learning) in the radial-arm maze. Neurotoxicology 13:429–442 (1992).
62. Schantz SL, Levin ED, Bowman RE. Long-term neurobehavioral effects of perinatal polychlorinated biphenyl (PCB) exposure in monkeys. Environ Toxicol Chem 10:747–756 (1991).
63. Bushnell PJ. Effects of delay, intertrial interval, delay behavior and trimethyltin on spatial delayed response in rats. Neurotoxicol Teratol 10:237–244 (1988).