Behavioral Toxicology, Risk Assessment, and Chlorinated Hydrocarbons

Ana Maria Evangelista de Duffard and Ricardo Duffard

Laboratorio de Toxicologia Experimental, Facultad de Ciencias, Bioquimicas y Farmaceuticas, Rosario, Santa Fe, Argentina

Behavioral end points are being used with greater frequency in neurotoxicology to detect and characterize the adverse effects of chemicals on the nervous system. Behavioral measures are particularly important for neurotoxicity risk assessment since many known neurotoxicants do not result in neuropathology. The chlorinated hydrocarbon class consists of a wide variety of chemicals including polychlorinated biphenyls, clioquinol, trichloroethylene, hexachlorophene, organochlorine insecticides (DDT, dicofol, chlordecone, dieldrin, and lindane), and phenoxyherbicides. Each of these chemicals has effects on motor, sensory, or cognitive function that are detectable using functional measures such as behavior. Furthermore, there is evidence that if exposure occurs during critical periods of development, many of the chlorinated hydrocarbons are developmental neurotoxicants. Developmental neurotoxicity is frequently expressed as alterations in motor function or cognitive abilities or changes in the ontogeny of sensorimotor reflexes. Neurotoxicity risk assessment should include assessments of the full range of possible neurotoxicological effects, including both structural and functional indicators of neurotoxicity. — Environ Health Perspect 104(Suppl 2):353–360 (1996)

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Introduction

It has been estimated that there are approximately 80,000 chemicals commercially available and that 1,100 to 1,500 new chemicals are submitted annually for premanufacture notification in the United States alone (*1*). Many, but not all of these chemicals undergo extensive toxicological evaluation before being approved for the market.

During the last two decades, there has been an increased interest in the nervous system as a target organ for toxicity (*2–5*). It is now well established that exposure to some chemicals used in agriculture and industry can produce neurotoxicity characterized by motor, sensory, cognitive, or autonomic nervous system dysfunction. For example, obvious signs of neurotoxicity including muscle weakness, loss of motor control and sensations in the periphery, tremors, visual dysfunction, and cognitive alterations have been reported by workers exposed to some herbicides and insecticides. In a review of signs and symptoms reported by humans exposed to chemicals, Anger and Johnson (*6*) identified more than 750 industrial chemicals as having neurotoxicity following acute or repeated exposure. It should also be noted that insidious problems of neurotoxicity may be undetected because the effects are incorrectly attributed to other conditions (e.g., advanced age, mood disorders) or simply misdiagnosed.

Until recently, screening chemicals for potential neurotoxicity depended heavily on the identification of adverse effects on the structure of the nervous system, i.e., neuropathology. Functional end points including chemical-induced changes in behavior, neurophysiology, and neurochemistry are being used with increasing frequency in the early phases of the risk assessment process. Functional measures are now viewed as a complement to the usual neuropathological assessment. The U.S. Environmental Protection Agency (U.S. EPA), for example, recently published a combined neurotoxicology screening protocol consisting of a functional observational battery, motor activity, and neuropathology (*7*).

Of the functional end points used in neurotoxicology risk assessment, behavioral measures are used with the most frequency. The U.S. EPA, for example, has published individual testing guidelines for a functional observational battery, motor activity, and schedule-controlled behavior, while behavioral end points figure prominently in guidelines to assess organophosphate-induced delayed neuropathy (*7*). Neurobehavioral effects are important because such changes are frequently associated with human neurotoxic disorders. The National Academy of Sciences (*8*) indicated that behavior is the net result of integrated sensory, motor, and cognitive function occurring in the nervous system that and chemical-induced changes in behavior may be a relatively sensitive indicator of nervous system dysfunction. The brain is an extremely complex organ, the function of which is to receive and integrate signals and then respond to them appropriately to maintain bodily functions. Moreover, it supports a diversity of complex processes including cognition, awareness, memory, attention, vigilance, and language, all of which are affected by exposure to chemicals (*6*). The complexity of the interactions of the nervous system with other organs provides a logical basis for the supposition that changes in nervous system functioning may occur on the dose–response curve of toxic effects at doses lower than those required to produce morphological or other changes.

Adverse behavioral effects were recognized as the outcome of exposure to chemicals by Weiss and Laties (*5*) over 20 years ago. Functional measures, especially behavioral end points, are now used routinely to detect and characterize potential neurotoxic effects of chemicals (*5,9,10*). As pointed out by Weiss (*11,12*), behavioral

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Address correspondence to Dr. Ana Maria Evangelista de Duffard, Laboratorio de Toxicologia Experimental, Facultad de Ciencias, Bioquimicas y Farmaceuticas, Universidad Nacional de Rosario, Suipacha 570 (2000) Rosario, Santa Fe, Argentina. Telephone: 54-041-382449. Fax: 54-041-389100.

Abbreviations used: CNS, central nervous system; PCBs, polychlorinated biphenyls; PERC, perchloroethylene; TCE, trichloroethylene; HCPH, hexachlorophene; BCH, benzene hexachloride; DDT, dichlorodiphenyl-trichloroethane; GABA, γ-aminobutyric acid; 2,4-D, 2,4-dichlorophenoxy acetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid.

assessment is important because often one of the earliest indications of exposure to neurotoxicants is subtle behavioral impairment such as paresthesia or short-term memory dysfunction. Frequently, such behavioral effects precede more obvious and frank neurological signs.

The purpose of this paper is to underscore the importance of functional measures, particularly behavioral end points, in neurotoxicity hazard evaluation. To illustrate this point, the neurotoxicological profile of the chlorinated hydrocarbon class of chemicals will be reviewed. There is considerable structural diversity in this class of agents, and they are used for a number of industrial and agricultural purposes. Many of these compounds share the ability to affect the nervous system; the effects are usually reproducible in animals and occur in most, if not all, subjects exposed to appropriate doses. There are, however, considerable qualitative differences in the symptomatologies and their effects on the central nervous system (CNS). They all have significant effects on the sensory, motor, or cognitive functioning of the nervous system.

Organopolychlorinated Compounds

Polychlorinated biphenyls (PCBs) are a group of biphenyl ring chemicals that contain from one to nine chlorine atoms per molecule, differing in the number and position of atoms on the two rings. They were formerly used in a wide range of industrial products including hydraulic fluids, plasticizers, adhesives, and dielectric fluids in capacitors and transformers. Commercial PCB mixtures are identified by their percent chlorine content (on a weight basis). Due to their chemical and thermal stability they persist in environmental media and accumulate in the food chain. PCBs are classified as pollutants by the U.S. EPA and are classified as 1 of the 100 most significant hazardous substances by the Centers for Disease Control (*13,14*).

Although PCBs were banned in the United States in the 1970s, and subsequently elsewhere, residues persist in air, soil, water, and sediment (*15*) and can be detected in biologic tissue in most residents of industrialized countries (*16–18*). Because of their persistence, they continue to be a health concern. Furthermore, they continue to be used commercially in some countries such as Argentina. Structurally related compounds such as the polychlorinated dibenzofurans (PCDFs) and polychlorodibenzo-*p*-dioxins (PCDDs) are highly toxic even at low doses, accumulate in a manner similar to PCBs, and frequently occur with PCBs in the environment.

Although there is little evidence to suggest that PCBs are directly neurotoxic in adults, there is considerable evidence indicating that these chemicals are developmental neurotoxicants (*19,20*). Initial evidence came from studies of pregnant women in Japan and Taiwan who had consumed cooking oil accidentally contaminated with large quantities of PCBs and PCDFs. In addition to having reduced birth size and dermatologic anomalies, children born to these women have shown poorer performance on standardized intelligence tests in follow-up studies (*20*).

In addition, those with highest transplacental exposure to PCBs showed hypotonia and hyporeflexia at birth and slowed motor development through 2 years of age, a defect in visual memory processing at 7 months, and defects in short-term memory at 4 years of age (*21,22*). Prenatal exposure (indicated by umbilical cord serum PCB level) predicted poorer short-term memory function on both verbal and quantitative tests in a dose-dependent fashion. These effects could not be attributed to a broad range of potential confounding variables, the impact of which was statistically evaluated (*22*).

Quantities of PCBs much larger than those received *in utero* are transferred to the nursing infant postnatally through breast-feeding because of the high lipid content of milk (*23*). This high liposolubility of organochlorides allows a high mother's milk–plasma ratio, thereby representing a risk for nursing infants (*24*). Although relatively small quantities of the PCBs may reach the fetus, the literature suggests the continuity of a toxic impact received *in utero* and observed initially during infancy. In general, these effects may be related to alterations in cognitive functioning fundamental to learning.

A review and a comparative evaluation of PCB effects were recently published (*19,25*). In general, developmental exposure to PCBs results in persistent neurobehavioral alterations in monkeys and nonprimates; similar neurobehavioral effects are observed across species, and such effects can occur in the absence of reduced body weights or gross signs of PCB intoxication. The most common finding in animal studies was that developmental exposure to PCBs results in behavioral hyperactivity and alterations in higher cognitive processes or learning. In humans, developmental delays and impaired cognitive function have also been reported.

The mechanism of PCB-induced neurotoxicity is not fully understood. Seegal and colleagues (*26,27*) have suggested that some congeners, i.e., *ortho*-substituted PCBs, may be neurotoxic. This observation has been supported in a recent paper by Kodavanti et al. (*28*). Other research (*29*) indicates that PCB-induced hypothyroxinemia may be related to some aspects of their developmental neurotoxicity.

Clioquinol

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) was initially produced as a topical antiseptic and marketed as an oral intestinal amebicide in 1934. Since then it has been used for a wide number of intestinal disorders including lambliasis, shigellosis, balantidirial dysentery, chronic nonspecific diarrheas, and "traveler's diarrhea" (*30*). Thirty-five years of worldwide acceptance as an inexpensive, mass-produced remedy for diarrhea with few recorded side effects reinforced the notion that clioquinol was a safe nonprescription drug.

However, in Japan between 1956 and 1972 (*31,32*), 10,000 cases of a subacute myelo-optic neuropathy caused by clioquinol were reported. Experimental animal studies were reported by Tateishi et al. (*33*), Lannek and Jonsson (*34*), and Heywood et al. (*35*), who reported that subchronic administration of clioquinol to beagle dogs produces an abnormal gait at one-third of its LD_{50}.

Chloroethylenes

Perchloroethylene (PERC) is a dry cleaning solvent and metal degreasing agent. Occupational exposure to PERC produces spontaneous abortion or perinatal death (*36,37*). With repeated exposure, prenarcotic effects, headaches, drowsiness, vertigo, and fatigue have been reported in humans; impairments of short-term memory and psychomotor function have also been reported (*38*).

Another chloroethylene is trichloroethylene (TCE), which is used as a dry cleaning agent, degreaser of metallic parts, cleaning fluid, and paint remover. Visual disturbances including peripheral visual-field constriction, abnormal visual evoked potential, and impairments in visuomotor performance have been observed following exposure to TCE. Longer term exposure has been reported to result in impaired visual performance (*39*). Case histories and experimental exposures in humans have not revealed a specific set of neurotoxicological

effects following acute or repeated exposure (*40*), but see Feldman and White (*41*).

Hexachlorophene

Hexachlorophene (HCPH) is an antibacterial agent widely used in soap and antiseptic solutions. Rats fed relatively large doses have been found to suffer damage to white matter and peripheral neuropathy (*42*). Motor dysfunction has been observed in adults exposed to HCPH (*43,44*). In addition, the synthesis of myelin was inhibited in HCPH-treated rats (*45*). HCPH is readily absorbed through the skin, and relatively high blood levels of HCPH have been found in children bathed in a 3% aqueous solution, which was equivalent to two-thirds of the amount causing a slight toxic effect in rats (*46*). In France, the deaths of more than 20 infants with neurological manifestations of encephalitis were attributed to the use of a talcum powder containing an excess 6% HCPH (*47*). Goldey and Taylor (*48*) have reported that rats exposed to HCBH *in utero* were hyperactive upon subsequent behavioral evaluation.

Organochlorinated Pesticides

Organochlorine pesticides are chlorinated hydrocarbons that have been widely used in agriculture and in the control of disease-bearing insects. These chemicals vary widely in structure and include hexachlorocyclohexanes or cycloparaffins (benzene hexachloride [BHC] and lindane, the gamma isomer of hexachlorocyclohexane [HCH]); chlorinated ethane derivatives or halogenated aromatic compounds (dichlorodiphenyltrichloroethane; DDT); cyclodienes (dieldrin, aldrin, endrin, hepatachlor, chlordane, chlordecone and mirex); and toxaphene, which is a mixture of chlorinated terpenes (*49*). Although most of these chemicals are insecticides, some are also used as rodenticides (BHC), acaricides (chlordimeform), fungicides (dichlorophen), or the herbicide chloroneb (1,4-dichloro-2,5-dimethoxybenzene). Many of them produce tremor or hyperresponsiveness to external stimulation (*50–52*). Other problems include headaches, irritability, insomnia, and a poorly described "neuroasthenic" or "asthenoautonomic" syndrome characterized by difficulty in thinking (*53*).

From the mid-1940s to the mid-1960s, the organochlorine insecticides were used widely in agriculture, soil and building insect control, and malaria control programs. As a class, however, they are used less frequently because they tend to persist in the environment and accumulate in biologic as well as nonbiologic media. They are, however, still important since they can cause systemic poisoning and they are still used in some countries (*54*).

In rats and mice, acute organochlorine-pesticide poisoning produces tremor, irregular muscle-jerking movements (myoclonus), hyperexcitability, irritability, hyperthermia, and clonic seizures, but the characteristics of the abnormal motor movements may differ with different organochlorine pesticides (*55*). Apprehension and excitability followed by various neurologic signs including twitching, tremors, mental disorientation, weakness, paresthesia, and convulsions, which are often epileptiform, are the symptoms and signs described in humans.

DDT

DDT is one of the best-known organochlorine insecticides and produces a neurotoxic syndrome in both vertebrate and invertebrate species. It is also a worldwide environmental contaminant still used in some countries (*56*). DDT is accumulated in the food chain and known to be transferred from mother to offspring via milk (*57,58*). In mice, DDT and some other environmental pollutants, such as PCBs and chlorinated paraffins, have been shown to be retained to a greater extent in the brain when given at 10 days of age than when given at other ages (*59,60*). The levels determined in human milk are more than 10 times higher than those in cow's milk (*61*). In many countries including Argentina (*62*), Panama, Brazil (*63,64*), Costa Rica (*65*), Guatemala and El Salvador (*66*), Mexico (*67*), Nigeria (*68*), and India (*69,70*), nursing infants potentially ingest organohalogens at a ratio many times that of the acceptable daily intake (ADI) as estimated by the Food and Agricultural Organization (*71*).

Neonatal exposure to a single low oral dose of DDT can lead to a permanent hyperactive condition in adult mice (*72*). The consequence of the early exposure is quite different from that reported for animals exposed to a single dose of DDT as adults. The signs of poisoning caused by DDT in adults are characterized by several behavioral manifestations including hyperactivity, ataxia, tremors, and paralysis. The principal neurophysiological mechanism of action of DDT is to slow the closing of the voltage-dependent sodium channel once it has been opened by the action potential, with the result that the hyperexcitable phase of the action potential is prolonged. This is exhibited at the organismic level in behavioral hyperexcitability (*52,73*).The dose of DDT required in adult animals to provoke signs and effects such as ataxia, tremor, increased activity in open-field test, and avoidance responding is more than 50 to 200 times the dose used for neonatal exposure. This amount of DDT is of physiological significance because it is of the same order of magnitude that humans can be exposed to during the lactation period.

Dicofol

Dicofol [bis(chlorophenyl)2,2,2-trichloroethanol] is an agricultural miticide for crops such as cotton, beans, citrus, and grapes. It is structurally related to DDT and shares many of its properties (*74,75*). In humans, volunteer studies and case reports have shown that exposure to high doses of dicofol results in disturbance of equilibrium, dizziness, confusion, headaches, tremors, fatigue, vomiting, twitching, seizures, and loss of consciousness (*74*). Lessenger and Riley (*76*) reported persistent cognitive and emotional difficulties in a young male exposed to a relatively high dose of dicofol.

Chlordecone

The pesticide chlordecone (decachlorooctahydro-1,3,4-metheno-2-H-cyclobutal [cd]-pentalen-2-one), a cyclopentadiene derivative, was manufactured in large quantities in the United States before 1975 (*77*). Hazardous conditions at a manufacturing plant in Hopewell, Virginia, led to epidemic poisoning of workers and contamination of the James River in 1976. The predominant signs of poisoning were motor incoordination, ataxia, tremors, opsoclonus (uncontrolled eye movements), muscular weakness, nervousness, pleuritic pain, joint pain, hepatomegaly, abnormal liver function, skin rash, sterility, and weight loss (*78–80*). As with other chlorinated hydrocarbon insecticides, the CNS is one of the major sites of chlordecone's effects.

Chlordecone's neurotoxicity has also been demonstrated in animal models. Dietz and McMillan (*81,82*), for example, compared the effects of daily administration of mirex and chlordecone on the performance of rats under several schedules of reinforcement, including a multiple fixed-interval 2-min fixed-ratio 12-response schedule. Both pesticides produced delayed disruption in performance, with the delay being inversely proportional to the dose administered daily. They reported that the disruption often occurred before the appearance of grossly observable signs of exposure. Chlordecone also produces

increased reactivity to external stimulation and tremor in rats (*50*).

An interruption of the estrous cycle in neonatal rats exposed to chlordecone was observed (*83*). The reproductive toxicity following exposure to chlorinated pesticides such as chlordecone and DDT has been attributed to their interaction with the intracellular estradiol receptor. In the female rodent, chlordecone disrupts both the preovulatory LH surge and sexual behavior. Chlordecone's toxicity includes production of tremor and severe attenuation of reproductive function and decreased sexual receptivity when treatment with the pesticide occurs on the day of proestrus in intact females (*84*). Chlordecone's ability to disrupt female reproductive behavior may involve its disturbance of the serotonin system, independent of its interaction with the CNS estradiol receptor (*85*). Tilson et al. (*86*) and Mactutus and Tilson (*87*) reported persistent alterations in learning and memory in rats exposed to chlordecone postnatally.

Dieldrin

In many countries outside the United States, dieldrin (a chlorinated cyclopentadiene derivative) is used as a broad spectrum insecticide to protect food crops and control disease vectors, locusts, and termites. Unlike chlordecone, signs of dieldrin poisoning in humans include severe tonic seizures usually of sudden onset, myoclonic jerks, loss of appetite, mental disorder including loss of memory and irritability, and headache. In several cases, seizures occurred many months after the last exposure to dieldrin (*88–90*). Offspring from mother rats fed dieldrin at levels often found in the environment and with a low protein diet showed altered behavioral effects (*91*).

Burt (*92*) reported that dieldrin affected the performance of rats and Japanese quail maintained under fixed-interval schedules of food reinforcement. Dieldrin decreased overall rates of fixed-interval responding and disrupted the within-interval pattern of responding in both species. The effects of dieldrin also persisted for at least 2 days in rats and 5 days in quail following acute administration.

Lindane

This organochlorine pesticide is used in both human and veterinary medicine to treat ectoparasites (*93,94*). In 1948, Wooldridge (*95*) successfully treated human scabies (skin disease caused by mites) with 1% lindane cream, and this treatment continues to be widely used. In addition, lindane shampoo is used for pediculosis (infestation with lice). While dermal absorption is generally low, there are exceptions. Lindane is also used as a general insecticide, particularly in countries outside the United States, to control structural pests such as termites although its environmental persistence may not be sufficient to make it satisfactory in this capacity (*94*). There have been numerous reports through the years of major toxicity and death associated with accidental or deliberate exposure to lindane (*96*). Lindane is a potent convulsant agent in humans and other mammals (*96*) as a result of a direct action on the CNS (*97*). In the most severe incident (*98*), epidemic poisoning occurred in India when lindane intended for preservation of seed grains was instead mixed with food grains and was consumed. The onset of signs of poisoning was sudden with seizures of the mixed type, i.e., grand mal, petit mal, and myoclonus, predominating. Other effects included intention tremors, memory impairment, irritability, and aggression.

Desi (*99*) found that repeated exposure to lindane increased the number of errors made in a food-reinforced maze. One interpretation of these results is that lindane may interfere directly with learning. Consistent with these data, it was reported that lindane has effects on long-term potentiation in the hippocampus, and it is possible that this effect may compete or interfere with the utilization of new information. The post-training administration of lindane did not affect retention. This suggests that the process of memory consolidation is not altered (*100*). Data from Tilson et al. (*101*) suggest that exposure to nonconvulsant doses of lindane can interfere with the ability to acquire and use new information and that these effects may be associated with alteration in GABA.

Although dieldrin and lindane have quite different chemical structures, the signs of poisoning that they produce are similar in both insects and mammals. Unlike the action of DDT, which is on the axonal membrane, the primary site of action of lindane and dieldrin is the synapse where both increase release of neurotransmitters (*102*). The action of lindane was first studied in insects and shown to be on the ganglia rather than on the axon. In the cockroach, lindane and dieldrin were both found to act on the cholinergic giant fiber system in the abdominal ganglion and to increase evoked and spontaneous release of acetylcholine (*103,104*). In addition, it has been shown that lindane interacts with the rat brain GABA receptor–ionophore complex at the picrotoxinin binding site (*105*); Matsumura and Ghiasuddin (*106*) demonstrated that dieldrin and lindane mimicked the action of picrotoxinin in inhibiting GABA-stimulated chloride uptake in cockroach muscle and competed directly with picrotoxinin for binding in rat brain synaptosomes.

Herbicides

The production and use of chemicals for destruction of noxious weeds have markedly increased worldwide during the last 30 years and exceed insecticides in quantity and value of sales. Until recently, it was widely believed that because plants differ from animals in their morphology and physiology, herbicides would be of relatively low risk to animals and humans. Recent experience with some herbicides, however, has indicated that this assumption is not valid. The chlorinated aromatic acid compounds, 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), have been widely used as herbicides both in the United States and in Vietnam as a component of Agent Orange. They have also been used as herbicides in agriculture and forestry throughout the world. It has been noted that the polychlorinated dibenzo-*p*-dioxins (PCDDs), particularly 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), may contaminate some phenoxyherbicide formulations.

Phenoxyherbicides are excreted in the urine, and 2,4,5-T and 2,4-D have been detected in the urine of children living in an area around a herbicide manufacturing plant in Arkansas (*107*). 2,4-D appears in the brain following *in ovo* or oral exposure (*108,109*), and it has been suggested that these agents might reach the brain by damaging the blood–brain barrier (*110,111*). Phenoxyherbicides are transported from the cerebrospinal fluid via the organic anion transport system, and inhibitors of this transport may block its elimination from the brain *in vivo*, just as they block its transport by the isolated choroid plexus (*112*).

Desi and Sos (*113*) and Desi et al. (*114,115*) observed, in acute and repeated exposure experiments in rats, cats, and dogs treated with 2,4-D, that cerebral electrical activity was disturbed, including a gradual slowing of the electroencephalogram. Demyelination in the dorsal portion of the spinal cord was observed in rats exposed to relatively large doses of 2,4-D. They concluded that the site of action was either in the cerebral cortex or in the reticular

formation. These authors did not report histological lesions in the CNS, although Duffard et al. (*116*) described CNS hypomyelination in 1-day-old chicks born from eggs externally treated with 2,4-D. Evangelista de Duffard et al. (*109*) reported that 2,4-D interfered with motor function of rats tested on a rotating rod; an increased brain level of 5-HT and 5-HIAA in adult rats exposed pre- and postnatally to 2,4-D was also detected (*117*).

Humans exposed to 2,4-D have reported neurologic symptoms that include numbness in the fingers and toes, muscle aches and fatigue, tetany of the limb muscles, and ataxia (*118*). CNS effects have also been reported and were manifested as aberrant spontaneous electrical activity of the cerebral cortex and reticular formation as measured by EEG (*119*). Peripheral neuropathies have also been ascribed to 2,4-D and 2,4,5-T. Singer et al. (*120*), for example, described an increased prevalence of slowed nerve conduction velocities among chemical workers exposed to the phenoxyherbicides 2,4,5-T and 2,4-D and related contaminants (chlorinated dioxins). The sural nerve seemed to be especially affected. However, human exposure to 2,4-D has often been obscured by simultaneous exposure to other xenobiotics. Alterations in motor function have also been observed in rats exposed to repeated doses of 2,4-D (*121*).

Gender and the physiological state of the animal appear to affect the manifestation of 2,4-D-induced neurotoxicity. Our laboratory demonstrated that oral administration of 2,4-D butyl ester (2,4-Dbe) to nulliparous females had no effect on either open field (OF) or rotarod performance (*109*). By contrast, dams treated with 2,4-Dbe during pregnancy exhibited impairments of activity and rotarod. Administration of 2,4-Dbe to 90-day-old intact male rats depressed spontaneous activity and rotarod endurance. Castration itself impaired performance in the rotarod test but did not alter OF activity significantly. The effects of castration were reversed by exogenous testosterone. In gonadectomized rats, 2,4-Dbe prevented the reversal of testosterone's effect on the influence of castration on behavior if given concomitantly with testosterone. However, when 2,4-Dbe treatment started 7 days after testosterone, the 2,4-Dbe effects on OF and rotarod were reinstated. Thus, the level of testosterone appears to be important for causing the toxic effects of 2,4-Dbe in rats (*109*).

Table 1. Comparison of behavioral toxicity of chlorinated hydrocarbons and related compounds.

Chemical or class	Neurobehavioral effects
Polychlorinated biphenyls	Developmental neurotoxicity, motor activity, cognitive function, developmental delays
Clioquinol	Motor dysfunction
Trichloroethylene	Visual neurotoxicant
Perchloroethylene	Cognitive dysfunction, vague symptoms of neurotoxicity
Hexachlorophene	Motor dysfunction, developmental neurotoxicity
Organochlorine pesticides	
DDT	Hyperexcitability, tremor in adults, developmental neurotoxicity
Dicofol	Hyperexcitability, tremor
Chlordecone	Hyperexcitability, tremor in adults, developmental neurotoxicity
Dieldrin	Convulsant in adults, developmental neurotoxicity
Lindane	Convulsant in adults
Phenoxyherbicides	
2,4-D	Motor dysfunction

Summary and Conclusions

The purpose of this paper is to illustrate the importance of behavioral measurements in assessing the neurotoxicity of chemical agents. The chlorinated hydrocarbons surveyed in this overview produce a wide spectrum of adverse effects on the nervous system of humans and animals. Such effects are summarized in Table 1 and include motor dysfunction (clioquinol, hexachlorophene, 2,4-D), visual impairment (trichloroethylene), tremors and hyperexcitability (DDT, dicofol, chlordecone), seizurigenic activity (dieldrin, lindane), and cognitive alterations (perchloroethylene). Several of the compounds are also developmental neurotoxicants (PCBs, hexachlorophene, DDT, chlordecone, and dieldrin). Chemical-induced neuropathology, however, is associated with only a few of these neurotoxicants including clioquinol, trichloroethylene, hexachlorophene, and 2,4-D. Chemical-induced changes in motor, sensory, or cognitive function are clearly cardinal indicators of exposure to the chlorinated hydrocarbons.

Unlike pharmacological agents and natural or synthetic toxins, many industrial and pesticidal agents are not designed to affect a specific biological function or interact with a specific receptor site at the cellular or molecular level. It is expected that many chemicals will have multiple mechanisms of toxic effect. Because many behavioral measures are apical tests, they may be better suited to assess chemicals with unknown or multiple mechanisms during the initial stages of hazard evaluation.

One of the main tasks of toxicology and risk assessment is to determine, through experiments with animals and documentation of adverse effects following accidental exposure of humans, safe limits of exposure to toxic chemicals. Since new chemicals are being released into the environment at a relatively steady pace, it is essential to use rapid and sensitive toxicological screening procedures for these and already existing chemicals. The survey of the literature presented in this paper supports the strategy of including behavioral tests of sensory, motor, and cognitive function in the initial phases of hazard identification. Once behavioral neurotoxic effects have been identified, it is important to improve the understanding of the mechanisms of neurotoxicity at the neurochemical, neurophysiological, cellular, and molecular levels of analysis. Neurotoxicity risk assessment will be improved by a more complete understanding of the interrelationships between the various levels of nervous system organization. Knowledge about the capability of humans and other organisms to cope behaviorally with conditions of their physical environment can add important information useful to decision makers and legislators concerned with environmental toxicology. Neurobehavioral toxicology contributes directly to this issue by systematically assessing the threshold and magnitude of exposure beyond which normal processes of nervous system functioning are significantly affected.

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