Lessons for Neurotoxicology from Selected Model Compounds: SGOMSEC Joint Report

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The ability to identify potential neurotoxicants depends upon the characteristics of our test instruments. The neurotoxic properties of lead, methylmercury, polychlorinated biphenyls, and organic solvents would all have been detected at some dose level by tests in current use, provided that the doses were high enough and administered at an appropriate time such as during gestation. The adequacy of animal studies, particularly rodent studies, to predict intake levels at which human health can be protected is disappointing, however. It is unlikely that the use of advanced behavioral methodology would alleviate the apparent lack of sensitivity of the rodent model for many agents. — Environ Health Perspect 104(Suppl 2):205–215 (1996)

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This paper on model compounds addresses the lessons that may be extracted from the intensive investigation over the last 15 years or so of selected agents (lead, methylmercury) or classes of agents (polychlorinated biphenyls [PCBs], solvents) identified as potent neurotoxins by environmental or industrial exposure of human populations. Such recognition in each case prompted extensive research in animal models to characterize effects and to predict potential neurotoxic end points in humans. Epidemiological investigation focused on characterizing effects in the human population and identifying no-effect levels. Much of the research on the effects of lead, methylmercury, and PCBs has focused on identifying behavioral and other neurotoxic effects produced as a result of developmental exposure, while most of the research on solvents has focused on exposure in adults. There are a number of questions that may be asked regarding what we have learned from these neurotoxic agents. How was neurotoxicity identified in the human population? How well are the effects characterized in humans, and how confident are we of our estimates of the intake or body burden necessary to produce effects? What do we know about the nature of effects in adults versus those in the developing organism? How good are animal models in identifying the neurotoxic potential of these agents? How well do effects in animals predict effects in humans? And finally, how well do the estimates of safe levels based on animal models actually predict what we know about intake necessary to produce human neurotoxicity?

Characterization of Effects

Lead

The recognition of lead as a neurotoxicant arose initially in the ancient world where the classic signs of lead poisoning—colic, constipation, pallor and palsy—were recognized by both the Greeks and Romans. This recognition reemerged in the 17th century and was brought to public attention periodically thereafter. Occupational exposure to lead still poses a threat to the health of workers, resulting in peripheral neuropathy and deficits in attention and cognitive function (1,2). Early in the 20th century, it was recognized that children represent a particularly vulnerable population, with exposure potentially resulting inencephalopathy and death. Despite this recognition, lead was used widely in paint and other industrial products and added to gasoline, ensuring worldwide and persistent distribution. Over the next several decades, physicians continued to report untoward effects as a result of lead exposure in children. As early as the 1940s there was a recognition that permanent neurological damage could result from exposure to lead at levels that had never produced overt signs of toxicity. In 1979, a landmark study by Needleman and colleagues in Boston (3) reported decreased intelligence quotient (IQ) and an increased incidence of distractibility and inattention in middle-class children with no identifiable source of lead exposure. The conclusion to be drawn from this study was that environmental sources of lead were producing intellectual impairment in children at levels that had come to be regarded as normal.

In the last decade and a half, there has been intense research into the health effects of lead in children and developing animals, such that probably more is known about the health effects of lead than any other noncarcinogenic environmental contaminant (4–8). The result in the United States has been that, over the last two decades, the blood level of lead considered safe for children has rapidly decreased to the present...
level of 10 µg/dl. Prospective studies in Boston, Cincinnati, Port Pirie, and New Zealand, as well as a number of cross-sectional studies, have demonstrated deficits in IQ as a function of increased body burden of lead after control for potential confounding variables. A 10 µg/dl increase in blood lead level is associated with about a 3 point deficit in IQ according to a recent meta-analysis (7). These effects have been documented from infancy through early-to-middle school age; historic lead levels have often been a better predictor of deficit than concurrent blood lead levels. Assessment of behavior by teachers or parents has revealed short attention span, increased distractibility, hyperactivity, and problems in following sequences of directions as a function of increased blood lead levels. Not surprisingly, deficits in school performance are also associated with an increased body burden of lead. Epidemiological studies in children have revealed deficits on vigilance tasks and increased reaction time, which may reflect increased distractibility and/or decreased attention span. Lead-exposed children also engage in perseverative behavior, continuing to respond in inappropriate ways.

The early animal research in the 1970s focused largely on the effects of exposure to high doses of lead on simple learning problems. Studies in which rats were prenatally exposed generally revealed lead-induced deficits, while postnatal or adult exposure generally produced no impairment (9). As behavioral methodology was refined, however, it became clear that the prenatal period was not the only period sensitive to lead-induced impairment. Lead research using animal models over the last 15 years has revealed lead-induced impairment at increasingly lower doses and on a wide range of behavioral tasks. Much of the more sophisticated work in rodents was performed at the University of Rochester by Cory-Slechta and colleagues using a postweaning exposure paradigm (8,10). Extensive research has also been performed in two species of macaque monkeys, the rhesus and cynomolgus (crab-eating), in three different laboratories (8). Exposure was prenatal, postnatal, or lifetime in various experiments. Robust deficits have been observed in monkeys exposed only prenatally or only postnatally and tested years after cessation of exposure, as well as in monkeys with low blood lead levels exposed over a lifetime. Consistent effects have been observed on complex tests of learning and memory. Analysis of error patterns responsible for lead-induced deficits has consistently revealed increased distractibility, perseveration, inability to inhibit inappropriate responding, and inability to change response strategy as hallmarks of developmental lead exposure in both rats and monkeys.

An interesting parallel in methodologies between the experimental and epidemiological literature addressed the effect of lead exposure on the ability to change response strategies from an established pattern to a new one. This issue was assessed in monkeys using a series of discrimination reversal tasks and in children using the Wisconsin Card Sort Test. The test in monkeys required them to learn a simple visual discrimination; once they learned the task, the formerly correct stimulus became the incorrect one, and vice versa (11,12). A series of such reversals was performed. Then the rules were changed in a different way: the relevant stimulus dimension changed from form (triangle vs cross) to color (red vs green). The triangle and cross were still superimposed on the colors; however, each appeared on the red or green in a balanced design. The monkey was required to learn to ignore the (formerly relevant) forms and to attend to the colors. After a series of reversals on this task, the relevant stimulus dimension was changed from color to form and the monkey was required to switch strategies again. In the Wisconsin Card Sort Test, the 10-year-old child was required to pick the card that went with a set of examples presented by the investigator (13). The relevant stimulus class could be color, number, or suit. The experimenter changed the relevant stimulus class at a number of points during the experiment. Thus, both of these tests required the subject to change an established response strategy and to figure out that a new rule was in effect. Both lead-exposed children and monkeys were impaired in their ability to do so: they stayed with an old strategy that was no longer useful.

Recent epidemiological studies suggest that children with blood lead levels as low as 10 µg/dl are impaired relative to children with lower blood lead levels (8). A recent meta-analysis concluded that there was no evidence for a threshold for lead-induced deficits down to a blood lead level of 1 µg/dl (7). These data are consistent with data from monkeys in which a group with blood lead levels of 11 µg/dl were impaired on a number of tasks compared to controls with blood lead levels below 5 µg/dl (8). Behavioral impairment has been observed in rats with blood lead levels of 20 µg/dl (114). A no-effect dose has not been observed in either the monkey or rat studies. It is probable that the very strong evidence from both the experimental and epidemiological studies concerning the deleterious effects of lead were necessary for the decision of the Centers for Disease Control and Prevention in the United States to set the current action level for children at 10 µg/dl. Lead levels have decreased in North America as a result of the removal of lead from gasoline in the 1970s, although leaded paint in old houses continues to present a hazard. In many other countries, lead is still allowed in gasoline and paint, and average blood lead levels in children are higher than they are in North America.

Blood lead levels in children typically peak at about 2 years of age and decrease thereafter. Of course, exposure to additional sources of lead may result in a temporary increase in lead body burden at any time during childhood. It is therefore important to determine whether these peaks in blood lead levels have lasting consequences. It was known as early as the 1940s that overt lead toxicity could produce permanent behavioral sequelae in children (8). Data from the modern prospective studies, in which behavioral performance at 5 to 10 years of age often correlated best with blood lead levels early in life, around the age of the peak in blood lead levels, are suggestive of long-lasting impairment, although the continued exposure to lead makes interpretation difficult. Experiments in which monkeys were exposed either in utero only or postnatally for a year or less have demonstrated clear deficits on a number of behavioral tasks when monkeys were adults (8). Thus, experimental research, in which there is the opportunity to control exposure conditions, was able to address an important issue that cannot be addressed directly epidemiologically.

The animal literature has also provided clarification to the epidemiological literature with regard to an issue that has proved contentious: the control of confounding variables in epidemiological studies. It is well known that IQ is affected by many variables: e.g., parental IQ, socioeconomic status, maternal ingestion of various drugs during pregnancy (including tobacco and alcohol), obstetric complications, and birth weight. In many of the lead studies, some of these variables, particularly socioeconomic status and maternal IQ, were highly correlated with children’s blood lead levels. In most studies, adjusting for various potential confounders decreased the statistical significance of the effect of lead. (This was not always the case, however. In the
prospective study in Boston, for example, socioeconomic status was higher in children with higher blood lead levels so that lead effects were more significant after covariate adjustment.) The argument has been made that effects attributed to lead were in fact due to some other unidentified factor that also covaried with lead levels. This assertion ignores the rather substantial animal literature reporting lead-induced deficits at roughly the same blood lead levels as those reported in children. The results from animal studies are not plagued by such potential confounders; subjects are randomly assigned to exposure groups, and all variables except exposure to lead are kept as constant as possible. The congruence of effects thus provides reassurance that the results of the epidemiological studies are not misleading.

The recognition of the potential for a profound effect on the population to be produced by a small effect has been highlighted by the decision to expose millions of children to lead. Generally, the effect of lead only accounts for a few percent of the variance on measures of IQ since IQ is influenced by many factors. It has been argued that the effects of lead are therefore unimportant. However, a 5 point decrement in IQ, identified in many prospective studies, will have a catastrophic effect on the characteristics of the population. It will result in a decrease in the number of people with IQs above 130 by more than half while concomitantly increasing those with IQs below 70, resulting in substantial economic and other consequences (15).

**Methylmercury**

Methylmercury is known as one of the most hazardous environmental pollutants, largely due to endemic disasters such as Minamata disease in Japan and methylmercury poisoning in Iraq (16). In both tragedies, infants exposed in utero were severely affected, even though their mothers may have had minimal symptoms of methylmercury poisoning. Thus, the developmental toxicity of methylmercury had become a focus of both human and animal studies. Even today there are several populations that are exposed to methylmercury through substantial fish consumption. Effects of in utero exposure to methylmercury in infants born to these populations are currently being evaluated.

Minamata disease was first identified among people living along Minamata Bay in Kyushu, Japan. The source of methylmercury was effluent from a chemical company where mercury was used as a catalyst. As a result of bioconcentration in the food chain, high concentrations of methylmercury accumulated in fish and shellfish. Abnormal gait, dysarthria, ataxia, deafness, and constriction of the visual field were the main symptoms. Cats living in the villagers’ homes showed signs of motor impairment similar to those manifested in humans. The early epidemiological investigation concluded that an unidentified toxic agent in fish and shellfish was responsible. It took almost 3 years to identify the causal agent by experimental pathology, clinical study, and chemical analyses of environmental samples. The report 3 years later finally concluded that “organic mercury is most suspected” (16).

Methylmercury easily crosses the placenta, and the developing brain can be severely affected by the compound. In the Minamata tragedy, affected infants manifested severe disease resembling cerebral palsy. Mental retardation, cerebellar ataxia, primitive reflex, dysarthria, seizure, and pyramidal signs were also observed. Because of the severity of signs in the infants, the typical symptoms such as constriction of the visual field could not be examined. The mothers of these children had seemed healthy at the time of parturition, although they developed symptoms later. Therefore, it was considered that the fetus is particularly vulnerable to methylmercury neurotoxicity.

In a subsequent episode in Iraq, people were exposed to methylmercury as a result of distribution of seed grain treated with a methylmercury fungicide. Rural people used the grain to make bread. The total number of official victims was 6530 including 459 deaths. Observed symptoms included paresthesia, malaise, ataxia, constriction of visual fields, and hearing impairment. Babies exposed in utero to methylmercury were investigated for physical and mental development. A scoring system of examination results was adopted in the investigation. Although individual scores exhibited variability, a dose–response relationship was observed between effects, such as retardation of walking and neurological signs, and maternal hair mercury concentration. From these analyses, an estimated lowest effect level was determined. Delays in speech development have also been observed following developmental methylmercury exposure in this population, although the possible contribution of hearing deficits is unknown.

In a study of 234 Cree children 12 to 30 months of age in Canada, the mother’s peak hair level was used as the index of exposure (17). Assessment of several neurological measures, in addition to the Denver developmental scale, revealed only abnormal muscle tone or reflexes and only in boys. There was not a clear dose-dependent relationship.

In a population-based study in New Zealand (18,19), mothers consumed fish on a frequent basis. Assessment on the Denver development scale revealed abnormal or questionable results at a higher frequency in 4-year-old children of mothers with hair levels of >6 μg/g during pregnancy compared to a matched control group. When these children were 6 to 7 years old, an average maternal hair concentration of 15 μg/g was associated with a poorer performance on the Wechsler Intelligence Scale for Children (WISC). However, the number of children tested in this study was small (60–70).

Methylmercury is also a potent neurotoxicant in animals. Emphasis has been placed on characterization of developmental neurotoxicity, although toxicity in adult animals has also been demonstrated. Spyker et al. (20) demonstrated that mice exposed to methylmercury in utero showed impaired swimming ability. Their result first confirmed fetal methylmercury poisoning in an animal model, although the impairment was less severe compared to fetal Minamata cases and was subtle before the mice were forced to swim. Subsequently, a large number of animal studies were performed using various methods; thus, a much wider range of effects has been examined in animal studies compared to the human studies.

As was the case for lead, considerable research on neurotoxicity produced by methylmercury has been performed in monkeys, presumably in response to the episodes of human poisoning. Research in adult monkeys replicated the constriction of visual fields and other visual deficits observed in adult humans as a result of methylmercury exposure (21); these findings of sensory system deficits have been extended in developmentally exposed monkeys in which visual, auditory, and somatosensory deficits have been observed (22). Early developmental exposure at high doses also produces the pattern of cerebral palsy and severe visual deficits including blindness (23,24) observed in humans.

Research in monkeys has also addressed the issue of cognitive impairment as a result of developmental methylmercury exposure. In utero exposure resulted in impairment of visual recognition memory (25) and retarded object permanence.
performance (26) when monkeys were tested during infancy. Interestingly, this same cohort of monkeys demonstrated facilitated performance on a delayed spatial alternation task when tested as adults (27). In utero plus postnatal exposure failed to produce deficits on a discrimination reversal task during both the infant and juvenile periods (28), even in a monkey with clear motor signs of methylmercury intoxication. It may be that the monkey does not provide a good model of the gross cognitive impairment observed after high-dose developmental exposure in humans.

Methylmercury also produces neurotoxicity in rodents, despite some differences in the neuropathology and pharmacokinetics between rodents and primates (including humans). The pattern of neuropathological damage in the brain is different in rodents than in species with deep sulci; specifically, methylmercury produces preferential damage to deep sulci such as calcarine fissure (subserving visual function) in adult humans (29), a pattern which is replicated in primates (30,31) but not in rodents. However, the pattern of damage after developmental exposure is more diffuse in all species (29).

There are also significant toxicokinetic differences between species, including blood and whole-body half-times, and brain: blood ratios (32). The rat is anomalous in having a very high red blood cell:plasma ratio, which undoubtedly contributes to the very low brain: blood ratio of 0.06, compared to a blood:brain ratio of 2 to 5 for monkeys and humans (22). The most obvious effect of methylmercury in adult rodents is motor deficit (33–36); reports of effects on activity are conflicting (37,38). In utero exposure at high doses reliably produces deficits in motor function. Very high doses may result in a decrease in locomotor activity (16), while lower doses often produce no effect (39–41). Both positive and negative results have been obtained on simple learning tests (16,22). The sensory system damage that is a hallmark of methylmercury toxicity in humans may not be produced by moderate exposure in rodents based on indirect evidence such as auditory startle response and visual discrimination performance, although high-dose in utero exposure produces changes in visual evoked potentials (22) or blindness (29). Direct assessment of auditory thresholds revealed no deficits in methylmercury-exposed rats (39).

**Polychlorinated Biphenyls**

Polychlorinated biphenyls (PCBs) are a family of chlorinated hydrocarbons containing 209 different isomers (congeners). Their major use was as a dielectric in transformers and capacitors, although they had other industrial uses as well. They were in widespread use from the 1930s until the 1970s; although PCBs were banned in the United States in the 1970s and subsequently elsewhere, they are presently a worldwide pollution problem. Residues persist in air, soil, water, and sediment and can be detected in biological tissue in most residents of industrialized countries. The chemicals are stored in fat and are not readily excreted except in breast milk.

In 1968 a tragic epidemic occurred in Japan as a result of contamination of rice oil with PCBs and small amounts of other contaminants. Infants born to mothers who consumed the contaminated oil had dark pigmentation of the skin, low birth weight, early eruption of the teeth, and swollen gums and eyelids (42). In another incident in Japan, affected children had hypotonic reflexes, were dull and apathetic, and had low IQs (43). Adults ingesting high levels of contaminated oil suffered chloracne, numbness and weakness of limbs, and decreased peripheral nerve conduction velocities. However, the developing fetus was much more sensitive than the mother. Children born to mothers exposed to a high, acute dose of PCBs in Taiwan have been followed for at least 6 years (44). These children exhibited delayed developmental milestones, deficits in intellectual functioning, and other behavioral problems. These effects were observed at exposure levels that produced overt signs including gum hypertrophy, deformed or pigmented nails, chloracne, hyperpigmentation, and hair loss.

An extensive prospective study involved Michigan children born to women who consumed fish from Lake Michigan (45–50). Reduced birth weight and head circumference were associated with consumption of contaminated fish. Fish consumption by the mothers was also associated with lower scores on the Brazelton neonatal development scale in the infants. Decreased visual recognition memory in this same set of infants at 7 months of age was associated with both maternal fish consumption and cord serum PCB levels (47); this task is reasonably predictive of IQ measured at school age. There was no association with postnatal exposure through nursing. Decreased weight and poorer short-term memory at 4 years of age was also associated with cord but not concurrent PCB levels (48–49). These measures were not associated with concurrent blood levels of PCBs, polybrominated biphenyls, lead, or dichlorodiphenyltrichloroethane (DDT). Hypoactivity was associated with concurrent blood PCB levels (49).

A prospective cohort of breast-fed infants was followed for 60 months in a North Carolina study (51–54). Mothers had no known excessive exposure to PCBs. Higher in utero PCB exposure, as assessed by maternal milk fat PCBs, was associated with hypotonicity and hyporeflexia; there was no association with birth weight or head circumference (54). Higher transplacental but not postnatal PCB exposure was associated with lower scores on the Bayley Scale of Intelligence at 6 and 12 months of age (51). There was no association between either prenatal or concurrent PCB levels and outcome on intelligence tests at 3 to 5 years.

Numerous developmental studies in both rodents and monkeys have demonstrated neurotoxicity as a result of PCB exposure. A recent review (55) summarized changes in activity levels, impaired neurodevelopmental, and impairment on simple learning tasks in rodents whose dams were exposed to various commercial PCB mixtures, often with dosage regimens that did not produce increased mortality or decreased weight in the pups. Dams were apparently unaffected. A series of studies in rhesus monkeys was performed at the University of Wisconsin. Maternal exposure to Aroclor 1016 at doses approximating 0.007 or 0.028 mg/kg/day (56,57) beginning prior to breeding and continuing until infants were weaned at 4 months of age resulted in hyperpigmentation in infants in both dose groups and decreased weight in the high-dose group (57). Impairment on a learning and memory task was also observed in the high-dose group during infancy. Further testing of cognitive function when these monkeys were juveniles revealed no impairment (56,57). In studies with Aroclor 1248, female monkeys were exposed to 0 or 1.0 ppm PCBs in the diet 3 days a week, or 0.5 ppm in the feed daily beginning prior to breeding and continuing until offspring were weaned at 4 months of age. Additional groups of females exposed to 2.5 ppm produced a number of sets of offspring: concurrent with exposure or in which exposure in the mothers ceased 1.0, 1.5, or 3.0 years prior to breeding. Concurrent exposure to 2.5 ppm resulted in reduced birth weight (58) and deficits in discrimination reversal learning (59). These monkeys were hyperactive when young (59) and hypoactive at
44 months of age (60). Monkeys exposed concurrently to 0.5 ppm were hyperactive at 12 months of age (61). The group born to mothers 1.0 year after cessation of exposure to 2.5 ppm showed facilitated performance on a shape discrimination-reversal task (56), which the authors interpreted as a deficit in the treated group’s ability to learn the irrelevance of the shape cue on a previous task. The performance of the 1.0 ppm group was not impaired on this task. Monkeys born to mothers 1.5 or 3.0 years after cessation of exposure to 2.5 ppm PCBs were impaired on a spatial alternation task at 4 to 6 years of age (57). The mothers did not exhibit signs of neurotoxicity.

There is excellent correspondence between the effects of developmental PCB exposure in the monkey and that observed in humans, including learning deficits, changes in activity, and hyperpigmentation. The rodent also provides a good model for effects in humans, with changes in activity levels and learning deficits observed as a result of perinatal exposure. It is not possible to determine whether behavioral processes underlying observed deficits are the same in animal models compared to humans because the issue has not been addressed.

### Predicted Ability of Standard Neurotoxicity Tests to Detect Neurotoxicity

There is a question central to the theme of this review: Knowing what we know today, if another agent that has effects similar to those of these model neurotoxicants was submitted to a governmental agency for marketing, would its neurotoxic potential be detected? Can we feel secure that the arsenal of methodology available to the experimental behavior toxicologist today would detect these agents as neurotoxicants? How well would these methodologies predict effects observed in humans? Would the methods generally required by regulatory agencies detect these agents as neurotoxic, or would their neurotoxic potential be missed? One of the difficulties in attempting to answer these questions is that different testing strategies are required by different governments, different agencies within the same government, or for different classes of agents depending upon chemical structure or intended use. In some instances, observation of adult animals would provide the only opportunity to detect neurotoxicity. Under other protocols, reproductive studies are required. For the purposes of the present exercise, protocols requiring acute exposure in adults, longer term exposure (e.g., 28 days) in adults, or reproductive/developmental exposure were considered. It was assumed that rats or mice would be the experimental model, and effects reported in both species were considered. For each type of protocol, an attempt was made to determine whether effects have been observed on end points included in the functional observation battery (FOB), motor activity, simple tests of learning/memory, or schedule-controlled operant behavior (SCOB). These tests have been recommended by various agencies as appropriate under various conditions. The results of this analysis are presented in Table 1.

One conclusion that may be drawn from this analysis is that all of the agents would have been identified as neurotoxic at some dosage level in some exposure protocol. It is probable that neither lead nor PCBs would have been identified as neurotoxicants following short-term adult exposure, while methylmercury would be identified on the basis of motor deficits. On the other hand, lead, PCBs, and methylmercury would have been identified as developmental neurotoxicants at high doses on the basis of screening procedures such as the FOB, although the effects of these agents on other measures at lower doses is a little less clear.

Behavioral analysis in rats exposed developmentally to lead has reliably

### Table 1. Predicted ability of standard tests to detect neurotoxicity of model agents.

| Adult acute | Lead | Methymercury | PCBs | Solvents |
|-------------|------|--------------|------|----------|
| FOB         | No motor effect (62) | No neurological signs (60,61) | None | Yes* |
| Locomotor activity | Motor impairment (63) | | | |
| Simple learning/SCOB | (3 days injection) | | | |
| Adult longer-term (e.g., 28 days) | FOB | No effect after 15 weeks (38) | Landing foot splay, weakness, irritability (36,66,68–70) | None | Yes |
| Locomotor activity | No effect until 6 weeks (38) | None, rat (38) | None | Yes* |
| Simple learning/SCOB | None (9,63) | Yes, mouse (69) | CAR, rat, effect after 9 weeks (38) | None | |
| Developmental (reproductive) | FOB | High dose, paraplegia, tremors (64) | Cerebral palsy, spasticity, seizures, at high doses (29,40,72) | Neurological signs, impaired incline screen, spinning (53)* | Yes/no* |
| Locomotor activity | Both increase and decrease (64,65) | Negative (39–41) | Positive (based on 6 labs) (73) | Increase or decrease (53)* | |
| Simple learning/SCOB | Numeroseffects (9,64) | Effect on water maze, avoidance (29,73) | No effect on learning, memory (39,74) | Avoidance, water maze, learning, memory (53,74,75) | |

Abbreviations: FOB, functional observation battery; SCOB, schedule-controlled operant behavior; CAR, conditioned avoidance response; FI, fixed interval; RAM, radial arm maze.

*Review article. *No mention of neurotoxicity in numerous studies. *Expected at high doses as a result of narcotic effects. *Expected as a result of known effects. *Expected effect, depending on agent.
revealed deficits in learning and performance at blood lead levels that are environmentally relevant (8,10,14). Such effects were detected in some instances on complex tasks using very sophisticated data analysis. However, lead-induced changes have also been detected using intermittent schedules of reinforcement. Such behavioral methodology is a standard part of the arsenal of behavioral tests available to the behavioral pharmacologist and toxicologist. It must be pointed out that these are not screening procedures, however; they require automated equipment and optimally a computer to control the experiment and collect data on line. If simple tests of learning that do not necessarily require automated equipment are considered, lead-induced deficits were revealed in general adult humans results only, even at high doses. At very high doses, prenatal exposure resulted in overt signs of toxicity in the pups including nervous system lesions and paralysis. It is clear, then, that high-dose effects in rodents mimic those observed following high-dose developmental exposure in humans. It is significant, however, that the intraperitoneal route of exposure in general resulted in negative results (9). It was necessary for the exposure route to be the same as that of human exposure, i.e., oral. It is also important to point out that, while effects on such screening tests as locomotor activity were sometimes (but not always) positive, results were inconsistent, with both increased and decreased locomotion observed. It might be tempting to conclude in such circumstances, if the agent in question were not already known to be neurotoxic, that such effects did not reflect neurotoxicity.

It is clear that perinatal exposure to methylmercury at high doses produces overt neurological effects that would be detected on the FOB. Methylmercury generally produces decreased locomotor activity in pups whose dams were exposed to high doses, while more recent studies at lower doses have been negative. The effects of methylmercury on simple tests of learning are equivocal, with both positive and negative results reported (22). Methylmercury reliably increased auditory startle in a collaborative study of six laboratories, with inconsistent effect on a discrimination task (73). No effects of developmental methylmercury exposure were observed on a battery developed at the U.S. Environmental Protection Agency (U.S. EPA). This battery included T-maze alternation, auditory startle, and olfactory discrimination (39).

In a European collaborative study, there were no effects on accuracy on visual discrimination reversal and spatial delayed alternation tasks, although changes in auditory startle were observed (74). Changes in latency to respond and failure to respond were observed, which would not necessarily be interpreted as cognitive deficits.

Developmental exposure to PCBs produces neurotoxic effects in rodents with good reliability. High doses result in neurological signs and impaired motor development, which would presumably be detected on an FOB. PCB exposure also produces changes in locomotor activity, although both increased and decreased activity have been observed. High doses also result in impairment on simple learning tasks such as active avoidance and water-maze performance. Effects have been observed on SCOB (76) and delayed alternation performance (77) but not on radial arm-maze performance (77). It seems clear that PCBs would have been identified as neurotoxic to the developing organism based on studies in rodents.

The ability of adult exposure paradigms to detect neurotoxicity in these three agents is less clear. Industrial exposure to lead in adult humans results in motor effects, as well as in psychiatric and cognitive disturbances following long-term exposure. It is generally recognized that the adult rodent is extremely resistant to lead-induced neurotoxicity (62). Administration of very high doses for a number of days may produce hind-limb weakness while repeated exposure to lower doses produced no effect on various measures included in the FOB. A 15-week exposure to high doses resulted in decreased body weight and motor activity, with no effect on body temperature, grip strength, negative geotaxis, startle, or conditioned avoidance response (CAR) (38). Effects became apparent 6 weeks after exposure started, so they would not have been detected in a 28-day study. Attempts to demonstrate impaired learning as a result of adult exposure have been largely negative (9). Other effects such as nephrotoxicity are apparent at doses lower than those needed to produce overt neurological signs. It is therefore unlikely that lead would have been regulated as a neurotoxicant on the basis of tests in adult rodents.

Methylmercury reliably produces gross neurological signs and changes in other measures of the FOB following repeated exposure in adult rodents; however, results after a single administration of methylmercury have been negative. Locomotor activity has been found to be affected in one study (69). There has been little research on the effects of methylmercury on learning tasks in the adult rodent, but effects that have been observed may be attributed to sensory or motor impairment (38).

For PCBs, little or no research has specifically addressed the issue of neurotoxicity as a result of exposure in adult animals. However, it is clear that other organ systems are more sensitive to PCB toxicity than the nervous system. The effects of PCBs in adult rodents at high doses include changes in body weight and impairment of liver, kidney, and immune function. Perusal of dozens of papers revealed no mention of overt neurotoxic effects. It is also clear that reproductive and developmental neurotoxicity are produced at doses that do not result in any overt toxicity in the mothers. It seems reasonable to assume, then, that if only adult exposure was used to assess PCB toxicity, the nervous system would not have been identified as a target.

The recognition that solvents represented a hazard at levels that did not produce narcosis arose from long-term industrial exposure. Therefore animal research focused on effects in adult animals. Screening tests would certainly detect the fact that solvents produce narcosis, and results of tests of motor activity or learning would be confounded by this effect, particularly at high doses. Little research has been performed on the effect of solvents on development.

**Congruence of Exposure Levels at Which Neurotoxicity Is Observed in Humans and Animals**

An additional issue worth addressing is the correspondence between the dose levels at which neurotoxicity has been observed in animals and the estimated intakes that produce neurotoxic effects in humans for these model agents. The protection provided by the ways in which animal data are used in the risk assessment process to protect human health may also be scrutinized. For this exercise, the rules by which reference doses (RfDs) are derived by the U.S. EPA will be used, since these or similar procedures are currently used by other agencies as well. The central question is this: From the results of the FOB, locomotor activity, and effects on simple learning tests or performance on SCOB, would the derived RfDs protect against neurotoxicity
LESSONS FROM MODEL COMPOUNDS

Table 2. Comparison of doses required to produce neurotoxicity in humans and rodents and calculated RfDs (mg/kg/day).

| Compounds | Exposure     | NOAEL          | RfD         | NOAEL    | RfD      |
|-----------|--------------|----------------|-------------|----------|----------|
| Lead      | Adult        | Occupational exposure | ?          | 5.2      | 5.2 x 10<sup>-2</sup> |
|           | Developmental| 5 x 10<sup>-3a</sup> | 5 x 10<sup>-4</sup>-1 x 10<sup>-3</sup> | 10(NOAE) - >100 | 10<sup>-2</sup>-1          |
| Methylmercury | Adult    | 3 x 10<sup>-3</sup> | 3 x 10<sup>-4</sup> | 0.7 - 1.6 | 7 x 10<sup>-3</sup>-16 x 10<sup>-2</sup> |
|           | Developmental| 7 x 10<sup>-4</sup>-1.2 x 10<sup>-3</sup> | 7 x 10<sup>-5</sup>-1.2 x 10<sup>-4</sup> | 5 x 10<sup>-3</sup>-2 x 10<sup>-2</sup> |
| PCBs      | Adult        | ?              |             | No effect |          |
|           | Developmental| 10<sup>-5</sup> | 10<sup>-6</sup> | 0.2 - 5 | 2 x 10<sup>-3</sup>-10<sup>-2</sup> |

*Based on estimated 50th percentiles for intake from food by children 2 years of age. Data from Beloian (78).

in humans? The conclusions are summarized in Table 2.

Lead produces neurotoxicity in adult rodents after repeated but not acute exposure, but only at doses that produce mortality or significant weight loss. The lowest observed adverse effect level (LOAEL) for decreased motor activity was 7.5 mg/kg, with a no observed adverse effect level (NOAEL) of 5.2 mg/kg (38). In general, learning has been found to be unaffected by lead exposure in adult rodents (9). The intake necessary to produce neurotoxic effects in adult humans has not been quantified. More information is available on the effects of developmental exposure to lead. The studies in which behavioral effects were detected at the lowest dose in rodents were performed by Cory-Slechta and colleagues using a post-weaning exposure paradigm (8). Neurotoxicity was detected at a dose of approximately 1 mg/kg (10), three orders of magnitude higher than the average lead intake by children, although assessment was not performed at lower doses in the rat studies. In those studies, effects were demonstrated in performance on schedules of reinforcement and complex learned behavior using sophisticated methodology. If a factor of 1000 is considered to be an appropriate safety factor—the procedure used for agents for which there is a LOAEL but not a NOAEL from animal data—the RfDs based on the animal data are in good agreement with estimates of human intake. Most studies, however, particularly earlier ones, detected neurotoxicity at much higher doses; in some studies doses over 100 mg/kg yielded negative results (9). RfDs based on these simple learning tests would yield RfDs greater than 1 mg/kg, which clearly greatly underestimates the toxicity of lead to the developing organism.

For methylmercury, the present U.S. EPA RfD of 0.3 μg/kg/day is based on paresthesias in adults. In a 15-week study in rats (38), 1.4 mg/kg/day produced effects on motor function after 5 months of exposure while 0.7 mg/kg/day produced no effect. A safety factor of 100 would yield an allowable intake of 7 μg/kg/day. Landing foot splay in the mouse was affected after exposure to 2.7 mg/kg/day for less than 28 days, while 1.6 mg/kg/day produced impairment after more than 60 days (36). An RfD based on a 28-day exposure would be 16 μg/kg/day. RfDs generated from these two studies are one to two orders of magnitude above the RfD based on adult human data. With respect to the developmental effects of methylmercury exposure, Stern (79) derived a reference dose of 0.07 μg/kg/day based on developmental neurotoxicity in humans, while the Agency for Toxic Substances and Disease Registry has determined a minimum risk level of 0.12 μg/kg/day based on the same data (80). It must be reiterated that the developmental data at this point consist of relatively crude end points, unlike the very extensive body of data on subtle behavioral deficits that exists for lead. Most rodent studies reported NOAELs between 50 and >2000 μg/kg/day depending on the study and end point examined (22). Tests that would typically be used in an assessment battery yielded values at the high end of this range. For example, in an interlaboratory study involving six laboratories (73), effects were observed at 6 mg/kg administered on gestation days (GD) 6 to 9 while effects were minimal or absent at 2 mg/kg on simple tests of activity and learning. In another study in which rats were dosed from GD 6 to 15, no effect was observed at 1 or 2 mg/kg using a testing battery developed at the U.S. EPA, including several tests of learning and activity (39). RfDs generated from these two studies would be 10<sup>-2</sup> mg/kg/day or higher, two to three orders of magnitude greater than the RfD calculated from the human data. In contrast, a study in which rats were required to perform on a differential reinforcement of high rate (DRH) schedule, which required the animal to emit a specific number of responses within a specified time, detected effects at a much lower level than other studies (81). A dose of 10 μg/kg during GD 6 to 9 produced effects, with a NOAEL of 5 μg/kg. With a safety factor of 100, the allowable intake would be 0.05 μg/kg/day, which is in very good agreement with estimates based on the human data. It is highly unlikely that an allowable intake for methylmercury in humans would have been based on a single apparently anomalous rodent study, however. The experimental design of most of these studies in which dams were dosed for only several days during pregnancy also presents problems in extrapolating the rodent data to humans.

While perinatal exposure to PCBs reliably produces a variety of behavioral effects in rodents, the doses at which these effects have been identified are considerably higher than those at which untoward effects apparently occur in humans. In their review, Tilson et al. (55) calculated RfDs based on rodent, monkey, and human data. RfDs from the human data were calculated to be approximately 10<sup>-5</sup>-10<sup>-6</sup> mg/kg/day based on behavioral data, using a safety factor of 10 below the estimated NOAEL. RfDs from the monkey data, based on motor activity and impairment on learning and memory tasks, were in the range of 10<sup>-5</sup>-mg/kg/day, which is in agreement with the human estimates. RfDs from most studies of developmental toxicity based on the rodent data were approximately 10<sup>-2</sup> mg/kg/day; the most sensitive indicator was motor activity, which yielded an RfD of 10<sup>-3</sup> mg/kg/day based on a NOAEL of 0.2 mg/kg/day and dividing by a safety factor of 100. This is three orders of magnitude higher than the estimates of intake that would protect against developmental neurotoxicity in humans. While the assumptions used to calculate the human RfDs may have resulted in an underestimation of the dose required to produce effects, it is doubtful that adjustments in the calculations would result in a change in the RfD by three orders of magnitude. Moreover, the calculations based on monkey data, which
are not subject to the same uncertainties in intake estimates, are in close agreement with the estimates based on the epidemiological studies. It therefore appears that the rodent data underestimate the intake that would protect against neurotoxicity in humans by about three orders of magnitude, based on currently accepted practices of estimating human risk from animal data.

An alternative strategy would be to base allowable intakes on body burden rather than dose. This would have the advantage of at least partially circumventing differences in toxicokinetics between humans and animal models. The most relevant comparison would be levels in target organ tissues, i.e., the nervous system. For example, in their review Burbacher et al. (29) point out that there is good congruence between signs of methylmercury toxicity between small mammals, primates, and humans based on brain mercury levels. Of course, such comparative data are not available for most agents. A readily accessible compartment is the blood compartment. For lead, clear effects in rats have been observed in experiments by Cory-Slechta and colleagues at blood lead levels of approximately 20 μg/dl (14); lower levels have not been studied. A recent meta-analysis of the epidemiological literature showed no evidence of a threshold for cognitive deficits produced by lead down to blood lead concentrations of 1 μg/dl (7). If one safety factor of 10 were eliminated for interspecies extrapolation because a measure of body burden was being compared directly between rats and humans, the allowable blood lead concentration based on these rodent data would be 0.2 μg/dl, which is in good agreement with the epidemiological conclusions. Blood levels associated with neurotoxicity are unknown for either methylmercury or PCBs in the rodent. However, methylmercury blood levels in the rat would undoubtedly be extremely misleading, given the huge difference in blood:brain ratios between rat and human. In addition, most current government regulatory protocols do not require measures of body burden; therefore, such data would be unavailable from animal studies. Moreover, if the agent being tested were a new chemical to which humans had not been exposed, relevant data from humans and particularly from children would also be lacking. The strategy of basing allowable intakes in humans on comparison of body burden between human and animal models is therefore not at all practical for new agents.

Conclusions
There are a number of conclusions that may be drawn from the exploration of effects in these model compounds. First, it is clear that the degree to which the effects of assessment of specific functions in animals predict effects in humans depends on both the agent and the developmental period at which exposure occurs. Developmental lead exposure in humans is characterized by deficits in IQ and other problems of cognitive functioning. Cognitive deficits have been reliably demonstrated in animal models including rodents and primates. In fact, the experimental and epidemiological literatures show remarkable congruence with regard to the behavioral processes underlying these deficits. The ability of effects in adult animals to predict effects produced by lead in humans is disappointing. It is difficult that the peripheral neuropathy and changes in cognitive function produced by occupational exposure would be predicted on the basis of rodent studies. While chronic lead exposure may produce changes in locomotor activity in rodents, studies of neurological function and learning have been almost universally negative. Methylmercury produces severe neurological impairment in humans as a result of adult exposure; these effects are replicable in animal models only after repeated exposure. One of the hallmarks of methylmercury poisoning in adults is constriction of visual fields and other visual effects. This probably would not have been predicted by rodent tests because of differences in the pattern of pathological damage between human and rodent brain and because of important differences in the visual systems between humans and rodents. Such effects have been demonstrated in monkeys exposed as adults, however. Developmental exposure to methylmercury in humans results in neurologic impairment at high levels of exposure and developmental delay and possibly cognitive impairment at lower exposure levels. Developmental exposure in animals clearly replicates the neurological dysfunction observed in human infants. On the other hand, results of tests of cognitive function in both rodents and monkeys is conflicting (22). Developmental PCB exposure produces behavioral delays or cognitive deficits in children. In good agreement with these findings, deficits on tests of learning and changes in activity have been demonstrated as a result of PCB exposure in both rodents and monkeys. Adult monkeys chronically exposed to PCBs develop the classic signs of PCB toxicity in humans, including chloracne, hair loss, and swelling of the eyelids (82,83); however, whether PCB exposure in adult monkeys also produces the paresthesias and weakness reported in humans has not been addressed. Neurotoxicity has not been reported in adult rodents as a result of PCB exposure.

The ability of animal studies to predict intake levels at which human health would be protected is less encouraging. It is clear from comparison of the human and rodent data that results from rodent studies often vastly underestimated intakes at which neurotoxicity was observed in humans. For PCBs, the difference in the estimated acceptable intake between human and rodent developmental data is 3 to 4 orders of magnitude, while for methylmercury the difference is two orders of magnitude or greater for most studies. For lead, deficits were revealed on activity and simple learning tests at doses that would also result in allowable intakes much higher than those at which cognitive impairment has been demonstrated in children. However, data from one laboratory using sophisticated analyses of behavior on operant schedules of reinforcement detected impairment at levels that would result in derivation of an RfD that would clearly indicate that human health was not protected at intake levels of many children in industrial societies. One conclusion that may be drawn from this analysis is that current methods of calculating acceptable intakes based on animal data, exemplified for the sake of discussion by current practices in the United States, are insufficient to protect the human population against behavioral toxicity. It may be argued that agents such as lead, methylmercury, and PCBs represent worst case scenarios because these agents have been released into the environment in huge quantities, are not degraded environmentally, accumulate in the food chain, and/or have very long biological half-lives in humans. Therefore the degree to which the lessons from these potent neurotoxicants may be extrapolated to other agents needs to be interpreted with some caution. However, the clarification of these issues for new agents would require extensive biological and environmental testing. It seems unlikely that such issues would be satisfactorily resolved for proposed new chemicals before their approval and use. (For example, it was argued that lead would not accumulate in the environment when industry proposed adding lead to gasoline.)
It might also be suggested that sophisticated behavioral testing, including developmental testing, be required for all chemicals suspected of producing neurotoxicity. A tiered approach has been suggested by a number of national and international agencies whereby detection of neurotoxicity at high doses would trigger assessment at lower doses using more sophisticated methodology. While such a strategy would undoubtedly aid in the characterization of effects as well as result in detection of neurotoxicity at lower doses, it is unlikely that this strategy would provide sufficient protection in all cases. For example, numerous reproductive studies using reasonable end points generated NOAELs as much as five orders of magnitude above those estimated from human data (Table 2). It is doubtful that any test done in rodents, no matter how sophisticated, would lower the dose at which impairment was detected by such an amount.

In conclusion, neurotoxicity would have been detected for all of these model agents only if both developmental and adult assessments had been performed. In addition, doses at which effects were observed in rodents were often several orders of magnitude higher than those at which effects were actually observed in humans. Whether these agents would have been approved for use would ultimately depend on decisions made subsequent to the detection of the fact that these agents were neurotoxic.

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