# Chapter 6

## Known and Suspected Developmental Neurotoxicants

A large number of chemical compounds interfere with normal brain development, including heavy metals, alcohol and other solvents, nicotine, opiates, cocaine, marijuana, some pharmaceuticals, pesticides, and others. As described in Chapter 2, neurodevelopmental toxicants may alter brain development and function in specific and permanent ways. A few have been extensively studied (e.g. lead, mercury, alcohol), while most others have undergone minimal examination.

The following profiles summarize what is known about the neurodevelopmental toxicity of some commonly encountered solvents, pesticides, nicotine, metals, and persistent organochlorine compounds. We also briefly discuss important controversies over the potential neurodevelopmental toxicity of compounds that are intentionally added to drinking water and food – fluoride and certain food additives.

Experimental toxicity testing usually involves examining one chemical at a time. Although this approach provides important information, it fails to inform us about the neurodevelopmental effects of exposures to mixtures of many different compounds. Every human body contains mixtures of heavy metals and synthetic organic chemicals in blood, bone and other organs, fat, breast milk, sperm and expired air. Epidemiological research is complicated by the fact that there are no unexposed people to serve as controls for comparison purposes. These limitations should be kept in mind when reading the following toxicity profiles.

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Finally, although the references cited do not exhaustively review the available literature, they are representative and include areas of uncertainty and controversy. Importantly, many chemical compounds with known or suspected neurological toxicity have never been tested for their effects on brain development and function. For them, there are no data to review.

## METALS

### Lead

- Increases in blood lead levels during infancy and childhood are associated with attention deficits, increased impulsiveness, reduced school performance, aggression, and delinquent behavior.

- Effects on learning are seen at blood lead levels below those currently considered "safe."

### Routes of Exposure

Since lead was removed from most of the nation's gasoline supply, most current environmental exposures in the US come from lead paint, lead contaminated dust, and drinking water. Occupational and hobby exposures also contribute to the lead levels of some adults. Lead tends to be stored in bones, and during pregnancy, accelerated maternal bone turnover results in mobilization of lead, leading to increased blood lead levels.

### Human Studies

Lead easily crosses the placenta and enters the fetal brain where it interferes with normal development. Many studies report adverse neuro-developmental impacts resulting from fetal or infant exposures to lead, including lowered intelligence, hyperactivity, learning and attention disorders, and changes in behavior. (for example, see [1][2][3]) Here we summarize results from several of the larger epidemiological studies, omitting most of a large body of animal research because of the relative wealth of human data.

In the 1940s, the consequences of lead poisoning, including poor school performance, impulsive behavior, short attention span, and restlessness, were reported.[4] Since then neurodevelopmental damage at lower levels of exposure has been well documented. In fact, there is no evidence of any threshold for lead-induced cognitive impairment resulting from early life exposures.[5]

In one of the earliest studies of lead effects on intelligence, investigators reported a 4-point difference in IQ, measured by the Wechsler Intelligence Scales for Children – Revised (WISC-R), between children with the highest and lowest deciduous tooth-lead levels.[6] Other studies have reached similar conclusions. In Boston, a cohort of children from middle and upper middle class homes has been followed for years.[7][8] Reduced performance on the Bayley Mental Development Index (MDI) was associated with elevated umbilical cord blood lead levels. The difference in scores between the high (mean, 14.6 microgm/dl) and low (mean, 1.8 microgm/dl) blood lead levels was 4-7 points at 6, 12, and 24 months of age. When the children were re-tested at 10 years of age, a 10 microgm/dl increase in blood lead at 24 months of age was associated with a 5.8-point decline in IQ as measured by WISC-R. Other studies show similar results.[9]

In the Boston cohort, teachers reported behavioral changes in children that correlated with lead levels. Children with the higher levels were more distractible, dependent, impulsive, easily frustrated, not persistent, and unable to follow directions. Attention-deficit

disorder also correlates with hair lead levels.[10] Increased blood level in infancy and early childhood may be manifest in older children and adolescents as decreased attention span, reading disabilities, and failure to graduate from high school[11] Two studies report that lead exposure correlates with aggressive, destructive, and delinquent behavior.[12] [13]

### Animal Studies

Animal studies support these conclusions from epidemiological data. Monkeys exposed to lead from birth, so that blood lead levels are maintained at about 15 microgms/dl, show increased distractibility , inappr opriate r esponses to stimuli, and difficulty adjusting response strategies. [14] A review of animal studies reports deficits in performance, learning, and attention associated with low-level lead exposures. [15]

### Mechanisms of Neurotoxicity

Several neurodevelopmental processes are altered by lead exposure, leading to abnormal brain development. Intrauterine neurodevelopmental effects of lead affect both the cellular structure of the brain and its chemistry.[16] Structural effects include altered cell proliferation, differentiation, synapse formation, and programmed cell death. Neurochemical effects include altered neurotransmitter levels (acetylcholine, dopamine, glutamate) and altered dopamine receptor density in various parts of the brain.[17] Lead is also a potent inhibitor of the NMDA (glutamate) receptor. The fetal brain may be particularly sensitive not only because

unique organizational processes are underway but also because of an immature blood-brain barrier. One study found greater uptake of lead in fetal brain during gestation than after birth in rats.[18]

## Mercury

- Freshwater fish are sufficiently contaminated with methylmercury in most of the US to necessitate fish consumption advisories warning pregnant women or women of reproductive age to avoid or limit consumption because of threats to fetal brain development

- Large fetal exposures to methylmercury cause mental retardation, gait and visual disturbances

- Smaller fetal exposures may cause lasting impairment of language, attention, and memory

- Fetal mercury and PCB exposures interact to result in magnified effects on neurological development

### Routes of Exposure

Mercury (Hg) may exist in a number of different chemical forms but is usually released into the environment as a metal or an inor ganic compound. The US EP A estimates that human activities are responsible for emissions of approximately 160 tons of mercury annually in the US.[19] Major sources are coal-fired power plants and municipal and medical waste inciner-ators. Atmospheric mercury often travels long distances before being deposited onto the ear th's sur face. Mer cury in sediments and water bodies is converted by bacteria into methylmer cury, which

## How Much Mercury In My Tuna Sandwich?

The EPA sets "safe" reference doses for the chemicals we are exposed to through our air, water, and food. Yet it is difficult to translate those levels, expressed in micrograms and parts per million, into information that is meaningful for our daily lives. For instance, how can I determine how much mercury I am exposed to each time I eat a tuna sandwich? Some basic information on equivalencies and abbreviations will help you do the math so you can determine how much of a chemical you may be exposed to.

*Spotlight*

The first step in determining exposure is converting the various measures into equivalent units. In the United States we often express our body weight in pounds or the amount of food we eat in ounces. Environmental concentrations and exposures, however, are usually calculated using metric units (grams, kilograms). Note the following equivalencies:

- 1 kilogram (kg) = 2.2 pounds (lb)
- 1 pound = 16 ounces = 454 grams
- 1 ounce = 28 grams (gm)

Because we are often concerned about exposures to very small quantities of chemicals, it is helpful to know the following units of measure that represent tiny subdivisions of the gram (gm):

- Milligram (mg) = 1/1000 gm (thousandth)
- Microgram (ug or microgm) = 1/1,000,000 gm (millionth)
- Nanogram (ng) = 1,000,000,000 gm (billionth)
- Picogram (pg) = 1,000,000,000,000 gm (trillionth)

For example, there are 1,000 milligrams in 1 gram, or 1 million micrograms in that same gram.

We are generally exposed to chemicals that are contained within another medium such as air, water or food. In order to calculate exposure we must first calculate the **concentration**, or the amount of the chemical that is contained in the water we drink or the food we eat. For example, if 1 gram of fish contains, on average, 1 microgram (ug) of mercury, we would express the concentration as 1microgm/gm. Since there are a million micrograms in a gram, another way to express this concentration is 1 part per million, or 1 ppm. The following chart outlines the equivalencies:

- Gm/kg = mg/g = parts per thousand = ppthousand (1/1000)
- Mg/kg = microgm/g = parts per million = ppm (1/1,000,000)
- Microgram/kg = ng/gm = parts per billion = ppb (1/1,000,000,000)
- Ng/kg = picogm/gm = parts per trillion = ppt (1/1,000,000,000,000)

Since we have determined the concentration of mercury in the tuna fish, we can determine how much mercury an individual is exposed to when eating the fish. With a few basic calculations, we can calculate the mercury exposure of a woman who consumes 7 ounces of tuna per week, given an average tuna mercury level of 0.2 ppm (Assume she does not eat any other fish or shellfish, or have any other significant exposures to mercury).

*How can I determine how much mercury I am exposed to each time I eat a tuna sandwich?*

- First we convert the ounces into metric units:

  7 oz = 196 gms fish

- Then we multiply the amount of fish consumed/week with the concentration of mercury in the fish to determine the mercury exposure  per week:

  196 gms fish/week  x 0.2 microgm mercury /gm fish = 39.2 microgm mercury/week

  How much mercury is that per day?

- Divide by 7, since there are 7 days in a week:

  39.2 microgm of mercury /week = 5.6 microgm of mercury/day = daily mercury exposure

Typically we standardize exposures by dividing the total exposure by the body mass. Expressing exposure on a "per kilogram" basis allows us to compare exposures among individuals of different sizes. If we assume the woman eating the sandwich is of average weight, (132 pounds, or 60 kg), we divide the total exposure by 60 kilograms:



   5.6 microgm/60 kg of mercury/day = 0.093 microgm/kg

We have determined that the mercury exposure of a 132 lb woman (60 kg) eating 7 ounces (196 grams) of tuna per week is 0.093 microgms/kg/day.  This level of exposure is just at the limit of EPA's "safe" reference dose of 0.1 microgrm/kg/day.
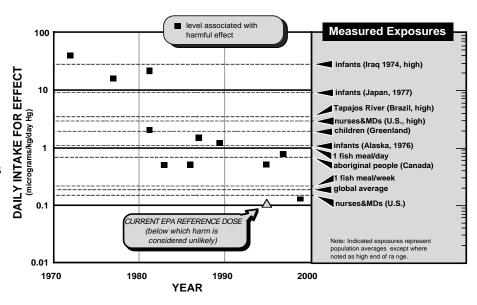
This calculation is based on the assumption that the woman weighs 132 lbs. What would the mercury exposure be if a 50 lb child consumed the same amount of tuna over the course of a week?  The child would be exposed to approximately 0.243 microgms/kg/day of mercury.

# Mercury:  Inadequate Margin of Safety

Mercury exposures associated with harmful effects have been recognized at progressively lower levels over the past several decades as research meth ods have improved.  EPA's current advised safe exposure limit, represented by the triangle, is exceeded by many groups. It is also exceeded by the global average mercury exposure. This average, based on a report of 559 hair samples from 32 locations around the world, reflects a cumulative average of levels of fish consumption, and degrees of fish contamination.[1][2][3][4][5][6][7][8][9][10][11] (All indicated exposures were assessed as hair or blood mercury levels, except US nurses and physicians, whose exposures were estimated by dietary survey.)

**Measured Exposures**

- infants (Iraq 1974, high)
- infants (Japan, 1977)
- Tapajos River (Brazil, high)
- nurses&MDs (U.S., high)
- children (Greenland)
- infants (Alaska, 1976)
- 1 fish meal/day
- aboriginal people (Canada)
- 1 fish meal/week
- global average
- nurses&MDs (U.S.)

Note: Indicated exposures represent population averages  except where noted as high end of ra nge.

DAILY INTAKE FOR EFFECT (micrograms/kg/day Hg)

■ level associated with harmful effect

CURRENT EPA REFERENCE DOSE (below which harm is considered unlikely)

YEAR

bioaccumulates as it passes up the food chain. As a result, fish consumed by pregnant women or women of reproductive age may be contaminated with methylmercury at levels that pose a threat to the uniquely vulnerable developing brain of the fetus. Forty states have issued fish advisories warning women of reproductive age to limit or avoid consuming fresh water fish because of mercury contamination. Large predator ocean fish, like swordfish and some tuna, may also be sufficiently contaminated to pose a risk, particularly when eaten regularly . According to EP A estimates, 1.16 million women of childbearing years eat sufficient amounts of mercury-contaminated fish to pose a risk of harm to their future children.

## Animal Studies

Studies in animals confirm the developmental neurotoxicity of organic mer cury. Four-month-old rats, exposed to 0.008 mg Hg/kg/day on gestational days 6-9 show significantly impaired

behavioral performance, as tested by rewarding for total lever presses.     [20] Fifty-60-day-old monkeys born to mothers that received 0.04 or 0.06 mg methylHg/kg/day for an average of 168 or 747 days prior to mating show impaired visual recognition memor y.[21] Autopsy studies in developmentally exposed animals show smaller brain sizes, dilated ventricles, and distorted cellular architecture.

## Human Studies

The devastating effects of methylmercury on the developing human brain after excessive exposure were tragically demonstrated in large-scale poisonings. In Minamata Bay   , Japan, during the 1950' s, residents r egularly consumed fish contaminated with methylmer cury from an industrial plant' s ef fluent in the bay   . Infants bor n in the late 1950's developed characteristic neuro-logical findings including mental retardation, disturbances of gait, speech, sucking, and swallowing, and abnormal reflexes.     [22] Mothers of affected children often showed no sign of mercury poisoning.

Another large scale poisoning occurred in Iraq in the 1970's when residents baked bread with grain intended for planting that had been treated with organic mercury as a fungicide. Unlike Minamata, this represented an acute rather than chronic poisoning. Symptoms were similar in the two circumstances, but visual disturbances in adults were more severe in Iraq with actual blindness in several instances. [23] The critical effect from prenatal exposure to methylmercury was psychomotor retardation with delays in learning to walk and an increased incidence of seizures. Using maternal hair mercury levels as a measure of prenatal exposure, investigators calculated that the lowest observed adverse effect level (LOAEL) for psychomotor retardation occurred when maternal hair levels of mercury were between 10-20 ppm. [24] Maternal hair mercury levels are thought to be a fairly accurate indicator of fetal mercury exposures during pregnancy.

More recently, epidemiological studies conducted in the Seychelle and Faroe Islands have attempted to identify more subtle developmental neurological effects of low-dose methylmercury exposure and to identify a threshold, if one exists, below which there is no discernable toxicity. These study populations were selected because their fish or marine mammal based diets regularly exposed them to low doses of methylmercury, and maternal hair levels of mercury in these populations bracketed the LOAEL identified in the Iraq study.

In the Seychelles, 738 children were followed with sequential detailed neurological testing. Maternal hair levels of mercury averaged 6.8 ppm. At age 2 years, more highly exposed boys scored significantly lower on activity level when tested by the Bayley Infant Behavior Record. [25] Among boys and girls combined, the effect of mercury on activity level was significant only at a maternal hair level greater than 12 ppm. Follow up testing at age 5 years showed no persistent effect of prenatal mercury exposure. [26] Neurological testing at 66 months of age included the McCarthy Scales of Children's Abilities, Preschool Language Scale, Woodcock-Johnson Applied Problems and Letter and Word Recognition Tests of Achievement, the Bender Gestalt Test, and the Child Behavior Checklist.

In the Faroe Islands, 917 newborn/mother pairs were tested at birth for maternal hair and umbilical cord blood mercury levels. Children whose mother's hair mercury levels were 10-20 ppm were compared with those whose hair levels were less than 3 ppm. Early examination of children showed that the most exposed children had subtle changes in the function of portions of the brain associated with hearing and motor skills. As they grew older, some deficits in learning capacity also became apparent. At age 7 years these children underwent extensive neurological testing including the Neurobehavioral Evaluation System (NES) Finger Tapping and Hand-Eye Coordination Test, Tactual Performance Test, NES

*According to EPA estimates, 1.16 million women of childbearing years eat sufficient amounts of mercury-contaminated fish to pose a risk of harm to their future children.*

Continual Performance Test, Wechsler Intelligence Scale for Children – Revised (WISC-R) Digit Spans, WISC-R Similarities, WISC-R Block Designs, Bender Gestalt Test, California Verbal Learning Test, Boston Naming Test, and the Nonverbal Analogue Profile of Mood States.[27] The studies showed a significant correlation between impairment in the areas of language, attention, and memory and prenatal mercury exposure.

Investigators in each study controlled for many potentially confounding factors including socioeconomic status, quality of the home environment, and breast feeding status, among others. The differing results may be explained by several different factors. First, some neurological effects do not become apparent until later in childhood when certain neurological functions begin to develop. This, however, becomes less likely to explain the discrepant findings as the Seychellois children approach age 7 and continue to show no lasting deficits. Second, the testing techniques used in the Faroes may be more sensitive than those used in the Seychelles. The Faroe investigators included examination of some specific areas of neurocognitive performance that are more easily and accurately detected by detailed computer analysis. Third, the exposure pattern is likely to have differed in the two groups. In the Seychelles, fish are contaminated with methylmercury at a relatively low level and mercury exposure is the result of a constant diet of fish. In the Faroe Islands, however, mercury exposure results from intermittent ingestion of pilot whale meat that contains about 10 times the

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mercury concentration of ocean fish. Consequently, it is likely that the Faroese experience intermittent spikes of mercury exposure that are higher than the Seychellois. The neurodevelopmental consequences of these two exposure patterns may differ. Fourth, pilot whale blubber is also contaminated with PCBs and other organochlorine chemicals, which also affect neurological development. Though methylmercury is largely contained in the whale meat, some residents also eat whale blubber, resulting in concomitant PCB exposures. PCB levels were measured in the Faroe Island study, and investigators used analytical statistical techniques to control for co-contaminants as they looked for effects of prenatal mercury exposure. However, some critics believe that the other contaminants may explain at least some of the findings. The Faroe Islands study team strongly disagrees and argues that they successfully controlled for PCB co-contamination.[28] Finally, the Faroe Islands study identified a relationship between neurodevelopment and cord blood levels of mercury, rather than maternal hair. Umbilical cord blood levels may better reflect actual fetal exposures.

Additional studies also show developmental neurotoxicity after oral exposure of humans and non-human primates to low doses of organic mercury.[29][30][31] In a New Zealand study, maternal hair mercury levels of 15 microgms/gm were associated with poorer performance on the Wechsler Intelligence Scale for Children.

Based on the Seychelles study, the Agency for Toxic Substances and Disease Registry (ATSDR) has established a

minimum risk level for oral exposure to methylmercury at 0.5 microgm/kg/day. However, the EPA has set the level at 0.1 microgm/kg/day. Based on dietary surveys, the EPA estimates that about 7% of women in the US of childbearing age consume methylmercury in excess of the "safe" dose.[32] However, among women who eat any fish at all, 50% of those of childbearing age consume excess methylmercury.

The Food and Drug Administration (FDA) established an "action level" for mercury in fish at 1 ppm in 1979. [33] However, the FDA's action level is a non-binding informal guideline, is not legally enforceable, and only serves as discretionary guidance to FDA and to states when deciding when seafood might be adulterated. Fish consumption has increased in the US since 1979, and critics have argued that this action level is not health-protective. Indeed, FDA was quoted in a 1991 General Accounting Office report as stating that the agency failed to consider reproductive and developmental toxicity when establishing the guideline. [34] Also in 1991, the National Academy of Sciences noted that the FDA guideline did not adequately protect sensitive populations, including fetuses, babies and young children.[35] (see Spotlight)

## Mechanisms of Neurotoxicity

Mercury has a high affinity for binding to specific chemical structures (e.g., sulfhydryl groups) on proteins, which is thought to explain many of its biological activities. [36] The result is diffuse alteration of cellular function, inhibition of protein synthesis, and formation of reactive oxygen species, which can damage DNA and disrupt cell division. [37] In the nervous system, mercury interferes with development of microtubules, which are small tubular structures in the neuronal skeleton. [38] Mercury also disrupts cell membrane integrity and alters the chemical characteristics of the surface of cells, making them more likely to adhere to one another . This may explain how cellular migration is affected during brain development. Mercury exposure also disrupts synaptic transmission.

In an in vitro study, methylmercury and polychlorinated biphenyls (PCBs) were reported to interact synergistically, with combined exposures resulting in lowering of dopamine levels in animal brain tissue to a greater degree than would have been predicted by adding the effects observed when the chemicals were used individually.[39] New data, as yet unpublished, from a long term ongoing study of children born to mothers consuming fish from Lake Ontario, show that prenatal PCB and mercury exposures also interacted to reduce performance of 3-year-old children on the McCarthy Scales of Children's Abilities.[40] Mercury exposures in this study were quite low, yet they combined with PCB exposures to increase adverse impacts on neuro-development. Together, these observations raise important questions about the adequacy of fish consumption advisories based on single chemical analyses. Freshwater fish in many areas of the US are contaminated with mercury, PCBs, dioxin, and other toxicants. Risk assessments or advisories that are based on single hazard analyses that define safe fish consumption limits are unlikely to be protective of public health.

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## Manganese

- Unlike many other metals, some manganese is essential as a catalyst in several critically important enzymatic processes

- However, several studies report a relationship between excessive childhood levels of manganese exposure and hyperactivity or learning disabilities

Unlike mercury and lead, which are not required for human health, the metal manganese is essential in trace amounts in order to promote several critical enzymatic reactions. Manganese deficiency may result in abnormalities of connective tissue, cartilage, and bone. In various species, too little dietary manganese causes impaired skeletal development and reproduction, abnormal carbohydrate and lipid metabolism, and movement disorders.

### Routes of Exposure

In non-occupational settings, most manganese exposure comes from food. The National Research Council estimates a safe and adequate daily dietary intake of 2-5 milligrams. The ordinary adult dietary intake ranges from 0.52-5.33 milligrams daily with an average of 3 milligrams.[41] Infant dietary intake of manganese varies dramatically with the source of food. Human breast milk contains about 6 micrograms/liter. Infant formula contains about 77 micrograms Mn/liter if no manganese has been added and about 100 micrograms Mn/liter if it has been supplemented.[42] Soybean plants efficiently extract manganese from soil,

and soy-based infant formula contains 200-300 micrograms Mn/liter.[43] Consequently, formula-fed infants ingest much more manganese than those who are breast-fed.

An organic form of manganese, methylcyclopentadienyl manganese tricarbonyl (MMT), is used in a portion of the nation's gasoline supply as an octane enhancer. When burned, MMT-supplemented gasoline releases several inorganic manganese compounds into the atmosphere, causing small but widespread inhalation exposures, as well as land and water deposition. Animal studies show that inhaled manganese compounds may travel along the olfactory nerve directly into the brain, bypassing the general circulation and the blood-brain barrier.[44] The relevance of this pathway of exposure in humans is uncertain.

In adults, only about 3-5%, or approximately 100 microgms, of ingested manganese is absorbed into the circulation. Much of this is immediately excreted into the bile so that adults retain only about 30 microgms daily.[45] Animal studies show that young animals absorb much more ingested manganese than adults – about 70% in young rats compared to 1-2% in adults.[46] Manganese balance studies in humans also show that infants and young children absorb more and excrete less ingested manganese than adults.[47] Moreover, the blood brain barrier, which keeps many blood-borne chemicals from entering the brains of older children and adults, is immature in infants, allowing proportionately more manganese to gain access to and lodge in the developing brain.

*Formula-fed infants ingest much more manganese than those who are breast-fed.*

*Infants and young children absorb more and excrete less ingested manganese than adults.*

## Animal Studies

Despite being an essential trace element, excessive exposures to manganese can be harmful to the brain, lungs, and reproductive system. Adverse reproductive effects, including testicular toxicity and reduced testosterone levels, occur in animals exposed to manganese during fetal development at levels that show no other toxic effects but that are considerably higher than normal human dietary intake. [48]

The more critical health effect, however, that may occur at much lower levels of exposure, is brain damage. Emerging evidence demonstrates that the brains of fetuses and newborns are more susceptible to the toxic effects of manganese than adults and that developmental exposures may result in unique neurological effects. A review of the published literature on manganese neurotoxicity in rodents identified seven studies in which animals were exposed during development. [49] Three studies investigated behavioral outcomes, and each reported increased activity levels in offspring.

## Human Studies

Respiratory symptoms, pneumonia, or bronchitis occur in workers with large inhalation exposures to manganese. Obvious neurological effects of manganese were first noted in workers in manganese mines, refineries, and smelters. "Manganism" includes tremor and movement disorders, often preceded by "manganese madness," characterized by compulsive running, fighting, and singing. The movement disorder of manganism has some similarity to Parkinsonism, though there are distinct differences.

Several investigators have attempted to detect early signs of neurological damage from manganese exposure in adults. One describes a continuum of dysfunction due to manganese exposure, including behavioral and emotional effects in addition to the well-known movement disorder.[50] Another used behavioral methods to look for early signs of manganese neurotoxicity after low-level exposures and concluded that there are effects on response speed, motor functions, and memory.[51]

Several studies have reported a relationship between manganese hair levels in children and hyperactivity or learning disabilities. One found that the concentration of manganese in the hair of formula-fed infants increased from 0.19 micrograms/gm of hair at birth to 0.965 microgms/gm at six weeks, declining to 0.685 microgms/gm at four months of age. In breast-fed infants, hair levels increased only to 0.330 microgms/gm at four months of age. In this study, hair levels of manganese in hyperactive children were 0.434 microgms/gm as compared to levels of 0.268 microgms/gm in age-matched controls who were not hyperactive. [52] Another study reported hair manganese levels of 0.83 microgms/gm in hyperactive children compared with 0.58 microgms/gm in controls. [53] This study also found elevated lead levels in hyperactive children. A third study also reports higher hair manganese levels in children with attention deficit hyperactivity disorder than in controls. [54]

*Emerging evidence demonstrates that the brains of fetuses and newborns are more susceptible to the toxic effects of manganese than adults and that developmental exposures may result in unique neurological effects.*

### Mechanisms of Neurotoxicity

Animals exposed to excessive manganese early in life show depressed levels of the neurotransmitters dopamine, norepinephrine, and serotonin.[55] One study shows that gestational serotonin depletion in rodents causes much more extensive structural change in the brains of offspring than similar depletions in adults, a result that is not surprising, in light of the important role of neuro-transmitters in brain development.[56]

### Conclusions

The susceptibility of the developing brain to manganese toxicity deserves further attention. Many infant formulas are regularly supplemented with manga-nese. Nutritional experts must have thought that human breast milk is deficient in this essential element and that supplements would not be harmful. Soy-based formulas contain even higher amounts of naturally-occurring manga-nese. But metabolic studies show that infants absorb more and excrete less manganese than adults. Furthermore, in infants, blood-borne manganese more readily enters the brain than in adults. Animal studies show that developmen-tal exposures to manganese are associ-ated with hyperactivity. Several studies show that hair manganese levels are higher in children with hyperactivity disorders than in controls. These obser-vations call into question the wisdom of supplementing infant formulas with this metal or adding MMT to gasoline, and make the case for urgent research to clarify areas of outstanding uncertain-ties. As we learned from boosting

*Some studies of children exposed to cadmium have shown hyperactivity and reduced verbal and performance IQ.*



gasoline octane ratings with lead, population-wide exposures, however low-level they may be, sometimes have serious, unintended consequences.

## Cadmium

- Studies of the neurological effects of developmental exposure to cadmium report mixed and sometimes conflicting results

- In animal tests, cadmium exposure causes a mixture of hyperactivity, reduced activity, and altered learning, depending on the timing, dose, route of exposure, and test methods

- Some studies of children exposed to cadmium have shown hyperactivity and reduced verbal and performance IQ

### Routes of Exposure

Cadmium is a metal with no essential biological function, but it may interfere with normal neurological development through a variety of mechanisms. Cadmium is released to the environment from fossil fuel burning, mining and manufacturing operations, sewage sludge, phosphate fertilizers, and medical and municipal waste incinerators. Cadmium is used for a variety of industrial

purposes including metal plating, paint pigments, plastic stabilizers, and nickel-cadmium batteries.

The largest source of most human exposure to cadmium is dietary with an average adult daily intake of 10-30 microgms. Soil cadmium is readily taken up by leafy vegetables and grain crops, creating the potential for significantly increasing levels in crops grown on soil treated with sewage sludge containing cadmium from industrial sources. [57] Domestic and laboratory animals fed plants grown on sludge-amended soil may develop cadmium toxicity. [58] Cadmium also tends to concentrate in shellfish found in contaminated coastal waters. Another important source of cadmium is cigarette smoke; smokers have blood levels of cadmium approximately twice that of non smokers. [59]

## Animal Studies

For several reasons studies of the neurological consequences of early life exposures to cadmium are more difficult to conduct than studies of lead, for example. Cadmium is rapidly removed from the blood and stored in the kidneys, liver, pancreas, and adrenal glands, making blood level measurements a poor indicator of exposure. Chronic cadmium exposure induces the production of a protein, metallothionein, which binds the metal and reduces its toxic effects. However, intermittent acute exposures to cadmium may escape this mechanism and lead to more severe toxic responses. In laboratory tests, even moderate levels of cadmium exposure may reduce animal weight gain, making it difficult to distin-guish between direct cadmium toxicity and nutritional deficiencies from decreased food and water intake. Finally, the effect of cadmium on the fetus may be largely an indirect result of impairment of placental function, enzyme inhibition, or alteration of other essential trace metals in the brain rather than a direct toxic effect on fetal tissues. For example, metallothionein induced by cadmium may also bind zinc, an essential trace element, resulting in manifestations of zinc deficiency, which include birth defects. Indirect neurodevelopmental effects are also inferred from the observation that studies of cadmium exposure during pregnancy usually fail to find evidence of elevated cadmium levels in the fetal brain.[60]

In animals exposed to cadmium prenatally, a mixture of, and sometimes conflicting, neurological effects are noted. Moreover, females seem to be more sensitive to neurodevelopmental effects than males, yet male animals are more often studied. Both hyperactivity and reduced activity are noted in offspring, depending on the exposure level, route of exposure, and tests used to measure activity levels. [61 62 63] The capacity of an animal to learn an avoidance task is also sometimes impaired. [64] In most cases, neurotoxicity is noted only when doses are sufficient to alter weight gain and growth. These studies have used maternal exposure levels in the range of 0.1-4.0 mg/kg daily during pregnancy via injection, diet, gastric lavage, or inhalation.

In contrast, neonatal exposure to cadmium is potentially more harmful than prenatal exposure because the

*Soil cadmium is readily taken up by leafy vegetables and grain crops, creating the potential for significantly increasing levels in crops grown on soil treated with sewage sludge containing cadmium from industrial sources.*

blood-brain barrier is not yet fully developed, and cadmium may have direct access to the developing brain. Microscopic studies show lesions in the brains of cadmium-treated neonatal rats that are not seen in the brains of treated adult rats, suggesting that the immature blood-brain barrier is an important factor in cadmium neurotoxicity. Here, too, animal studies show a mixture of hyperactivity, reduced activity, and altered learning in young animals, depending on test methods, dose, and route of exposure.

## Human Studies

Several human studies have attempted to examine the neurological consequences of early exposures to cadmium. These are complicated by the correlation of lead and cadmium exposures, making it difficult to determine the relative contribution of each metal to observed effects. One study found a significant correlation between elevated hair cadmium and lead levels and hyperactivity in children.[65] Another study of a rural population of 149 children 5-16 years old found a correlation between hair lead and cadmium levels and reduced verbal and performance IQ when tested by the Wechsler Intelligence Scale for Children.[66] This study controlled for gender, age, race, and socioeconomic status. Interestingly, lead and cadmium seemed to affect different aspects of intelligence. Lead levels were more highly correlated with reduced performance IQ while cadmium levels correlated better with reduced verbal IQ.

In a prospective study, a hair sample was taken from 26 newborn children and their mothers and analyzed for lead and cadmium.[67] Six years later, the children were tested by the McCarthy Scales of Children's Abilities. Cadmium hair levels in children correlated with reduced perceptual and motor performance. Cadmium hair levels in mothers correlated with poorer child performance in general cognitive, perceptual, quantitative, and motor function. Lead levels also correlated with reduced perceptual performance, motor, and quantitative scores.

## Mechanisms of Action

Cadmium may be directly or indirectly toxic to the brain of the developing child. During pregnancy, cadmium may interfere with placental and essential enzyme function or the availability of essential trace elements or other nutrients. Neonatal exposures alter neurotransmitter levels, including norepinephrine, dopamine, serotonin, and acetylcholine.[68] Cadmium exposure is also associated with increased free radical production in tissues resulting in cell membrane damage and changes in a variety of other physiological functions.

## Tobacco Smoke and Nicotine

- Children born to women who smoke during pregnancy are at risk for IQ deficits, learning disorders, and attention deficits

- Children born to women who are passively exposed to cigarette smoke are also at risk for impaired speech, language skills, and intelligence

### Routes of Exposure

Cigarette smoke and one of its components, nicotine, are among the most studied neurodevelopmental toxicants. Many animal studies are conducted with pure nicotine, which easily crosses the placenta, while human epidemiological studies examine the effects of exposure to the complex mixture of chemicals in tobacco smoke, including nicotine. Nicotine exposure in animals, however, produces some of the same effects in offspring as those seen in children whose mothers smoked during pregnancy, and nicotine is, therefore, likely to be a substantial contributor to the observed effects.

### Animal Studies

In animals and humans, nicotine and tobacco smoke exposure cause growth retardation and other complications of pr egnancy (pr ematurity , placental abnormalities, respiratory distress syndrome).[69] In order to examine for neurological effects of prenatal nicotine exposure that are due solely to toxic effects on the developing brain and not due to generally retarded growth, it is important to conduct animal testing at relatively low-levels of exposure. Larger doses that cause decreased oxygen delivery to the fetus may cause retarded growth and are less informative about exclusively neurotoxic effects. Therefore, animal studies done with low-dose infusion pumps that better mimic the level of human fetal exposure to nicotine due to maternal smoking give extremely relevant information.

In rats, prenatal nicotine exposure by maternal low-dose infusion, causes hyperactivity in young offspring. [70] The effect is most pronounced in males. Results of testing for effects on learning and memory are mixed. Normally rodents tend to show increasing interest in exploring novel environments as they age from infancy to adulthood. Rats exposed to low doses of nicotine in utero showed an opposite effect in that they tend to explore novel environments more readily in infancy but less after puber ty.[71] Similar changes wer e seen in other maze tests. [72] These tests also show that complicating the task by changing the testing context sometimes uncovers nicotine-induced behavioral changes that would not otherwise be apparent.

### Human Studies

A number of studies of children whose mothers smoked during pregnancy report adverse effects, including diminished intellectual capacity and achievement into adulthood. [73 74 75 76] Effects are apparent immediately after birth. For example, one study reports that, using Brazelton Neonatal Behavioral Assessment Scales, infants born to smokers score significantly lower at 2, 3, and 14 days postpartum than unexposed infants. [77] Hearing seems to be particularly affected. Nicotine exposed infants were able to adapt to sounds normally but were less able to orient toward the source of the sound. This finding persisted at 2 weeks of age.

A large study of 12,000 children followed from birth to 11 years of age showed that those whose mothers

*A number of studies of children whose mothers smoked during pregnancy report adverse effects, including diminished intellectual capacity and achievement into adulthood.*

smoked more than 10 cigarettes daily during pregnancy were 3-5 months retarded in general ability, reading and math skills at age 11.[78] Investigators corrected for socioeconomic and biological variables in the study population.

One study that followed a cohort of children into adulthood found that, by age 23, offspring of mothers who smoked during pregnancy had significantly lower academic achievement than unexposed children.[79] This study controlled for social class, size of family, and birth weight. It did not control for maternal academic achievement.

Maternal and/or childhood exposure to environmental tobacco smoke ("passive smoking") also seems to have adverse effects.[80] For example, after correcting for confounding variables, children at ages 6-9, tested for speech and language skills, intelligence, and visual/spatial abilities, whose mothers were exposed to passive cigarette smoke during pregnancy, performed intermediate between children of smoking mothers and those unexposed.[81] Investigators noted attention deficits and information processing problems in exposed children. Testing included the Wechsler Intelligence Scale for children with three-factor scores including verbal comprehension, perceptual organization, and freedom from distractibility. In an animal study of the effects of environmental tobacco smoke, rats exposed only post-natally and not pre-natally had reduced DNA content in their brains when compared to unexposed animals.[82]

## Mechanisms of Neurotoxicity

Animal studies show that gestational exposure to nicotine at levels that do not cause growth retardation increases the number of cholinergic nicotinic neuro-receptor sites in the fetus and neonate, an effect that persists through the postnatal period of synapse formation.[83] Prenatal nicotine exposure also causes subnormal levels of the neurotransmitters dopamine and norepinephrine in the postnatal period.[84] Changes in norepinephrine utilization persist in some areas of the brain in adulthood.

A study of fetal and neonatal rats exposed to nicotine showed reduced DNA synthesis in the brain.[85] This was particularly marked in areas of the brain with higher concentrations of nicotinic receptors and in areas undergoing rapid cell division.

Cigarette smoke, however, is chemically complex and includes carbon monoxide and cyanide. In addition to the direct action of nicotine on the developing brain, other potential mechanisms of toxicity of smoke include low oxygen levels from carbon monoxide and impaired transfer of nutrients across the placenta, resulting in generally retarded fetal growth.

## Conclusions

Tobacco smoke is a complex mixture of chemicals including nicotine, a neurotoxic substance with lasting effects on neurological function after fetal exposures. Offspring of animals and humans exposed to nicotine in utero are hyperactive and experience increased tremors and impaired auditory responsiveness. Children exposed to

nicotine and other contaminants of cigarette smoke during gestation show persistent intellectual impairment that affects performance on neurological testing and is associated with lower academic achievement. Environmental tobacco smoke ("passive smoking") also interferes with brain development.

## DIOXINS AND PCBS

- Monkeys exposed to dioxin as fetuses show evidence of learning disabilities

- Humans and animals exposed as fetuses to low levels of PCBs have learning disabilities

- Children exposed to PCBs during fetal life show IQ deficits, hyperactivity, and attention deficits when tested years later

Dioxins are a family of chemical compounds unintentionally produced during a variety of industrial processes, including municipal and medical waste incineration, secondary copper smelting, hazardous waste incineration, and chlorine-based pulp and paper bleaching, among others. Dioxins consist of two benzene rings, joined by two oxygen atoms, with varying numbers of chlorine atoms distributed around the periphery. The toxicity of a given dioxin molecule varies with the number and position of chlorine atoms. Most of the toxic manifestations of dioxin exposure are mediated through attachment of the dioxin molecule to a cellular receptor (the Ah receptor), although some neurodevelopmental effects may be unrelated to Ah receptor activation. Within the dioxin family of compounds,

2,3,7,8 – tetrachlorodibenzo-p-dioxin (with chlorine atoms in the 2,3,7,8 positions) has the highest affinity for the Ah receptor and is the most potent trigger of Ah receptor-mediated effects.

Polychlorinated biphenyls (PCBs) are industrial chemicals that were intentionally produced for many years and used for a variety of purposes including as lubricants, coatings, and insulating material in electrical transformers. In the US, and in most other countries, PCB production has been

### Current Dietary Dioxin Exposures

| ● = 1 pg/kg/day (the advised limit for chronic exposure) |
|---|

| AGE GROUP | EXPOSURE | |
|---|---|---|
| Over 20 years | ●● | 2X |
| 10-14 years | ●●●●●●●● | 1-16X |
| 5-9 years | ●●●●●●●●●●●● | 1-27X |
| 1-4 years | ●●●●●●●●●●●●●●●● | 1-32X |
| Breast-fed Infant | ●●●●●●●●●●●●●●●●●●●●●● ●●●●●●●●●●●●●●●●●●●●● | 34-53X |

\* Based on a *minimal risk* level defined by ATSDR as a level at or below which adverse health effects are not expected to occur in humans. *Chronic exposure* is defined as an exposure lasting 1 year or longer.

Dioxin is concentrated in animal fat, and accumulates at higher levels in long-lived animals, and animals higher in the food chain. Because human food sources vary with age, dioxin intake also varies with age.[1] [2] Because dioxin is concentrated in breast milk, the intake of breast-feeding infants is highest, exceeding ATSDR's recommended limit for chronic exposure (one year or longer) by a factor of 34-53. This limit is exceeded to lesser degrees in all age groups. According to EPA, if one were to calculate, based on all human and animal data, a dioxin exposure limit that would protect against noncancer effects, (incorporating uncertainty factors to account for species differences and sensitive populations, such as the fetus), this exposure limit would be "on the order of 10 to 100 times below the current estimates of daily intake in the general population."[3]

banned because of their environmental persistence, tendency to bioaccumulate, and toxicity. However, PCBs still exist in many electrical transformers, in landfills, and hazardous waste sites. PCBs are structurally similar to dioxins but lack the oxygen atoms between the benzene rings.

### Routes of Exposure

Exposure to dioxins and PCBs is largely through dietary sources. Both dioxins and PCBs are environmentally persistent and tend to bioaccumulate in fatty tissue. Consequently, concentrations of each are highest at the top of the food chain, including beef, pork, dairy products, and fish. Breast milk contains among the highest levels of any human tissue because of its high fat content, which explains why a nursing infant is exposed to a substantial portion of a total lifetime dose of each of these families of chemicals during the first few months of life.

*An adequate margin of safety requires several orders of magnitude between the range of human exposures and the lowest level of adverse health effects. Adverse health effects of dioxin,[1][2][3][4] however, have been demonstrated in animals at levels of exposure that approximate the upper range of human exposures. This demonstrates the urgent need to reduce human exposures by reducing or eliminating dioxin production and release.*
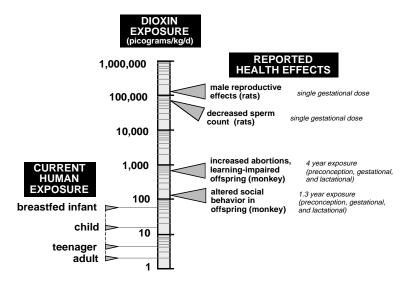
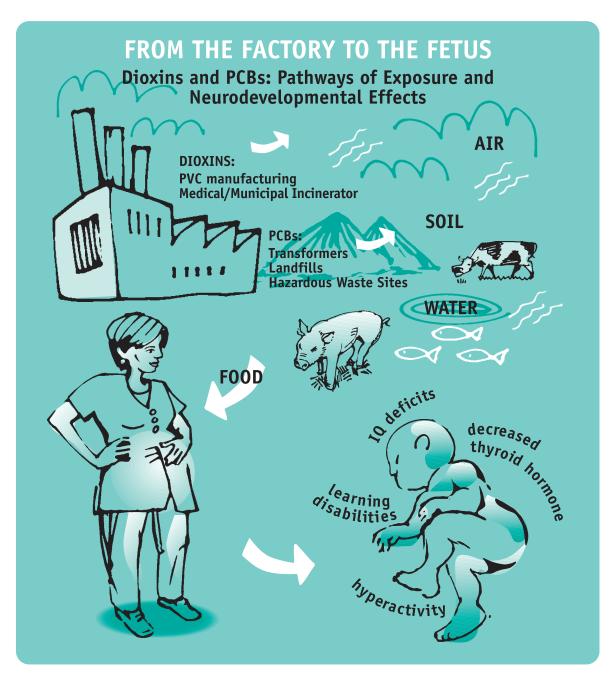### Dioxin: Inadequate Margin of Safety



### Animal Studies

Monkeys exposed gestationally to dioxin through a maternal diet containing 5-25 ppt dioxin, within the range of human breast milk contamination, show deficits in discrimination-reversal learning (retarded learning of shape reversals).[86] In this test, animals initially learn to respond correctly to a particular shape, form, color, or position. Then the correct answer is reversed so that the previous incorrect response now becomes correct. This requires changing a response strategy, a task more difficult than simply learning to discriminate initially.

Monkeys fed from birth to age twenty weeks with a PCB mixture and concentration representative of PCBs typically found in human breast milk showed significantly impaired learning and performance skills when tested between 2.5 and 5 years of age.[87]  In addition to retarded learning, exposed monkeys showed perseverative behavior (constant repetition) and an inability to inhibit inappropriate responses.[88] The affected monkeys had blood PCB levels of 2-3 ppb, similar to levels in the general human population. Other investigators report similar effects on learning and behavior in monkeys exposed to PCBs shortly after birth, including hyperactivity.[89][90]

Rats exposed to PCBs prenatally show reduced visual discrimination, increases and decreases in activity level, and impaired learning.  [91][92]  Depending on the particular PCB(s) used in the study, effects are seen at maternal doses as low as 2 microgms/kg/day every second day from day 10-20 of gestation, with no no-effect level identified.

# FROM THE FACTORY TO THE FETUS

## Dioxins and PCBs: Pathways of Exposure and Neurodevelopmental Effects

AIR

**DIOXINS:**
PVC manufacturing
Medical/Municipal Incinerator

SOIL

**PCBs:**
Transformers
Landfills
Hazardous Waste Sites

WATER

FOOD

IQ deficits

decreased thyroid hormone

learning disabilities

hyperactivity

Dioxin is unintentionally produced in a variety of industrial processes, including municipal and medical waste incineration.[1] Once emitted into the air, dioxin often travels more than a thousand miles before settling on pastures and water bodies that produce the global food supply.[2] PCBs were produced predominantly from the 1920's to the 1980's, for use in a variety of products including transformers, capacitors, and lubricant oils.[3] While PCB production has been banned in most countries, approximately two-thirds of the total amount produced has not yet been released to the environment.[4] PCBs have been introduced into the environment through careless disposal, leakage from industrial facilities and waste disposal sites, and from products in use.[5] [6] PCBs introduced to land or water bind to soil and sediment particles, evaporate at various rates, and, like dioxin, undergo long range atmospheric transport.[7] [8]

Because of their similar chemical properties, PCBs and dioxin have similar patterns of long range atmospheric transport resulting in widespread deposition. Both accumulate in the cattle and fish feeding on contaminated vegetation, and concentrate further in species eating high on the food chain, including humans. PCBs and dioxin can remain in soil for many years.[9] [10] Laboratory studies in animals have demonstrated significant dermal absorption of PCBs, but not of dioxin, following contact with contaminated soil.[11] [12] [13] However, most human exposure to both PCBs and dioxins occurs through food consumption.[14] [15] Because dioxin and PCBs are carried by fat, they are passed during pregnancy from mother to fetus, the most vulnerable stage of human development, and continue to be transmitted during breast feeding. Dioxin and PCBs thus illustrate one of the unforeseen pathways by which industrial chemicals may travel from the factory to the fetus.

## Human Studies

In the late 1960s and early 1970s two episodes of accidental human exposure to PCB-contaminated rice oil in Japan and Taiwan resulted in tragic developmental effects in children born to exposed mothers. [93] The developing fetus was much more sensitive than mothers, and numerous abnormalities were observed including low birth weight, hyperpigmented skin, swollen gums and eyelids, and early tooth eruption. Neurological abnormalities were among the most significant findings, including mental retardation among some of the most highly exposed. Delayed brain development and behavioral abnormalities in the children persist for years after the incidents. Exposed children have deficits on IQ testing, and according to teachers, are hyperactive and exhibit more behavioral problems than those unexposed. [94]

Although these tragic incidents exposed children to obviously toxic amounts of PCBs, other studies have examined the neurodevelopmental effects of exposures to levels of PCBs found in the ambient environment. One group of 212 children in Michigan has been followed for years. Children were classified as offspring of fish-eating mothers if maternal Lake Michigan fish consumption was at least 6-8 oz/month. Some of the study families, however, were not fish eaters. In most cases, but not all, fetal and nursing PCB exposure correlated with maternal Lake Michigan fish consumption before and during pregnancy. The children most highly exposed to PCBs prenatally showed delayed or reduced psychomotor development and poorer performance on a visual recognition memory test. [95] These children have now been followed for more than 11 years. Prenatal PCB exposure remains associated with lower IQ scores after controlling for other factors, including socioeconomic status. [96] Compared with the low exposure group, the most highly exposed children were more than three times as likely to perform poorly on IQ tests and tests designed to measure their attention span. They were more than twice as likely to be at least two years behind in word comprehension in reading. According to the investigators, the most frequent manifestations of neurodevelopmental toxicity of PCBs are disturbances in neuromotor activity and attention (deficits in focused and sustained attention), impairments of higher cognitive functions and learning, and neurodevelopmental delays. These disturbances seem likely to persist throughout the school years.

Another group of children in North Carolina shows similar results. Higher fetal PCB exposures, as measured by PCB levels in maternal blood, were associated with lower scores on psychomotor development tests (Brazelton) at six and twelve months of age than those with lower exposures. [97]

In a New York study of several hundred newborn children whose mothers ate PCB-contaminated fish from Lake Ontario, those with the higher exposures showed abnormal reflexes and startle responses and
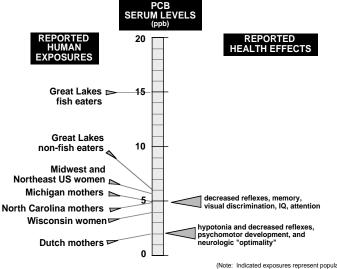
## PCBs: Inadequate Margin of Safety

decreased visual recognition when compared with the less exposed. [98] Recently, investigators reported that, at 12 months of age, prenatal PCB exposure was associated with poorer performance on the Fagan Test of Infant Intelligence and at 3 years of age with poorer performance on the McCarthy Scales of Children's Abilities. [99]

The development of another group of 418 children has been studied prospectively for several years in the Netherlands, after measuring PCB/dioxin levels in maternal blood during the last month of pregnancy, in umbilical cord blood, and in breast milk. These exposures were all at ambient environmental levels and not the result of a large accidental exposure or of excessive fish consumption. Cognitive abilities were assessed in 395 of these children with the Kaufman Assessment Battery for Children at 42 months of age. [100] After adjustment for co-variables, maternal PCB blood levels were significantly associated with lower scores on the overall cognitive and sequential and simultaneous processing scales of this battery. Lactational exposures and current exposure to PCBs and dioxin were not related to 42-month cognitive performance, indicating that the adverse effect is the result of fetal exposure to PCBs.

The investigators also report that umbilical cord and maternal PCB blood levels are significantly associated with less time at high level play. Blood PCB levels in 42-month old children are associated with slower reaction times and more signs of hyperactivity as

reported by parents. This study also reported small but significant reductions in thyroid hormone levels at 2 weeks and 3 months of age in the children with the highest PCB/dioxin fetal exposures. [101]

### Mechanisms of Neurotoxicity

The mechanisms of action of dioxins and PCBs on early neurological development are incompletely understood. Dioxins and some PCBs share one mechanism of action but differ in others. However, because their chemical characteristics are similar, they tend to co-exist in biological tissues, making it difficult to distinguish between their toxic effects in human epidemiological studies.

Dioxins and dioxin-like PCBs (so-called coplanar or non-ortho PCBs) share a common mechanism of action by binding to the Ah receptor. This complex is then further processed and passes into the cell nucleus where it binds to DNA, influencing the production and metabolism of a variety growth factors, hormones, and hormone receptors. However, many non-coplanar or ortho-PCBs that do not readily attach to the Ah receptor also have biological activity, which substan-

Prenatal exposure to background levels of PCBs has been shown to adversely effect reflexes, memory and neurological function as assessed by physical exam in infants and toddlers. Adverse effects on attention, memory, intelligence and reading comprehension have been demonstrated in children followed up to age 11.[1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22]



**PCB SERUM LEVELS (ppb)**

REPORTED HUMAN EXPOSURES | REPORTED HEALTH EFFECTS

- Great Lakes fish eaters — 15
- Great Lakes non-fish eaters — 10
- Midwest and Northeast US women
- Michigan mothers
- North Carolina mothers — 5 — decreased reflexes, memory, visual discrimination, IQ, attention
- Wisconsin women
- Dutch mothers — hypotonia and decreased reflexes, psychomotor development, and neurologic "optimality"
- 0

(Note: Indicated exposures represent population averages except where noted as high end of range.)

tially contributes to their neurodevelopmental toxicity . At least some of this toxicity may result from interference with thyroid hormone function.

PCBs may interfere with thyroid hormone in a variety of ways. In animal tests, some PCBs displace thyroxine from its carrier protein, transthyretin, in the circulation. In many animals, thyroxine, attached to transthyretin, is the form by which thyroid hormone gains access to the fetal brain. Any chemical that interferes with this binding has the potential to alter normal brain development. However, in humans, another protein, thyroid binding globulin, is the main carrier protein for thyroxine, and their binding is less affected by PCBs.

Dioxin and PCBs may also interfere with thyroid hormone function by increasing the turnover of thyroxine through induction of an enzyme, which facilitates the metabolism and excretion of the hormone. [102] PCBs may also interfere with thyroid-hormone-mediated gene transcription. [103] A recent report, however , shows that, although pr enatal PCB exposure does reduce thyroxine levels, thyroid-dependent protein synthesis in the brain is not affected by the doses used. [104] This finding implies that the neurodevelopmental effects of prenatal PCB exposures are not exclusively due to decreased thyroid hormone levels.

Some PCBs also alter normal brain neurotransmitter levels, although the nature of change depends on PCB structure. [105] For example, ortho-PCBs

decrease dopamine synthesis while non-ortho PCBs increase dopamine levels after in utero and lactational exposure in rats. [106] This effect may also be related to the neurodevelopmental delays described in humans exposed to PCBs in utero.

## Conclusion

Dioxins and PCBs adversely affect brain development and function at ambient levels of exposure. The effects of prenatal exposure to PCBs appear to be permanent. Psychomotor developmental delays, attention deficits, changes in play behavior, and cognitive impairment, including IQ deficits, have been described in large human study populations. The mechanism(s) by which these chemicals exert their neurotoxic effects are not fully understood but probably include alterations in neurotransmitter levels and thyroid hormone function.

## PESTICIDES

- Animal tests of pesticides belonging to the commonly used organophosphate class of chemicals show that small single doses on a critical day of development can cause permanent changes in neurotransmitter receptor levels in the brain and hyperactivity

- One of the most commonly used organophosphates, chlorpyrifos (Dursban), decreases DNA synthesis in the developing brain, resulting in deficits in cell numbers

- Some pyrethroids, another commonly used class of pesticides, also cause permanent hyperactivity in animals exposed to small doses on a single critical day of development

- Children exposed to a variety of pesticides in an agricultural community in Mexico show impaired stamina, coordination, memor y, and capacity to r epr esent familiar subjects in drawings

Many pesticides kill insects because they are neurotoxic. For example, the organophosphates and carbamates inhibit acetylcholinesterase, the enzyme responsible for breaking down the neurotransmitter acetylcholine. Other families of pesticides including pyrethroids, pyrethrins, and organochlorines also exert their toxic action by interfering with nerve cell function.     Routes of exposure to pesticides are discussed in Chapter 7.

## Organophosphates

Organophosphates are widely used for pest control in the home, on the lawn and garden, and on the commercial food supply.

### Animal Tests

Studies in neonatal mice show that a single dose of an organophosphate pesticide (1.5 mg diisopropylfluoro-phosphate [DFP]/kg body wgt) on postnatal day 10 causes permanent decreases in muscarinic cholinergic receptors in the cerebral cortex and hyperactivity at 4 mos. of age.     [107] Exposed animals showed persistently increased locomotion (horizontal movement) and total activity (all types of movement) when compared to untreated controls.

Chlorpyrifos (Dursban), one of the most heavily used organophosphates, also causes neurochemical and

behavioral effects in rats exposed during gestation. Pregnant rats given chlorpyrifos (6.25, 12.5, or 25 mg/kg/day by injection, gestational days 12-19) had offspring with fewer muscarinic cholinergic receptors in their brains and markedly altered righting reflex and cliff avoidance tests.     [108] When maternal rats are treated with 5 mg chlorpyrifos/kg/day by gavage from gestational day 6-postnatal day 11, offspring have a decreased auditory startle response and decreased brain weight.     [109] (For comparison purposes, the current reference dose [RfD] for chlorpyrifos, the human dose below which no adverse ef fects ar e consider ed likely , is 3 microgm/kg/day)

Another organophosphate, diazinon, was given to pregnant mice at 0, 0.18, or 9.0 mg/kg/day thoughout pr egnancy , and the development of their offspring was evaluated.     [110] Offspring of the mothers receiving the highest dose grew more slowly than those in the lower exposure groups. Although offspring of those receiving the lowest dose grew nor mally , behavioral testing r evealed delays in development of the contact placing r eflex and sexual maturity    . Adult offspring of mothers exposed at either dose showed impaired endurance and coordination.  The RfD for diazinon is under r eview by EP A.

## Organochlorines

DDT is an organochlorine pesticide no longer used in the US but heavily used in some parts of the world both in agriculture and for disease vector control. DDT was banned in the US and

*Studies in neonatal mice show that a single dose of an organophosphate pesticide on postnatal day 10 causes hyperactivity at 4 mos. of age.*

other countries because of toxicity to wildlife and its capacity to bioaccumulate and persist in the environment for years. DDT also exerts its toxic action by interfering with the stability of nerve cell membranes, resulting in overstimulation of the nervous system in exposed animals.

### Animal Tests

Newborn mice were given a single dose of 0.5 mg DDT/kg on day 3, 10, or 19 of life and examined at 4 months of age for activity level and muscarinic cholinergic receptors in the brain cortex. [111] Those animals exposed to DDT on day 10 showed significant increases in activity level and decreases in receptor levels at that age. Mice exposed on days 3 or 19 did not show significant changes. These results highlight a short but significant window of vulnerability to a neurotoxic chemical when exposure may have lifelong effects on brain structure and function.

### Human Studies

Reports of the neurological evaluation of children exposed to pesticides are few and are usually limited to the acute effects of exposures. However, a recent study of children in Mexico, who are regularly exposed to a mixture of pesticides in their largely agricultural community, suggests that many different brain functions may be impaired by pesticide exposure during child development. [112] Researchers compared two different groups of 4-5 year old children who came from very similar genetic, social, and cultural backgrounds. However, one group lived

in a community where pesticides were regularly used in agriculture whereas the other came from a community with a non-chemical agricultural system. A variety of organochlorine pesticides were measured in the umbilical cord blood and breast milk of individuals in the pesticide-exposed community, though exposure to other classes of pesticides were also likely.

Children in the exposed community showed significantly diminished stamina and coordination when asked to catch a ball, stand on one foot as long as possible, jump in place, and drop raisins into a bottle cap from a distance of 15 cm. Memory in the pesticide-exposed children was also impaired. They were less able to recall what they had been promised as a reward (a red balloon) before testing started. Exposed children were also impaired in their ability to draw recognizable representations of people and objects. When asked to draw a person, exposed children averaged 1.6 body parts/drawing in drawings considerably more distorted than those

of the unexposed children that averaged 4.4 body parts/drawing. Houses and trees drawn by pesticide exposed children were also more distorted and difficult to interpret. Exposed children appeared to be less creative in their play activity.
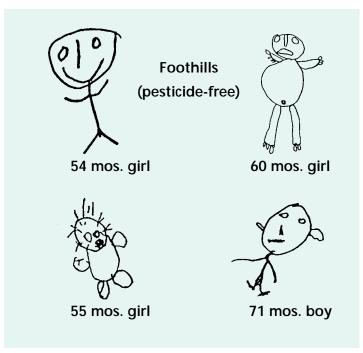
## Pyrethroids

Naturally-occurring pyrethrins or synthetic pyrethroids are insecticides that also exert their toxic action by interfering with the electrical activity of nerve cells. They are sometimes divided into Type I and Type II compounds. Type I cause repetitive firing of nerve cells while Type II cause nerve inexcitability by blocking cell depolarization.
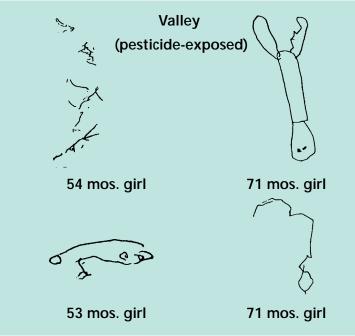
### Animal Tests

Mice given small doses of bioallethrin (Type I) or deltamethrin (Type II) on day 10 of life also show reduction in muscarinic cholinergic receptor levels in the brain cortex as adults, along with hyperactivity.[113] In an attempt to better define the dose-response curve, investigators used doses of bioallethrin at 0.21, 0.42, 0.70, and 42 mg/kg on day 10 of life. The hyperactivity of the mice as adults increased with increasing levels of exposure through the 0.70 mg/kg dose, but then fell sharply with the 42 mg/kg dose. This observation is particularly important for pesticide testing in that testing at higher doses of exposure may fail to identify an adverse effect seen only at lower levels of exposure. Current methods for dose selection for pesticide regulatory testing purposes may miss this effect and should be re-examined.

## Drawings of a Person
### by Yaqui children (by age and gender)



Foothills (pesticide-free)

54 mos. girl     60 mos. girl

55 mos. girl     71 mos. boy

Valley (pesticide-exposed)

54 mos. girl     71 mos. girl

53 mos. girl     71 mos. girl

Illustrations are those by Mexican Yaqui Indian children drawn during a study of the effects of pesticide exposure on neurological development. The study was conducted by Elizabeth A. Guillette, PhD, University of Arizona. Illustrations used with permission.

Another study of two pyrethroids, fenvalerate and cypermethrin, examined the effect on neurotransmitter levels in offspring of rats after gestational and lactational exposures.[114] Alterations in levels of neurotransmitter enzymes (monamine oxidase and acetylcholinesterase) were noted. Dopamine receptor levels in the brain were decreased after exposure to each of the chemicals and muscarinic cholinergic receptor levels were markedly decreased only after cypermethrin.

### Mechanisms of Neurotoxicity

Organophosphates and carbamates inhibit acetylcholinesterase, the enzyme responsible for breaking down the neurotransmitter acetylcholine at nerve synapses or at the junction of nerves with muscles. The result is twofold. In the short term, the synapse or neuromuscular junction is overstimulated and clinical symptoms result. But in the developing organism, as previously noted, neuro-transmitters also play important roles in orchestrating cell proliferation, migration, differentiation, synapse formation and apoptosis. Alterations in neurotransmitter levels during development have significant effects on the brain that do not occur after adult exposures.

Several different mechanisms help explain the neurodevelopmental effects of organophosphates. First, by altering neurotransmitter levels (acetylcholine and others secondarily) these chemicals interfere with cell replication and differentiation. Second, acetylcholines-terase itself appears to have a role in

*The low concentrations of chlorpyrifos necessary to impair DNA synthesis and cell division are actually lower than exposure levels of children under some pesticide home-use conditions.*

brain development, independent of its serving as an enzyme to break down the neurotransmitter, acetylcholine. Research shows that the enzyme facilitates neurite outgrowth from neurons and that deficiency of the enzyme reduces neurite outgrowth.[115] In addition, chlorpyrifos decreases DNA synthesis, independent of its cholinergic mechanism, resulting in deficits in numbers of cells in the developing brain.[116] [117] This latter obser-vation is particularly important for two reasons. First, the potential for toxicity of organophosphates is often inferred from the degree of cholinesterase inhibition, but the effects of chlorpyrifos on DNA synthesis and cell numbers show that no general conclusions may be drawn from anticholinesterase activity alone. Neuro-toxicity testing has not generally been designed to measure the effects of organo-phosphates on cell proliferation and differentiation. The presumption has been that cholinesterase inhibition is the most sensitive endpoint. Second, the low concen-trations of chlorpyrifos necessary to impair DNA synthesis and cell division are actually lower than exposure levels of children under some pesticide home-use conditions.[118] [119]

Pyrethroids, pyrethrins, and organochlorines also exert their toxic action by interfering with nerve cell function. By modifying the permeability of nerve cell membranes to various ions they may either increase or decrease the excitability of nerve cells causing repetitive firing or prolonged inactivity. Studies done in developing animals show that each of these classes of insecticides

may also permanently alter neuroreceptor levels in portions of the brain and modify animal behavior as a result.

### Conclusions

Several different classes of pesticides show unique neurodevelopmental toxicity in animals exposed during gestation or at particular windows of vulnerability in the neonatal period. Small exposures during those periods of susceptibility permanently alter brain neuroreceptor levels and cause hyper-activity in the animals as adults. These adverse effects are distinctly unlike those seen after adult animal exposures. It is important to note that the stage of brain development in rodents at age 10 days is similar to the same stage in humans during the last trimester of pregnancy. These results, therefore, suggest the potential for similar effects in the off-

spring of women who are exposed to these types of chemicals during the latter part of pregnancy. One study of children exposed to a mixture of pesticides during development shows adverse impacts on a variety of neurological functions, including stamina, coordination, memory, and ability to conceptualize and draw. These results confirm the need for comprehensive neurodevelopmental testing of pesticides before they are licensed for commercial use. Under current law, the US EPA is authorized to require such testing but, with rare exceptions, has failed to exercise that authority. (see chapter 7)

### SOLVENTS

- Exposure to organic solvents during development may cause a spectrum of disorders including structural birth defects, hyperactivity, attention deficits, reduced IQ, learning and memory deficiencies.

- As little as one alcoholic drink a day during pregnancy may cause impulsive behavior and lasting deficits in memory, IQ, school performance, and social adaptability in offspring.

- Animal and limited human studies show that exposures to common chemicals like toluene, trichloro-ethylene, styrene, and xylene during pregnancy can also cause learning deficiencies and altered behavior in offspring, though fairly large exposures may be necessary.

## Routes of Exposure

Organic solvents are widely used in consumer products, hobbies, and industry. Releases of some organic solvents to the environment from large industrial sources are reported on the Toxics Release Inventory (TRI). For example, in 1997 over 115 million pounds of toluene, 75 million pounds of xylene, 46 million pounds of styrene, and 21 million pounds of trichloroethylene were released to air, water, and land by the largest industries required to report their toxic emissions. Ethanol is consumed in alcoholic beverages. Toluene and xylene are in gasoline and its vapors, as well as other consumer products. Trichloroethylene is commonly used as a degreaser and is a common drinking water contaminant at low concentrations. Because many solvents are volatile, inhalation exposures are particularly important.

## Ethanol (alcohol)

The neurodevelopmental effects of ethanol have been extensively studied. The term "fetal alcohol syndrome" (FAS) was first coined in 1973 to describe malformations in the offspring of chronic alcoholic women. [120] However, the consequences of fetal alcohol exposure had been known long before. Affected children show a mixture of craniofacial, limb, and cardiovascular defects associated with growth and developmental delays, though the degree of impairment can vary considerably. Cognitive functions may vary from normal to severely disrupted while physical features may independently vary from normal to obviously abnormal.

The Institute of Medicine of the National Academy of Sciences Committee to Study Fetal Alcohol Syndrome has proposed five diagnostic categories for fetal alcohol related abnormalities: [121] 1) diagnosis of FAS and a confirmed history of maternal alcohol exposure, 2) diagnosis of FAS without a confirmed history of maternal alcohol exposure, 3) partial FAS with confirmed alcohol exposure, 4) alcohol related birth defects, 5) alcohol related neurodevelopmental disorders.

The spectrum of clinical abnormalities probably reflects differences in timing, duration, and level of alcohol exposure during gestation, although the timing of periods of vulnerability for each of the various disorders is not well known. First trimester exposure is probably necessary for the distinctive physical facial abnormalities seen in FAS. [122] Alcohol exposure during the second and third trimester alters neuronal circuitry. The third trimester is a particularly vulnerable time for brain injury as a result of alcohol exposure. [123] Alcohol's effects on the fetus are more related to the maternal peak blood alcohol level than to total alcohol consumed, so that binge drinking is likely to be more harmful than equal amounts consumed over a longer period of time. [124] One study finds a threshold effect at an average of 0.5 oz. absolute alcohol per day. [125]

Clinical manifestations of fetal alcohol exposure include hyperactivity and attention deficit. [126] Memory, speed of information processing, and arithmetic functioning are also

*Clinical manifestations of fetal alcohol exposure include hyperactivity and attention deficit.*

adversely affected.[127] Eating disorders, bedwetting, sleep disorders, speech delay, anxiety, depression, and psychotic symptoms may also occur. Although there is a higher likelihood of cognitive disorders and mental retardation in FAS children, mental function varies and may be normal.

A study of 16 pairs of twins heavily exposed to alcohol prenatally found concordance for fetal alcohol syndrome in five pairs of monozygotic twins and in 7 of 11 pairs of dizygotic twins. [128] Genetically-determined variations in maternal metabolism of alcohol also influence the likelihood of FAS in offspring, since one of the metabolites of alcohol, acetaldehyde, is thought to contribute substantially to the condition. [129] These observations demonstrate the interaction of genetic factors with a well-known neurodevelopmental toxicant.

One of the difficulties encountered in studying the results of fetal alcohol exposure is the frequent co-occurrence of poor maternal nutrition, delayed prenatal care, and other maternal substance abuse, including tobacco. These factors complicate efforts to tease out the clinical features that are solely due to alcohol. Moreover, eating and sleep disturbances, behavioral difficulties, and impaired cognitive functioning and attention often adversely impact the mother-infant relationship. Thus, it is difficult to know how much future disability is attributable to fetal alcohol exposure and how much is due to social factors during infancy and early childhood.

## Mechanisms of Neurotoxicity

Animal studies show that fetal alcohol exposure causes reduction in brain weight, selective loss of certain cells, impaired maturation of cells, and retarded synaptic development.[130] [131] Several different mechanisms probably contribute to alcohol toxicity. They include disruption of cell-cell interactions by interfering with cell adhesion molecules[132], reduction of placental transport of amino acids, glucose, and other nutrients as a result of reduced oxygen supply[133], and abnormalities of synaptic transmission.[134]

## Other Solvents

### Human Studies

Compared to ethanol, much less is known about the effects of other solvents on brain development and function. Occupational exposures to solvents may cause both peripheral and central nervous system effects in adult workers and are also associated with birth defects, including abnormalities of the central nervous system, in their offspring. [135] [136] However, little information is available about the neurological development of children whose mothers were exposed to solvents during pregnancy. One study examined neurological development of children at an average age of 3 years whose mothers had been occupationally exposed to solvents during at least some portion of pregnancy.[137] No significant effect was found on evaluation for attention, behavior, sociability, activity, or learning. Developmental milestones were the same in exposed and unexposed groups, with

the exception of delayed onset of walking in the children exposed throughout pregnancy as compared to unexposed children. (13.3 mos. vs. 12.2 mos.) This finding is of uncertain significance since the children of mothers exposed for only the first or first and second trimesters of pregnancy actually began walking sooner than the unexposed. (10.8, 11.6 mos vs. 12.2) Maternal exposures in this study were not actually measured, and no attempt was made to correlate developmental outcomes with specific solvents. It may, therefore, be misleading to draw any firm conclusions from this single study.

Toluene is an organic solvent used in glues, inks, paints, cleaning agents, and gasoline. After large exposures, such as encountered with maternal glue sniffing during pregnancy, offspring may be born with craniofacial abnormalities resembling those of FAS.[138] Follow up studies show growth retardation and persistent deficits in cognition, speech, and motor skills. It is unknown whether or not a threshold level of exposure to toluene exists, below which no neurodevelopmental effects occur in humans. The developmental effects of toluene so closely resemble those of alcohol, that some investigators believe the mechanism of toxicity is similar.[139] As with alcohol, it may be the case that even relatively small exposures to toluene have subtle but important effects on neurocognitive development, though this has not been studied well in humans.

## Animal Studies

Animal studies also show neurobehavioral consequences of intermittent large prenatal exposures to toluene. Pregnant mice were exposed to 200, 400, or 2000 ppm (parts per million) toluene by inhalation for 60 minutes, 3 times a day, on gestational days 12-17. Offspring from the highest exposure group performed more poorly on behavioral tests of righting reflex, grip strength, and inverted screen.[140] Rats exposed to 1800 ppm toluene 6 hrs/day by inhalation on days 7-20 gestation gave birth to offspring with impaired learning when tested in a Morris water maze.[141] Occupational safety limits for toluene in the US allow 200 ppm exposure for a 40 hr. work week. (Occupational safety limits may not be enforced. The values are presented here only for purposes of comparison to experimental data.) Mice supplied with drinking water containing 16, 80, or 400 mg. toluene/liter (ppm) during pregnancy and lactation gave birth to offspring with deficient motor skills (rotorod performance).[142] The highest exposed group showed decreased habituation in open-field activity. The EPA's maximum contaminant level (MCL) for toluene in drinking water is 1.0 mg/l (ppm).

The offspring of rats supplied with drinking water containing 312, 625, or 1250 mg trichloroethylene/liter (ppm) throughout gestation and lactation were studied.[143] Exploratory behavior was increased in 60- and 90-day old male rats exposed at any level. Locomotor

activity (running wheel) was higher in rats exposed to 1250 ppm trichloroethylene. The EPA's drinking water MCL for trichloroethylene is 0.005 mg/l (ppm) The offspring of rats exposed to 1800 ppm trichloroethylene by inhalation 6 hr/day, 5 days per week for 2 weeks before mating had reduced body weight but no evidence of behavior abnormalities. [144] The offspring of those exposed throughout pregnancy had marginally reduced activity levels. Occupational safety limits in the US allow trichloroethylene exposure at 100 ppm for a 40 hr. work week.

Xylene exposure by inhalation at 500 ppm, 6 hrs/day, on gestational days 7-20 resulted in rat offspring with decreased brain weight and diminished motor performance (rotorod) and learning and memory (Morris water maze).[145] 500 mg/m3 xylene is equivalent to 115 ppm. In another study, the offspring of rats exposed to xylene at 500 mg/m3, 6 hrs/day, 5 days/wk, throughout pregnancy showed reduced horizontal movement in open field testing and structural changes in brain, heart, liver, and kidneys. [146] At 50 mg/m3 offspring showed retarded growth and skeletal abnormalities. Occupational safety limits in the US allow xylene exposure at 100 ppm for a 40 hr. work week.

Young rats (1-48 days of age) exposed to styrene at 25 and 50 ppm 7 hrs/day, 6 days/wk. showed significant delays in weight gain, decreased activity in open field testing, and decreased avoidance behavior.[147] Occupational safety limits for styrene exposure in the

US allow 50 ppm for a 40 hr. work week. Another study shows an important interaction between prenatal styrene exposure and dietary protein deficiency.[148] Rats given a protein deficient diet and styrene (100 mg/kg/day) during pregnancy gave birth to offspring with lower brain weights and a marked increase in amphetamine-induced hyperactivity when compared to controls, including those exposed to just styrene or only a low protein diet.

## Conclusions

In summary, many studies show that fetal exposure to relatively small amounts of alcohol disrupts normal brain development, resulting in hyperactivity, attention and learning disorders, and impaired memory. The magnitude of risk of fetal alcohol syndrome depends on both genetic and environmental factors and their interactions. Large inhalation exposures to toluene during pregnancy (glue sniffing) also carries the risk of devastating effects on fetal brain development, as well as causing structural birth defects. The effects of smaller exposures on fetal brain development are unknown. Other solvents that may be encountered in the workplace or in consumer products have the potential for disrupting normal brain development but usually at relatively high exposure levels. However, animal tests suggest that, at levels at or below those allowed in the workplace, xylene and styrene may alter learning, behavior, motor skills, and activity levels after fetal exposure. Since volatile solvents are

*Animal tests suggest that, at levels at or below those allowed in the workplace, xylene and styrene may alter learning, behavior, motor skills, and activity levels after fetal exposure.*

often present in consumer products, excessive hobby and home exposures are possible, particularly when products are used in confined or poorly ventilated areas. Nutritional factors may also contribute to neurodevelopmental impacts of solvent exposure.

## Additional Chemicals of Concern

Although assessments for developmental neurotoxicity are missing for many chemicals, two very different kinds of substances deserve particular mention because they are intentionally added to water or food, thereby exposing large populations on a lifetime daily basis. Whenever entire populations are exposed to any chemical substance through the food or water supply, exhaustive safety evaluations should be essential prior to initiation of exposure and as new data become available.

### Fluoride

Since the 1950's, in many communities throughout the US and other areas of the world, fluoride has been added to community drinking water supplies with the intention of reducing tooth decay. Controversy about the safety of that practice centers around concerns about increased risks of tooth staining and brittleness (dental fluorosis), bone brittleness (skeletal fluorosis), bone cancer, hormone disruption (melatonin), premature puberty, and altered neurological developmental. In addition, some critics argue that fluoridating the water supply has a minimal impact on tooth decay. The practice has been staunchly defended by the American Dental



Association and heralded by the Centers for Disease Control and Prevention as one of the major public health success stories of the 20th century. We do not intend to review the entire controversy here. Recent reviews are found elsewhere.[149][150][151] Rather, here we comment briefly on concerns about neurodevelopmental impacts of prenatal exposure to fluoride.

The US EPA sets a Recommended Maximum Contaminant Level of 4.0 ppm fluoride in drinking water. The National Institute for Dental Research considers fluoride at 1 ppm optimal for preventing dental caries. This level may be exceeded in some communities. Additional sources of fluoride, including topical fluoride treatments, fluoride tablets, and fluoride toothpaste, add to the total fluoride burden.

In an animal study, pregnant rats were given 0.13 mg sodium fluoride/kg by injection on 9 separate occasions from days 14-18 or 17-19 during pregnancy.[152] Offspring of treated animals and controls were monitored by videotape that was then computer-analyzed in order to quantify various behavioral characteristics. Offspring

exposed to fluoride on days 17-19 of pregnancy showed significant hyperactivity. They tended to move from one activity to another more frequently than unexposed animals. This study has been criticized for using excessive fluoride exposures. The authors respond by noting that the blood levels of fluoride in the treated animals were similar to the levels measured in people who are exposed through fluoridated water. Another criticism centered on the lack of biological plausibility that the results would differ in the two groups exposed at similar times during pregnancy.[153] The authors, however, point out that vulnerable developmental stages change rapidly during this time window and argue that the findings are entirely plausible.[154]

Another study found that the offspring of rats given 5, 15, 50 ppm fluoride in drinking water during pregnancy and lactation had significantly elevated acetylcholinesterase levels when tested at 80 days of age.[155] Maternal acetylcholinesterase levels were also increased. Though not measured in this study, a likely result of elevated acetylcholinesterase activity is decreased acetylcholine levels. As we have noted, the enzyme, acetylcholinesterase, and the neurotransmitter, acetylcholine, play important roles in brain development. Changes in the concentrations of any neurotransmitter during development may have permanent neurological consequences. The largest effect was seen at 5 ppm, decreasing at the higher levels.

Two reports from China identify significantly lower childhood IQs in communities where fluoride exposure is elevated. In one community, where drinking water naturally contains 4.12 ppm fluoride, IQs were significantly lower than in a nearby community with fluoride levels at 0.91 ppm. (average IQ 98 vs. 105)[156] This difference persisted when the study population was controlled for parental educational level. The authors describe similar occupations, living standards, and social customs in the two communities. The ecologic design of this study imposes some limits on the conclusions that may be drawn since the exposure (fluoride) and outcome (IQ) were compared on a population-wide basis without any attempt to associate individual fluoride exposure levels with individual IQs. Nonetheless, an IQ shift of 7 points in an entire population has large population-wide implications, as well as impacting individual members, and these results deserve close attention.

In the other study, investigators used dental fluorosis and urinary fluoride levels to stratify children into four quartiles.[157] Elevated fluoride exposures were associated with decreased IQs in this population. That is, the distribution of IQ scores in children in each quartile of fluoride exposure shifted progressively downward as the fluoride exposures increased.

## Conclusion

Studies in animals and human populations suggest that fluoride exposure, at levels that are experienced by a significant proportion of the

*Studies in animals and human populations suggest that fluoride exposure, at levels that are experienced by a significant proportion of the population whose drinking water is fluoridated, may have adverse impacts on the developing brain.*

population whose drinking water is fluoridated, may have adverse impacts on the developing brain. Though no final conclusions may be reached from available data, the findings are provocative and of significant public health concern. Perhaps most surprising is the relative sparseness of data addressing the central question of whether or not this chemical, which is intentionally added to drinking water, may interfere with normal brain development and function. Focused research should address this important matter urgently.

## Food Additives

The potential for certain food additives to alter neurological development, behavior, and learning capacity has been the subject of lively debate and controversy for many years. Food additives of concern are 1) the amino acid, glutamate, present naturally in many proteins and added to many processed foods, 2) the artificial sweetener, aspartame, which is metabolized into the two amino acids, aspartate and phenylalanine, and 3) food colorings and dyes.

One focus of concern centers around the observation that glutamate and aspartate are the major excitatory amino acid neurotransmitters in the mammalian brain and that large amounts of glutamate administered to pregnant rhesus monkeys late in gestation result in damage to the fetal brain.[158] [159] Damage to the hypothalamus, the portion of the brain responsible for sending chemical messages to the underlying pituitary gland, and an essential link in hormonal regulatory processes, has been most extensively studied.

It is important to note that most of the adult brain is protected by the blood-brain barrier, whereas the blood-brain barrier is not complete in the developing human brain until about six months of age (3 weeks in rats, an important difference when considering the design of neurotoxicity testing). However, the hypothalamus is not protected by a blood-brain barrier at any time during life and remains in contact with any potentially neurotoxic substances circulating in the blood.[160] Destruction of hypothalamic cells would be expected to disrupt the intricate chemical messenger (hormonal) feedback loops among the hypothalamus, pituitary, and testes or ovaries.

Indeed, studies show that rats treated in the neonatal period with large doses of monosodium glutamate (MSG) have significantly smaller accessory sexual organs and lower concentrations of testosterone.[161] However, the doses of MSG used in these studies are often 2-5 gms/kg on several consecutive days, levels known to cause destruction of hypothalamic neurons, whereas the upper bound of human dietary daily intake of MSG is approximately 35 mg/kg.[162] [163] [164] However, Olney argues that blood glutamate levels, after an oral dose in adult humans, rise 20 times higher than a comparable dose in adult monkeys and five times higher than in mice.[165] Therefore, he says, the margin of safety is not what it appears from animal testing.

One study shows that the offspring of rats fed diets containing 2%, 4%, or 6% aspartame during pregnancy and lactation showed delays in eye opening, swimming, righting, startle response, and walking. [166] Effects were seen at each exposure level. Exposure during nursing had more effect than prenatal exposure. These exposures are approximately 3-9 gms/kg/day, which is about a thousand times higher than expected human exposure levels. However, it is important to remember the lessons from lead, mercury, and PCBs - that animal studies commonly underestimate human sensitivity to developmental neurotoxicants by 100-10,000 fold.

The second focus of concern centers on the apparent capacity of food dyes and additives to alter behavior in some children diagnosed with ADHD or other attentional disorders. In the 1970's, Benjamin Feingold sparked considerable interest when he linked food additives to behavior changes in children with hyperactivity, mood, and behavioral disorders.[167] Since then, the topic has remained highly controversial. A recent report from the Center for Science in the Public Interest reviewed 23 controlled studies, some of which were blinded, and found 17 with evidence that some children's behavior significantly worsens after they consume artificial colors or certain foods. [168] The authors also note that the National Institute of Mental Health largely dismiss diet as a treatment approach and that the Food and Drug Administration denies the effect of diet on behavior. This topic is an instructive intersection of published scientific studies, anecdotal reports, regulators, a publicly funded research institution, burdens of proof, and uncertainty.

## Conclusions

For about 25 years considerable controversy has swirled around the degree to which food additives, including artificial sweeteners, flavor enhancers, colorings, and dyes, may influence children's brain function. Studies show that exposures substantially higher than those in the human diet are necessary to cause observable adverse effects in animals. Historical reviews show, however, that animal tests frequently underestimate the sensitivity of the human brain. Human studies also show that at least some children appear to be particularly sensitive to dietary exposures to these additives, with hyperactivity and decreased attention spans.

The degree to which these food additives contribute to attentional and behavioral disorders in the general population remains uncertain, though it seems clear that some children respond with behavioral changes recognized by parents, teachers, and healthcare providers. The link between diet and behavior in children with ADHD is uncertain and remains a matter of considerable disagreement. A substantial body of literature concludes that the link exists in some children and raises questions about the origins of a heightened sensitivity to these dietary exposures. Genetic and early-life environmental factors must be considered as these questions are explored.

# Toxicants and their Health Effects

| Toxicant | Health Effects/ Characteristics |
|---|---|
| **Metals** | |
| Cadmium – H, A | Learning disabilities<br>Decreased IQ<br>Motor dysfunction<br>Hyperactivity<br>Hypoactivity |
| Lead – H, A | Learning disabilities<br>IQ deficit<br>Attention deficit<br>Impulsivity<br>Violence<br>Hyperactivity<br>Aggression |
| Manganese – H, A | Brain damage<br>Motor dysfunction<br>Compulsive behavior<br>Memory impairment<br>Hyperactivity<br>Learning disabilities<br>Attention deficit |
| Mercury – H, A | Visual impairment<br>Learning disabilities<br>Attention deficit<br>Motor dysfunction<br>Memory impairment<br>(minimal)<br>*At higher levels:*<br>Smaller brain size,<br>cellular distortions<br>in brain<br>Mental retardation |
| **Solvents** | |
| Ethanol (Alcohol) – H, A | Learning disabilities<br>Attention deficits<br>Memory impairment<br>Behavioral disorders<br>Eating and<br>  sleeping disorders<br>Lower brain weight<br>Craniofacial, limb and<br>cardiovascular<br>abnormalities<br>associated with various<br>growth and<br>developmental delays<br>Mental retardation |
| Styrene - A | Decreased activity<br>Decreased avoidance<br>behavior<br>*In conjunction*<br>*with dietary protein*<br>*deficiency:* Lower brain<br>weight, Hyperactivity |

| Toxicant | Health Effects/ Characteristics |
|---|---|
| **Solvents** | |
| Toluene – H, A | Learning disabilities<br>Speech deficits<br>Motor dysfunction<br>Craniofacial<br>abnormalities |
| Trichloroethylene - A | Increased exploratory<br>behavior<br>Hyperactivity |
| Xylene - A | Motor dysfunction<br>Learning disabilities<br>Memory impairment<br>Decreased brain weight |
| **Pesticides** | |
| Organochlorines<br>DDT - A<br>Mixture – H | Hyperactivity<br>Decreased stamina<br>Decreased coordination<br>Decreased memory<br>Decreased ability to<br>draw familiar objects |
| Organophosphates<br>(including DFP,<br>chlorpyrifos (Dursban),<br>diazinon) - A | Developmental delays<br>Hyperactivity<br>Behavioral disorders<br>Motor dysfunction |
| Pyrethroids (including<br>bioallethrin, deltamethrin,<br>cypermethrin) - A | Hyperactivity |
| **Other** | |
| Nicotine – H, A | Hyperactivity<br>Learning disabilities<br>Developmental delays<br>in cognitive functions |
| Dioxins – H, A | Learning disabilities |
| PCBs – H, A | Learning disabilities<br>Attention deficit<br>Memory impairment<br>Hyperactivity<br>Psychomotor dysfunction |
| Fluoride –<br>A<br>H | Hyperactivity<br>Decreased IQ<br>(ecologic studies) |

H= Human studies,
A= Animal studies

**Notes:**

1. Learning disabilities include dysfunctions in listening, speaking, reading, writing, spelling or calculations.

2. Only neurodevelopmental, learning or behavioral effects of toxicants, or physical impairments that lead to them, are listed.

3. Chart information is synthesized from Chapter 6. Please see this chapter for references to studies on these chemicals.

## GLOSSARY OF TESTS OF NEUROLOGICAL DEVELOPMENT IN RODENT STUDIES

Many different tests are used to assess the neurological development of experimental animals. It is important that these tests are validated and that they do, in fact, serve as useful indicators of normal or abnormal neurological function. Test validation has several components. Investigators must conduct testing in very similar conditions using standardized testing protocols. If those conditions are met, similar results should be observed in multiple laboratories and should be replicable in a given laboratory. Any test that is not replicable or that shows wide and unexplained variability in results has little utility for neurological testing. Finally, predictive power and species variability (species concordance) must be considered. These issues are particularly important when using animal tests to predict effects in humans.

The following descriptions are intended to provide the reader with a skeletal description only of the tests mentioned in this report. Standardized protocols for administering the tests are not included.

EARLY REFLEXES:

**Placing response –** the animal is held by the nape of the neck and touched on the chin with a stretched wire. Normally the animal grips the wire with its forelimbs. This response develops within a few days of birth

**Righting reflex** – the animal is placed on its back and observed for speed and ability to turn upright

AVOIDANCE:

**Cliff avoidance** – the animal is placed on a raised platform; the normal response is to turn away when visual stimulus suggests dangerous depth (height)

STRENGTH:

**Grip strength –** the strength with which the animal grips a wire with forelimbs against resistance

LOCOMOTOR ACTIVITY:

**Open field activity** – the animal is placed in the middle of a transparent plastic cage marked off into squares. Numbers of squares entered (total activity), horizontal and vertical movements (rearing), duration of inactivity, description of gait or any abnormal movements, habituation, and response to novel environments may be observed.

**Rotorod performance** – tests the ability of the animal to maintain its balance on a small horizontal cylinder that has a rubberized surface and is rotated by a motor at different standardized speeds. This tests balancing reactions and motor coordination.

**Running wheel** – the animal is observed running inside a rotating wheel

LEARNING AND MEMORY:

**Morris water maze** – the animal capable of swimming is placed in a tank of water with a small platform submerged just below the surface at a specific place in the tank. The animal finds the platform and can stand on it. This can be repeated at various intervals to test learning and memory. The position of the platform can be changed in order to examine the animal's tendency to perseverate or ability to re-learn.

**T maze tests –** the animal is placed in a T shaped maze and learns to find the reward in one arm of the maze. Maze tests, like other tests that require choices, can be reversed so that the animal must learn to change response strategies in order to be rewarded. Changing response strategies is more complex than learning the correct response initially and may be a more sensitive indicator of impaired learning.

**Visual recognition** – various tests are designed to test the animal's ability to recognize shapes, colors, or positions, by rewarding a correct response. Discrimination-reversal learning may then be tested by reversing the correct answer so that the previous correct answer is now incorrect.

**Footnotes to diagrams**

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