Exposures of Children to Organophosphate Pesticides and Their Potential Adverse Health Effects

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Recent studies show that young children can be exposed to pesticides during normal oral exploration of their environment and their level of dermal contact with floors and other surfaces. Children living in agricultural areas may be exposed to higher pesticide levels than other children because of pesticides tracked into their homes by household members, by pesticide drift, by breast milk from their farmworker mother, or by playing in nearby fields. Nevertheless, few studies have assessed the extent of children's pesticide exposure, and no studies have examined whether there are adverse health effects of chronic exposure. There is substantial toxicologic evidence that repeated low-level exposure to organophosphate (OP) pesticides may affect neurodevelopment and growth in developing animals. For example, animal studies have reported neurobehavorial effects such as impairment on maze performance, locomotion, and balance in neonates exposed *in utero* and during early postnatal life. Possible mechanisms for these effects include inhibition of brain acetylcholinesterase, downregulation of muscarinic receptors, decreased brain DNA synthesis, and reduced brain weight in offspring. Research findings also suggest that it is biologically plausible that OP exposure may be related to respiratory disease in children through dysregulation of the autonomic nervous system. The University of California Berkeley Center for Children's Environmental Health Research is working to build a community–university partnership to study the environmental health of rural children. This Center for the Health Assessment of Mothers and Children of Salinas, or CHAMACOS in Monterey County, California, will assess *in utero* and postnatal OP pesticide exposure and the relationship of exposure to neurodevelopment, growth, and symptoms of respiratory illness in children. The ultimate goal of the center is to translate research findings into a reduction of children's exposure to pesticides and other environmental agents, and thereby reduce the incidence of environmentally related disease. *Key words*: asthma, children, environment, exposure, growth, neurodevelopment, organophosphate, pesticide.
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Recent studies have demonstrated that home environments throughout the United States are commonly contaminated with pesticides, including organophosphate (OP), carbamate, organochlorine, pyrethroid, and herbicide compounds (*1–12*). Young children may be highly exposed to these pesticides because of their normal tendency to explore their environment orally, combined with their proximity to potentially contaminated floors, surfaces, and air. Physiologic characteristics of young children, such as high intake of food, water, and air per unit of body weight, may also increase their exposures (*13*). Because children are developmentally immature, they may also be at higher risk for adverse health effects (*13*). This paper reviews information on children's exposure to OP pesticides and potential adverse health effects. We also outline our planned research in Monterey County, California, to investigate exposures to children living in an agricultural area and possible effects on growth, neurobehavioral development, and respiratory disease.

Nationally, approximately 750–800 million pounds of conventional pesticides are used annually in agriculture, excluding sulfur, oils and repellants (*14*). Total conventional pesticide use, including home, structural, and other applications, averages about 1 billion pounds in the United States. During the mid-1990s, national pesticide use levels have been stable (*15*), although trends vary by region. In California, which has the largest agricultural output of all 50 states, approximately 200 million pounds of pesticidal active ingredient are used annually in agriculture (*16*). Pesticide use data for California suggest a trend of increasing use between 1991 and 1995 for production agriculture, postharvest treatment, structural fumigation, and landscape maintenance (*16*). These changes may be due, in part, to unique meteorologic and economic factors, including heavy rains, shifts to lower toxicity compounds that require higher volumes, and changes in planted acreage (*16–18*). Agricultural use of neurotoxic pesticides, including the OPs chlorpyrifos and diazinon, was also higher in 1995 than in 1991, most likely due to increased use on cotton, and to a lesser extent on oranges, alfalfa, apples, and broccoli (*16,17*). Overall, pesticide use in California appears to be stable or increasing, with annual fluctuations making it difficult to identify long-term trends.

Pesticide residue in food may also contribute to children's exposures. In response to concern about low-level exposure, the Food Quality Protection Act of 1996 (P.L. 104–170) (*19*) was unanimously passed by the U.S. Congress to address pesticide food safety issues raised by the seminal 1993 National Academy of Sciences report Pesticides in the Diets of Infants and Children (*13*). This report drew the public's attention to the specific vulnerability of children to many pesticides. The National Academy of Sciences committee found that current tolerances for pesticide levels in food are not health based and may not adequately protect children. Congress specifically directed the U.S. Environmental Protection Agency (U.S. EPA) to reevaluate food tolerances and establish health-based standards that account for children's unique sensitivity to environmental toxicants. The law requires the U.S. EPA to consider all nonoccupational sources of pesticide exposure, especially exposure to compounds with similar mechanisms of toxicity. The National Academy of Sciences recommended in 1993 that the U.S. EPA modify its

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decision-making process for setting pesticide tolerances to reflect the unique characteristics of the diets of infants and children and account also for all nondietary intake of pesticides (*13*). Findings from several small, cross-sectional studies (*3–6,8,12*) indicate that nondietary exposures to young children from residential contamination may be an important component of total pesticide exposure. Unfortunately, our knowledge about the actual levels of pesticide exposures of children from food and environmental exposures and their potential health effects is extremely limited.

Children's Exposure to Pesticides

National population-based surveys of pesticide urinary metabolites in adults indicate widespread exposure to pesticides, including organophosphates, carbamates, wood preservatives, and fungicides (*20,21*). For example, Hill et al. (*20*) detected chlorpyrifos, an OP pesticide, in 82% of 993 adults tested through the National Health and Nutrition Examination Survey, and found a 5-fold increase in the proportion of adults with levels over 5 μg/L compared to earlier surveys, suggesting increasing exposure in the general population. OPs are eliminated from the body after 3–6 days (*22*), so the widespread detection of these compounds indicates continuing exposure.

Biologic information on children's pesticide exposure is very limited. Hill et al. (*23*) reported detections of dichlorobenzene and wood preservatives in 96% and 100%, respectively, of 197 Arkansas children, whereas phenoxy herbicide metabolites were found in 20% of all samples. Preliminary results from the federal Agricultural Health Study indicate detectable pesticide residues in children's urine (*8*). Loewenherz et al. (*24*), working in Washington state, found that 44% of children of pesticide applicators and 27% of nonfarm, rural children had detectable OP residues. In preliminary data from Arizona, chlorpyrifos was detected in 100% of about 40 children >6 years of age sampled in a population-based survey, and approximately 25% of 150 children ≤6 years of age sampled in an agricultural area. Detection limits in the second survey were higher (*25*). Comparison of these studies to each other and to data reported by Hill et al. (*20*) is difficult because of differences in detection limits, sample type (spot samples vs first morning void), and ages of participants. Overall, these studies suggest the potential for widespread low-level pesticide exposure in children and the need for population-based studies to establish norms.

Pesticides enter children's bodies via dermal absorption, ingestion, and inhalation. Exposure in the home depends on the frequency, duration, and nature (i.e., dermal contact, hand-to-mouth behavior) of the child's interaction with contaminated media such as house dust. Children may have higher exposure to pesticides than other residents living in the same contaminated environment, in part because young children spend more of their time indoors at home (*26,27*). Thus, they are likely to spend more time in proximity to any pesticides present in their immediate environment. The importance of specific exposure-related behaviors, such as hand-to-mouth activity, will be age dependent, suggesting that consequent exposure levels and pathways will vary with age, as has been observed for lead exposure (*28*). For example, children younger than 6 months of age may receive their greatest exposures through breast milk or inhalation, but dermal absorption and ingestion may be the major pathway of exposure when children begin crawling and placing their hands on dusty surfaces and increasing their hand-to-mouth behavior. The level of exposure may continue to increase, given that the normal tendency of young children to explore their environment orally increases through 2 years of age. The actual dose to the child will depend on environmental concentrations and the efficiency of pesticide uptake for the different types of exposure routes, i.e., dermal contact versus ingestion. To date, direct observation and quantification of children's exposure-related activity patterns and their interaction with their environment is very limited. Time–activity analysis thus could provide information about age-specific exposure pathways.

To assess time–activity patterns, most researchers have preferred self-administered time diaries and interviews (*29,30*). However, these diaries are subject to inaccurate recall and thus have limited validity (*31–33*). Moreover, they fail to document microactivities such as dermal and hand-to-mouth contacts, which are important pathways of exposure in young children. Observational techniques are more detailed and accurate than conventional methods of questionnaires and diaries for recording such microscopic data (*34–37*). Leckie and Zartarian and co-workers (*36–39*) have successfully developed videotaping methodologies and video translation software to quantify children's activity patterns for dermal and nondietary contacts, and have piloted these techniques on 2- to 4-year-old children of Mexican American farmworkers in California (*36*). More extensive data collection is needed on children of various ages to assess the changing pathways and routes of exposure as children develop.

Exposures of Farmworkers and Their Families

Nationally, an estimated 5 million farmworkers work on America's farms, including approximately 1 million California residents (*40*). A growing body of literature indicates that resident farm families, hired farmworkers, and their children are among those most highly exposed to pesticides (*5,6,8,10, 12,24,41–49*). These studies suggest that farm children can be exposed by the same pathways as other children, namely through consumption of contaminated food, by household use of pesticides, as a result of drift from nearby agricultural applications, by contaminated breast milk from their farmworker mothers, by playing in the fields, and through pesticides tracked into their homes by their parents or other household members working in fields (*5,6,8,10, 12,24,41–43,50,51*). For example, preliminary data from pilot studies conducted for the Agricultural Health Study in North Carolina and Iowa indicate elevated levels of recently applied pesticides in the food, homes, and bodies of farm families and their children (*8,10,12,43,44*). In a study of 88 children in the Yakima Valley, Washington, Loewenherz et al. (*24*) reported more frequent detection of OP metabolites in children of pesticide applicators compared to nonapplicators, particularly those living less than 200 ft from orchards. Trends of increasing exposure with decreasing age also suggested that child activity is an important exposure variable.

Simcox et al. (*5*) studied 59 families in the Yakima Valley, and compared levels of four organophosphate (OP) pesticides in the homes of hired farmworkers, families residing on farms, and nonagricultural families. Chlorpyrifos was detected in 95% of the homes. House dust concentrations were consistently higher for agricultural families than for nonagricultural families, and pesticide applicators tended to have higher house dust concentrations compared to nonapplicators. There was a 3-fold difference in median chlorpyrifos house dust concentration between farmworkers who did not directly handle pesticides and reference families of nonfarmworkers living in agricultural communities (median

=172 ng/g for farmworker vs 53 ng/g for nonagricultural families).

Bradman et al. (*6*) conducted a pilot study of pesticide exposures to children of migrant farmworkers and nonfarmworkers living in a largely Latino community in California's Central Valley. Floor dust samples and child hand wipes were collected from the homes of 10 families, 5 of which had at least one resident farmworker. Higher levels of the OPs diazinon (maximum = 160 ppm), chlorpyrifos (maximum = 33 ppm), and malathion (maximum = 1.6 ppm) were found in house dust in farmworker homes (*6*). Residues of diazinon and chlorpyrifos were detected on the hands of two and three farmworker toddlers, respectively, who also lived in the homes with the highest dust concentrations. A preliminary risk assessment suggested that diazinon exposures in children could exceed the U.S. EPA Office of Pesticide Program's oral reference dose of 9E-5 mg/kg/day.

Overall, the findings from these studies suggest that inadvertent carry-homes of occupational pesticides are occurring and that contamination in the homes of farm families are likely to be higher than in other homes. Further, a significant source of exposure to farmworker families may derive from their residential proximity to fields.

Potential Health Effects of Exposure to OP Pesticides

Studies of the effects of pesticide exposure on children's health have been limited to those of birth defects, childhood cancer, and acute poisonings following ingestion. Several case–control studies have associated parental exposure to pesticides or pesticide use in the home with childhood brain tumors, leukemias and lymphomas, testicular cancers, and other cancers (*52–55*). Other studies have reported that parental exposure to pesticides or application of pesticides in the home is associated with certain birth defects including neural tube and other birth defects (*56,57*).

To date, only one small ecologic study has examined whether low-level chronic exposure of children to pesticides can lead to adverse health consequences (*58*). This study of Yaqui children in Mexico found that children 4–5 years of age (n = 33) living in an agricultural valley with presumably higher pesticide exposure had deficits in tests of stamina, coordination, recall, and ability to draw a person, compared to children (n = 17) living in the foothills where there was mostly ranching. This study, although suggestive of an effect of pesticides, is limited by small sample size, utilization of a convenience sample, the lack of individual exposure data, and no statistical control of potential confounders. At present, the only prospective study investigating pesticides and adverse health effects is the National Cancer Institute/U.S. EPA Agricultural Health Study, which is a large cohort study of cancer in midwestern and eastern farmers and their families (*49*). In spite of the paucity of information concerning the potential health effects in children of chronic low-level exposure to organophosphates, there is substantial evidence in developing rodents and limited evidence in adult humans who have been chronically exposed to OPs that low-level chronic exposure to organophosphates may affect neurologic functioning, neurodevelopment, and growth. Because OP exposure may cause dysregulation of the autonomic control of airways, it is biologically plausible that exposure may be related to the occurrence of asthma in children.

Effects of Acute Exposure to OP and Carbamate Pesticides in Children

The primary effects of OP and carbamate acute exposure are on the parasympathetic, sympathetic, and central nervous system. These pesticides interfere with the metabolism of acetylcholine (ACh) by inhibiting the enzyme that hydrolyzes it, acetylcholinesterase (AChE) (*59*). ACh accumulates at the neuronal junctions, resulting in the continued stimulation and then suppression of neurotransmission to organs. ACh is the chemical transmitter of somatic motor neurons to skeletal muscle, postganglionic parasympathetic nerve fibers, preganglionic fibers of both sympathetic and parasympathetic nerves, and some fibers in the central nervous system. The accumulation of ACh at the motor nerves results in weakness, fatigue, muscle cramps, fasciculations, and muscular weakness of respiratory muscles. Accumulation at the autonomic ganglia results in increased heartbeat and blood pressure, pallor, and hypoglycemia. Accumulation of ACh at muscarinic receptors results in visual disturbances, tightness in the chest and wheezing due to bronchoconstriction and increased bronchial secretions, and increased salivation, lacrimation, sweating, peristalsis (resulting in nausea, vomiting, cramps, diarrhea), and urination. Central nervous system effects from ACh accumulation include anxiety, headache, confusion, convulsions, ataxia, depression of respiration and circulation, slurred speech, tremor, and generalized weakness (*59,60*). Carbamates, unlike OPs, do not irreversibly inhibit ACHE. Thus, their activity is quickly reversed after excretion of the pesticide (*61*). Pregnancy may pose a time of increased risk because plasma AChE activity is already reduced, at least during the first two trimesters (*62,63*).

The most frequent acute symptoms of OP poisoning in children include miosis, excessive salivation, nausea and vomiting, lethargy, muscle weakness, tachycardia, hyporeflexia, and hypertonia, and respiratory distress (*60,64*). Duration of symptoms depends on the dose, with the highest doses resulting in death. In one study, pneumonitis developed in about one-third of poisoned children (*64*). OP-induced delayed onset peripheral neuropathy (OPIDN), reported for adults, has not been reported in children.

Long-Term Sequelae of Acute Exposure to OP and Carbamate Pesticides in Adults

Although no studies have examined the long-term sequelae of acute pesticide poisoning in children, some studies in adults suggest that there are residual effects. Neuropsychologic investigations of poisoned farmworkers, pest control workers, and industrial workers tested a number of months to years after acute exposure to various OP pesticides have revealed deficits in overall abstraction, verbal and visual attention, visual memory, visuomotor speed, sequencing, visuomotor problem solving, motor steadiness, motor dexterity, and fine motor speed (*65–68*). These workers report anxiety, depression, irritability, confusion, and impaired concentration and memory. On neurologic exam, lower vibrotactile sensitivity has been reported (*68,69*). High acute or subchronic exposures to OPs may also result in delayed neurotoxicity or OPIDN (*59,70,71*). OPIDN usually manifests 1 to 6 weeks after exposure and may result in moderate to severe peripheral neuropathies lasting months, years, or indefinitely (*59*).

Effects of Chronic Exposure to OP and Carbamate Pesticides in Adults

Although there are no studies in children on the neuropsychologic effects of chronic pesticide exposure, small studies of chronic low-level exposures of farmers or pest

control workers who had levels of AChE within normal limits found no differences in tests of their neurobehavioral functioning compared to those of unexposed controls (*72*) or in a pre/post exposure comparison (*73*). Results of other studies in adults indicate that there may be mild peripheral effects of chronic lower level exposure as indicated by slower reaction times (*74*), impaired proprioception (postural sway) (*75*), decreased conduction velocities in motor (median and peroneal) and sensory (median and sural) nerves (*76*), wider two-point discrimination (*77*), as well as some neurobehavioral effects such as increased anxiety (*78*), decreased visuomotor speed (*65,79,80*), and short-term verbal memory (*79*). Daily exposure to OPs that are insufficient to cause signs and symptoms of acute poisoning may also produce an influenza-type illness characterized by weakness, anorexia, and malaise (*81*). In chronic lower level exposures, depression of cholinesterase activity may be cumulative, and there is no predictable correlation between the severity of symptoms and the degree of cholinesterase inhibition.

Animal Evidence for Neurodevelopmental Effects of Exposure to OP Pesticides

There is a strong and growing body of evidence linking exposure to OP pesticides during gestation or the early postnatal period and neurodevelopmental effects in animals. These effects may be due to the direct impact of OPs on the cholinergic system of the fetus, although effects on cellular intermediates such as adenylyl cyclase (*82*) and altered DNA synthesis in the brain through noncholinergic mechanisms (*83–85*) have been hypothesized. Table 1 summarizes animal studies investigating different organophosphate pesticides, dosing regimes, and exposure routes and their impacts on the developing nervous system.

Chlorpyrifos

Neurobehavioral tests given postnatally found that animals exposed *in utero* demonstrated decreased balance (*86*), increased righting reflex time, and poorer cliff avoidance (*87,88*). When exposure occurred in the early postnatal period, there was a lowered threshold for convulsions (*89*) as well as increased gait abnormalities and tremors (*90*) and deficits in delayed alternation on mazes (*91*).

Some studies suggest that early gestation may be a critical period for the neurodevelopmental effects of certain pesticides. Muto et al. (*86*) studied the effects of exposure to chlorpyrifos in rats occurring both during gestation (gestation days 0–7 and 7–21) and the postnatal period. They reported that early prenatal exposures were more likely to result in poorer performance on the rotorod test than exposures during later gestation, which was in turn, more likely to result in deficits than those occurring postnatally.

A number of mechanisms have been proposed to explain the observed neurobehavioral effects in animals. Chanda and Pope (*88*) found that repeated exposure of rodents to low levels of chlorpyrifos during gestation was related to inhibited levels of AChE and downregulated muscarinic receptors in the fetal brain. Transient brain AChE inhibition also has been consistently reported in neonates postnatally exposed to chlorpyrifos (*90–94*). Other effects of chlorpyrifos that, in part, could explain the neurobehavioral impairment include decreased muscarinic receptor binding (*90,94,95*), altered brain RNA concentrations (*96*), and inhibition of brain DNA synthesis (*84,85,97*). For example, after treating rats on postnatal days 1–4 with a dosage that produced minimal AChE inhibition (1 mg/kg), Dam et al. (*85*) reported large deficits in DNA synthesis in the brain stem and forebrain, with lesser effects on the cerebellum. Similar deficits in DNA synthesis were observed after a single early postnatal exposure but at a slightly higher dose (*97*). Early postnatal exposure to chlorpyrifos (postnatal days 1–4 or 11–45) also altered RNA concentrations in the brain stem and forebrain of rats (*96*). By targeting RNA, the macromolecule that controls postmitotic processes of cell differentiation and growth, the chemical may evoke alterations in cell function and number in developing organisms (*96*).

The results of these studies indicate that OP pesticides could contribute to behavioral abnormalities in young animals by producing cellular deficits in the developing brain. Recently, Campbell et al. (*84*) concluded that regions rich in cholinergic projections, such as the brain stem and forebrain may be more affected than the less cholinergic regions such as the cerebellum. However, the maturational timetable of each region (brain stem then forebrain then cerebellum) may be an important factor in determining relative vulnerability. Nevertheless, there is reasonable evidence that even subtoxic exposure to chlorpyrifos during the critical period of brain development could produce cellular, synaptic, and neurobehavioral aberrations in animals (*97*).

Other OP Pesticides

OPs other than chlorpyrifos have been associated with lowered AChE in the brain of rodents exposed prenatally [bromophos (*98*), dichlorvos (*99*), dimethoate (*100*), methyl parathion (*101*), quinalphos (*102*)]. Studies in which animals were exposed early in the postnatal period to these other organophosphate pesticides have also reported inhibition of brain AChE [dichlorvos (*103*), diisopropylfluorophosphate (*104*), quinalphos (*105*), parathion (*92,93,95,106*)] and downregulation of muscarinic receptors [diisopropylfluorophosphate (*104,107*), parathion (*95,106*)]. In addition, evidence from a single *in vitro* study suggests that prenatal exposure to organophosphates could alter human fetal brain AChE levels. For example, Banerjee et al. (*108*) reported a dose-dependent inhibition of cerebellar AChE activity in human fetal brain cells (8–10 weeks gestation) treated with 5×10^{-11} – 5×10^{-8} M diisopropylfluorophosphate. Further research in rodents has found reductions in brain weight, most pronounced in the cerebellum and brainstem, following OP exposure during gestation [dichlorvos (*109*), trichlorfon (*109–111*)].

Neurobehavioral deficits such as impaired maze performance [dichlorvos (*99*), diazinon (*112*)], decreased open-field activity [sumithion (*113*)], impaired locomotion or swimming [trichlofon (*110*), diazinon (*112*)], and reduced time on the rotorod test [diazinon (*112*), sumithion (*113*)] have also been associated with prenatal organophosphate exposure. In addition, permanent alterations in spontaneous motor behavior (i.e., locomotion, rearing, and total activity) have been observed in mice exposed to a single subsymptomal dose of diisopropylfluorophosphate early in the postnatal period (*107*).

Other Potential Developmental Effects of OP Pesticides

Decreased Birth Weight and Altered Growth

A number of the animal studies reported above have demonstrated a decrease in birth weight or body weight in developing animals exposed to OPs. Anticholinesterase agents such as OPs may have a nonspecific regulatory effect on growth, perhaps by an influence on placental transport of nutrients (*112,114*) or by altering the activity and reactivity of the adenylyl cyclase

Table 1. Review of the literature of the effects of organophosphate pesticides on neurobehavioral functioning in developing animals.[a]

Author, year	Species	Agent (dose mg/kg) Route	Exposure period	Neurodevelopmental effects
Spyker and Avery, 1977 (*112*)	Mouse	Diazinon (0.18, 9) Oral	Throughout gestation	Lower birth weight Decreased rate of postnatal weight gain Balance (rotorod), swimming, and maze (speed) effects No differences in auditory startle, visual cliff response, or open-field motor activity
Crowder et al., 1980 (*132*)	Rat	Methyl parathion (1.0) Gavage	GD 7–15	Slight changes in learning ability as measured by simple two-choice maze Effects on open-field activity
Maslinska et al., 1981 (*103*)	Rabbit	Dichlorvos (9.0) Gavage	PND 6–16 or 16	Inhibited AChE activity in all brain regions tested; recovery slower in animals exposed over 10 days than after a single dose Decreased serotonin concentration in brainstem (22%), mesencephalon (26%), and hippocampus (59%) after prolonged exposure
Gupta et al., 1985 (*101*)	Rat	Methyl parathion (1.0, 1.5) Oral	GD 6–12	Altered postnatal development of brain cholinergic neurons Reduced AChE activity in all brain regions (1.5 mg/kg) Increased choline acetyltransferse activities in all brain regions (1.5 mg/kg) Subtle alterations in selected behaviors: impaired cage emergence, accommodated locomotor activity, and operant behavior in a mixed paradigm No morphologic changes in hippocampal or cerebellar tissue
Berge et al., 1986 (*110*)	Guinea pig	Trichlorfon (100) Gavage	GD 36–38 or 51–53	Disturbances of locomotion Reduced brain weight, particularly cerebellum, hippocampus, thalamus, and colliculi
Pope et al., 1986 (*133*)	Pig	Trichlorfon (60) Oral	GD 55 or 55 and 70	Dose-related cerebellar hypoplasia Ataxia not observed in neonates
Stamper et al., 1988 (*95*)	Rat	Parathion (1.3, 1.9) Subcutaneous	PND 5–20	Dose-dependent reductions in AChE activity and muscarinic receptor binding in the cortex No differences in most reflex measures, eye opening, or incisor eruption during the preweanling period Small deficits in tests of spatial memory in both the T-maze and the radial arm maze during the postweanling period
Lehotzky et al., 1989 (*113*)	Rat	Sumithion (5, 10, 15) Gavage	GD 7–15	Dose-related decrease in open-field activity and motor coordination (rotorod) Alterations in acquisition and extinction of a conditioned escape response Increased social interactions No significant behavioral effects at lowest dose (5 mg/kg)
Clemens et al., 1990 (*134*)	Rat	METASYSTOX-R (Methyl demeton) (0.5, 1.5, 4.5) Oral	GD 6–15	No differences in fetal brain AChE No differences in neonatal survival, growth, and development No alteration of sensory or reflex functions, maze learning ability, or open-field activity
Veronesi and Pope, 1990 (*106*)	Rat	Parathion (0.882) Subcutaneous	PND 5–20	Cellular disruption and necrosis in the dentate gyrus and CA4 regions of the hippocampus Depressed hippocampal AChE (73%) and muscarinic [^{3}H] QNB binding (36%) at PND 12
Pope et al., 1991 (*92*)	Rat	Methyl parathion (7.8) (adult: 18) Parathion (2.1) (adult: 18) Chlorpyrifos (45) (adult: 279) Subcutaneous	PND 7 and adult	ChE inhibition in whole brain, plasma, and erythrocytes (transient) Maximal brain ChE inhibition (>78%) similar in both age groups ChE activity recovered faster in neonate
Muto et al., 1992 (*86*)	Rat	Chlorpyrifos (0.03, 0.1, 0.3) (0.1, 0.3) Intraperitoneal	GD 0–7 or 7–21 PND 3, 10 or 12, 6–10	Lower body weight Balance effects (rotorod) Effects on open-field motor behavior Effects early gestation > late gestation > postnatal
Pope and Chakraborti, 1992 (*93*)	Rat	Methyl parathion (< 7.8) (adult: < 18)[b] Parathion (< 2.1) (adult: < 18)[b] Chlorpyrifos (< 45) (adult: < 279)[b] Subcutaneous	PND 7 and adult	Inhibition of brain and plasma ChE activity in both neonate and adult Good correlation between brain ChE (r = 0.93) or plasma ChE (r = 0.99) inhibitory potency and acute toxicity

(Continued)

Table 1. *Continued.*

Author/Year	Species	Agent (dose mg/kg) Route	Exposure period	Neurodevelopmental effects
Srivastava et al., 1992 (*102*)	Rat	Quinalphos (0.5, 1.5) Gavage	GD 6–20	Reduced AChE activity in fetal brain (0.5–1.5 mg/kg) and placenta (1.5 mg/kg) No differences in fetal weight or anomalies
Balduini et al., 1993 (*104*)	Rat	Diisopropyl-fluorophosphate (0.5–1.0) Subcutaneous	PND 4–9 or 4–20	Inhibition of AChE Downregulation of muscarinic receptor recognition sites These alterations may delay the maturation of the cholinergic system and may account for some long-lasting neurotropic effects observed after developmental exposure
Wurpel et al., 1993 (*89*)	Rat	Chlorpyrifos (0.3–10) Subcutaneous	PND 16 or 17	More rapid amygdala kindling in treated animals Proconvulsant effect was dose related Increased excitability of the amygdala
Chakraborti et al., 1993 (*94*)	Rat	Chlorpyrifos (40) Subcutaneous	PND 7–10	AChE activity 55–60% controls Less inhibition of AChE in neonate relative to adult Muscarinic [^{3}H] QNB receptor binding in cortex, hippocampus, and striatum marginally affected (5–11% reduction) in neonate Basal motor activity levels not affected
Mehl et al., 1994 (*109*)	Guinea pig	Dichlorvos (15–30) Trichlorfon (125) Subcutaneous	GD 42–46	Reduction in brain weight Most pronounced in cerebellum, medulla, thalamus, hypothalamus, and quadrigeminal plate
Santhoshkumar and Shivanandappa, 1994 (*98*)	Rat	Bromophos (500) Gavage	GD 18	AChE inhibition in fetal brain started at 2 hr and reached a maximum at 16 hr postexposure (transient) Recovery almost complete by PND 1 Sensitivity of ChE inhibition *in vivo*: maternal serum > maternal brain > fetal brain
Stanton et al., l994 (*91*)	Rat	Chlorpyrifos (90, 120, 240) Subcutaneous	PND 21	Signs of severe poisoning prevented behavioral testing at highest dose Transient memory impairment on maze (120 mg/kg) Dose-related inhibition of brain AChE but transient Reduced muscarinic binding of [^{3}H]QNB in frontal cortex (240 mg/kg)
Ahlbom et al., 1995 (*107*)	Mouse	Diisopropyl-fluorophosphate (1.5) Gavage	PND 3, 10, or 19	Altered spontaneous motor behavior (increased locomotion, rearing, and total activity) observed at adult age of 4 months Decreased muscarinic receptor density at adult age Persistent effects found in adult mice exposed to single subsymptomal dose on PND 3 or 10; not in animals exposed on PND 19
Chanda et al., 1995 (*87*)	Rat	Chlorpyrifos (200) Subcutaneous	GD 12	Decreased brain AChE activity in both dams (85–88%) and fetuses (42–44%) By PND 3, brain AChE still inhibited in pups (30%); less than for dams (82%) *In vitro* inhibition of maternal and fetal brain AChE activity indicated that prenatal AChE activity was somewhat more sensitive Righting reflex time was increased in PND 1 pups No differences in righting reflex at PND 3
Nagymajtenyi et al., 1995 (*135*)	Rat	Dimethoate (7, 10.5, 14, 28) Dichlorovos (1, 1.5, 2, 3.9) Methyl parathion (0.2, 0.3, 0.4, 0.9) Gavage	Three generations Males and non-pregnant females: 5 days/week Pregnant females throughout gestation and lactation	Altered electrophysiological function in primary somatosensory, visual, and auditory cortex Increased mean frequency and EEG index, and decreased mean amplitude dose dependent Changes more expressed in second and third generations AChE inhibition in brain (significant at highest dosages)
Schulz et al., 1995 (*99*)	Rat	Dichlorvos (0.97–3.88) Oral	Throughout gestation and lactation	Increased maze running time and errors AChE in brain 40–65% control Changes were dose related
Whitney et al., 1995 (*97*)	Rat	Chlorpyrifos (2.0) Subcutaneous	PND 1	Inhibition of DNA synthesis within 4 hr of treatment and at 8 days of age in all brain regions Concluded that low doses target the developing brain during critical period in which cell division is occurring, effects that may produce eventual cellular, synaptic, and behavioral aberrations after repeated or prolonged subtoxic exposures
Breslin et al., 1996 (*115*)	Rat	Chlorpyrifos (0.1, 3, 15) gavage (0.1, 1, 5) oral	GD 6–15 Two generations: 5 days/week	No teratogenic effects found in animals exposed on gestational days 6–15 Two generation study: AChE inhibition in brain (52% control) and decreased body weight in F1 litters (5 mg/kg)

(Continued)

Table 1. *Continued.*

Author/Year	Species	Agent (dose mg/kg) Route	Exposure period	Neurodevelopmental effects
Chanda and Pope, 1996 (*88*)	Rat	Chlorpyrifos (6.25–25) Subcutaneous	GD 12–19	AChE inhibition Righting reflex and cliff avoidance affected Dose-related downregulation of muscarinic receptors Low-level, repeated exposures caused extensive neurochemical and neurobehavioral changes
Srivastava and Raizada, 1996 (*100*)	Rats	Dimethoate (3.75, 7.5, 15, 30) Gavage	GD 6–20	Dams exposed to 15 and 30 mg/kg produced 20 and 60% mortality, respectively; highest dosage group not considered for toxicity evaluation Inhibition of AChE activity in fetal brain and placenta dose dependent; less than for dams Reduced fetal weight No teratogenic effects
Campbell et al., 1997 (*84*)	Rat	Chlorpyrifos (1, 5) Subcutaneous	PND 1–4 or 11–14	Significant mortality and severe cell loss in brainstem at higher dose (PND 1–4) Cell loss in forebrain (PND 11–14); loss occurred after treatment DNA levels elevated in cerebellum after exposure, then subnormal
Song et al., 1997 (*82*)	Rat	Chlorpyrifos (1, 5) Subcutaneous	PND 1–4 or 10–14	Deficits in adenylyl cyclase cascade in forebrain, cerebellum, and heart Disrupted signaling function in transduction of both cholinergic and adrenergic signals Concluded that disruption of cell development is not restricted to cholinergic targets, nor even to the central nervous system
Dam et al., 1998 (*85*)	Rat	Chlorpyrifos (1.0) Subcutaneous	PND 1–4	Decreased DNA synthesis in brainstem and forebrain on PND 5 (24 hr after last treatment) No effects seen 4 hr after treatment Smaller deficits in cerebellum No differences in RNA or protein synthesis
Gupta et al., 1998 (*105*)	Rat	Quinalphos (0.5) Oral	PND 10–21 or 10–45	Decreased AChE in the brain and blood but transient Increased superoxide radical generation in brain: 43% by PND 21 and 59% by PND 45 Enhanced brain lipid peroxidation: 28% by PND 45 Produced cerebral oxidative stress, which may affect central nervous system function
Hjelde et al., 1998 (*111*)	Guinea pig	Trichlorfon (25–200) Gavage or subcutaneous	GD 42–44 or 34–53	Reduced weight in almost all regions of the brain, especially cerebellum, medulla, and hypothalamus Minimum dose required for effect was 100 mg/kg over 3 consecutive days Suggested alkylation of DNA or effects on its repair capability as possible mechanisms
Johnson et al., 1998 (*96*)	Rat	Chlorpyrifos (1, 5) Subcutaneous	PND 1–4 or 11–14	Altered RNA concentrations in the brain stem and forebrain at subtoxic doses Targeted macromolecules that control cell differentiation during critical postmitotic period; may elicit delayed developmental neurotoxicity as a result Concluded that the developing brain is a selective target for chlorpyrifos
Moser and Pedilla, 1998 (*90*)	Rat	Chlorpyrifos (15, 80) Gavage	PND 17 and adult	Behavioral effects (lowered activity, gait abnormalities, tremors, smacking) and ChE inhibition at 5-fold lower dose than adult Maximal effects occurred at 6.5 hr after dosing Blood and brain ChE recovery by PND 24 Decreased muscarinic receptor (QNB) binding

Abbreviations: AChE, acetylcholinesterase; ChE, cholinesterase; GD, gestational day; PND, postnatal day; QNB, quinuclidinyl benzilate. [a]All reviewed studies contain an unexposed control group for comparison. [b]Animals were treated with graded doses up to the maximum tolerated dose (MTD).

signaling cascade, which would disrupt cell development in all areas of the body, not only those cholinergically regulated (*82*).

Muto et al. (*86*) reported lower body weight in rats exposed during the first 7 days of gestation to chlorpyrifos (0.03 mg/kg); with higher doses they found a decrease in the length of the limbs (0.1 and 0.3 mg/kg) and head circumference (0.3 mg/kg). Other studies have reported a decrease in pup weight (*115*) and a decrease in weight gain postnatally following exposure to chlorpyrifos (*88*) or diazinon (*112*) during gestation. Spyker and Avery (*112*) also reported lower birth weight and a slower rate of postnatal weight gain in mice exposed to diazinon (9 mg/kg) throughout gestation. In rats, low levels of two carbamates and a triazine herbicide administered postnatally interacted to increase thyroxine levels and alter levels of somatotropin, hormones that regulate growth (*116*).

Potential Respiratory Health Effects

Much of the animal literature reviewed here has focused on the central nervous system effects of organophosphate exposure. Because OP pesticides exert their pharmacologic effects through inhibition of AChE, both short- and long-term effects on autonomic regulation are prominent features in the toxicology of this class of pesticides (*76,117*). No previous work has addressed the autonomic sequelae of pesticide exposure per se, yet disorders of autonomic regulation may be one of the earliest and most sensitive measures of long-term physiologic effects of exposures in infants and young children.

Similarly, the parasympathetic nervous system provides the principal neural control of lung airway tone. There are considerable data indicating that dysregulation of both parasympathetic (cholinergic) and sympathetic autonomic control of airways, such as by pesticide exposure, may be important in the occurrence of asthma and its severity (*118*). Dysregulation of parasympathetic function, as measured by respiratory sinus arrhythmia, predicts the onset of wheezing in adults (*119*). Although there are few direct studies of the effects of OP and carbamate pesticide exposure on asthma risk, farmworkers' exposure to carbamate pesticides has been associated with the occurrence of asthma after adjustment for other relevant factors (*120*). Professional fumigators reportedly have an increased occurrence of allergy and asthma in parallel with a higher risk of a > 20% decrease in red blood cell AchE (*121*). Exposure to chlorpyrifos has also been associated with an increase in the occurrence of atopic conditions (*122*). Although none of these studies involved children, they raise the prospect that pesticide exposure could be important etiologic and morbidity-modifying factors in the occurrence of childhood asthma.

Biologic Plausibility for the Effects of Low-Level Chronic Pesticide Exposure in Children

Tests on young rodents demonstrate a progressive decrease in susceptibility to OP pesticides with increasing age (*13,123–125*). In some cases, the lethal dose in immature animals is only 1% of the adult lethal dose (*92,93,97*). A study of rats found that animals 1 and 7 days of age tolerated only 4% and 17% of the adult dose, respectively (*92,93,97*). Seven-day-old rats were 2.3, 8.6, and 6.2 times more sensitive than adults to the acute toxicity of methyl parathion, parathion, and chlorpyrifos, respectively (*92*). In humans, children have had higher fatality rates than adults in several cases of OP poisoning (*13*).

Young animals may be more susceptible to the toxic effects of organophosphates due to lower activity of detoxifying enzymes such as paraoxonase that deactivate active OP metabolites (e.g., paraoxon, chlorpyrifos–oxon) (*123,126–131*). For example, Mortensen et al. (*126*) reported markedly lower plasma and liver chlorpyrifos–oxonase levels in neonate compared to adult rat tissue. They concluded that the higher sensitivity of young rats to acute chlorpyrifos toxicity may not be explained by increased sensitivity of the target enzyme, brain AChE, but it may be partially explained by a deficiency of chlorpyrifos–oxonase activity (*126*).

Although young animals are more sensitive than adults to the acute toxic effects of chlorpyrifos, some researchers have suggested that lower level chlorpyrifos exposures may produce more extensive neurobehavioral effects in the adult rat than in the neonate (*94*). In addition, more extensive changes have been found in cholinergic parameters in the maternal brain compared to the fetus or neonate (*88*). Developing animals also appear to recover more quickly from cholinesterase inhibition than the adult (*92,98*), and may be less susceptible to developing OPIDN (*71*). However, repeated low-dose chlorpyrifos exposure during gestation has been associated with persistent neurochemical and neurobehavioral changes in developing rodents (*88*).

In summary, young children may be especially vulnerable to pesticides because of the sensitivity of their developing organ systems combined with a limited ability to enzymatically detoxify these chemicals (*13,123,126–131*). According to the National Academy of Sciences (*13*), children's OP exposures are of special concern because "exposure to neurotoxic compounds at levels believed to be safe for adults could result in permanent loss of brain function if it occurred during the prenatal and early childhood period of brain development" (*13*). Because there is so little information available on the levels and routes of children's pesticide exposure, it is not feasible to conduct a risk assessment predicting the likelihood of adverse effects based on animal studies. Thus far, there are no data in children to support or refute the hypothesized health effects of chronic low-level pesticide exposure.

Future Directions

There is clearly a lack of information on the sources, pathways, and levels of pesticide exposures of children, and in particular, of those children at highest risk because they live in agricultural communities. Similarly, there is a dearth of information on whether low-level chronic exposure to pesticides is associated with adverse health effects. The goal of the Center for Children's Environmental Health Research at the University of California, Berkeley is to address these questions by conducting a longitudinal cohort study of approximately 500 pregnant women and their children who live in a rural agricultural community in the Salinas Valley of Monterey County, California. The specific aims of this Center for the Health Assessment of Mothers and Children of Salinas, or CHAMACOS (which means "little child" in Chicano Spanish), are to *a*) characterize OP exposure levels and pathways in pregnant women and their children; *b*) determine the predictors of OP levels in the body and home; *c*) describe the exposure-prone behavior of children at different developmental stages using time–activity analysis; and *d*) follow up the children to 3 years of age to determine whether exposure *in utero* and/or during the postnatal period is associated with poor neurodevelopment (assessed by tests of the central and autonomic nervous system), slower and stunted growth, and increased prevalence of respiratory symptoms and disease. Our ultimate goal is to involve community partners in planning, coordinating, and conducting an intervention to reduce pesticide exposures to young children in this agricultural community and to evaluate the efficacy and sustainability of the intervention. To accomplish our goals, we have established a multidisciplinary partnership comprised of farmworkers, health care providers, growers, journalists, scientists, educators, and representatives of community groups and state and county health and agricultural departments. We are hopeful that the results of this study will benefit this community and agricultural communities, in general, and will directly contribute to the information necessary for the implementation of the Food Quality Protection Act.

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