Pediatric RESEARCH

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REVIEW ARTICLE

Children's low-level pesticide exposure and associations with autism and ADHD: a review

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Pesticides are chemicals that are designed specifically for the purpose of killing or suppressing another living organism. Human toxicity is possible with any pesticide, and a growing body of literature has investigated possible associations with neurodevelopmental disorders. Attention deficit disorder with or without hyperactivity (ADHD) and autism spectrum disorder (ASD) are two of these specific disorders that have garnered particular interest. Exposure to toxic chemicals during critical windows of brain development is a biologically plausible mechanism. This review describes the basic laboratory science including controlled pesticide dosing experiments in animals that supports a mechanistic relationship in the development of ADHD and/or ASD. Epidemiological relationships are also described for low-level pesticide exposure and ADHD and/or ASD. The available evidence supports the hypothesis that pesticide exposure at levels that do not cause acute toxicity may be among the multifactorial causes of ADHD and ASD, though further study is needed, especially for some of the newer pesticides.

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INTRODUCTION

The term pesticide is broadly categorical and includes insecticides, herbicides, rodenticides, fumigants, fungicides, and disinfectants. All classes of pesticides share the quality that each active ingredient is developed with the intention to kill or suppress some living organism. We can expect all to have some degree of toxicity to humans. Many pesticides are devised to affect the nervous system of animal hosts, particularly insects and rodents. Others, such as herbicides and fungicides, though not designed as neurotoxicants, have been shown to have neurological effects on exposed animals. It is for this reason that one of the primary goals of animal testing with the goal of risk management in registration of pesticides by United States Environmental Protection Agency (USEPA) is to detect neurotoxicity. The symptoms of acute exposures vary according to the inherent toxicity of the agent, mode of action, and dosage. Both acute and chronic or low-dose exposures have been implicated in injury to children. These effects are often persistent.[1]

Neurocognitive disorders have received considerable attention in research during the past several decades with a particular focus on potential environmental causation. Autism spectrum disorder (ASD) and attention deficit disorder with or without hyperactivity (ADHD) are among the most well-known and extensively studied neurocognitive disorders affecting children. Additional neurocognitive disorders of interest such as learning disability (LD), conduct disorder, intellectual disability, cerebral palsy, and impaired vision and hearing will commonly share some other neurocognitive symptoms or diagnoses. For instance, LD is often a co-morbid condition in patients with ADHD, and intellectual disability with ASD. The focus of this report is to evaluate the existing evidence regarding the relationship between pesticide exposure and two of the most important neurological problems of early childhood, ASD and ADHD.

Since both disorders manifest themselves in very early childhood, it is axiomatic that the causes(s) stem from very early in life, perhaps even in utero. Research into the causes of ASD and ADHD and their relationship to pesticide exposure has proceeded along several parallel and linked lines. There is considerable in vitro and animal research regarding the mechanisms of toxicity and effect. Multiple cross-sectional and longitudinal epidemiological studies of pesticide-exposed humans have been designed to detect a variety of neurological injuries.

While it could be tempting to conclude that these are simply genetically determined diseases, there is considerable support for the view that environmental factors interact with genetic predisposition to result in these disorders. To the extent that early life or in utero environmental factors influence development of these entities, it seems reasonable to evaluate injury in the early stages of neurodevelopment as being responsible by brief exposure leading to lifelong impairment. Alternatively, it is possible that epigenetic mechanisms alter the genome to more indirectly influence the early stages of neurodevelopment. It has been difficult to model ASD in experimental animals. Due to the complex nature of ASD and ADHD it has not been feasible to conduct mechanistic studies in human subjects. Likewise, dosing studies in humans have been considered unethical. This is particularly true for studies in children. However, the processes of development of the nervous system in small mammals and humans are qualitatively very similar, though it proceeds at differing rates in small mammals compared to humans. This allows evidence from in vitro and animal studies to be linked to evidence from human epidemiological studies.

Several important and comprehensive reviews have been published that examine various relationships between pesticides and neurodevelopmental effects.[2–7] Most of the reviews include studies that assess additional or non-specific outcomes such as

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developmental or cognitive delays. One review is specific only to the organophosphate (OP) insecticide chlorpyrifos and is the most comprehensive review of this particular insecticide to date.[4] Most but not all reviews have documented or interpreted adverse effects following pesticide exposure. In a larger context, over the past 70 years there has been a progression of multiple generations of new pesticides. Each has been introduced with the hope that they will be safer and more specific to intended targets. This has generally been a false hope as each pesticide generation has been more toxic to children and adults than initially thought.[3] This article differs from other more extensive reviews in that we attempted to provide a concise discussion limited to ASD and ADHD. This type of review article differs substantially from a systematic review, which carries a study methodology all of its own and is designed to produce a consolidated outcome measure based on outcome measures from similar studies. In this case, we are summarizing available evidence at three levels: in vitro laboratory, animal studies, and human epidemiology in order to develop a reasonable framework for future studies in this area.

In the process of searching for the evidence, we combined search terms for ADHD and pesticides, autism and pesticides, and pesticides and developmental delay. The latter combination was to find any potential studies that may have observed autism or ADHD while primarily listing developmental delay as an outcome. The search resulted in 249 articles, some of which were duplicates. Among these were 115 human studies, of which 15 were review articles, 95 studies that reported an association between pesticides and autism and/or ADHD or other developmental/cognitive outcomes, and 5 that reported no associations. We report on those articles specific to ADHD and ASD.

ADHD PREVALENCE

Data from the Centers for Disease Control and Prevention (CDC) indicate a rise in prevalence of ADHD from 6.3% in 1997 to 9.5% in 2010. ADHD is more common in boys with the prevalence being 12.4% compared to 5.7% for girls during the time period of 2007–2010. For children living below the poverty level, the prevalence was 11.3%, compared to 8.6% of children from families living at or above the poverty level. Race/ethnicity variations were also present, with 11.7% for all other races, 10.7% white, 10.2% black, 4.8% Hispanic, and 1.7% Asian children being reported to have ADHD. The prevalence varies by demographics, with a slightly higher prevalence among practices with a high percentage of black children, and with low socio-economic status.[8] A recent study in a population-based sample of elementary school children in a county in North Carolina using the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition criteria suggested a prevalence of ADHD as high as 15.5%.[9]

ASD PREVALENCE

The rates of diagnosis of ASD suggest a dramatic increase in prevalence over the past two decades. A proportion of this increase may be attributed to changes in diagnostic criteria with the inclusion of Asperger's syndrome and pervasive developmental delay in ASD. While that expansion in the spectrum has identified more children, it does not entirely explain the rising frequency of ASD. The prevalence of ASD changed from 0.1% of all 5–17-year-old children in 1997 to 1% in 2010. Boys are more likely to have ASD than girls, at a 3:1 ratio. There appears to be no relationship based on family income.[8] (https://www.epa.gov/ace, accessed on 09/8/2018) Separate data from the CDC reported that in 2014 the incidence of ASD among its 11 Autism and Developmental Disabilities Monitoring sites was 16.8 per 1000 (one in 59) children aged 8 years.[10] There was considerable variance among sites reported by the CDC, from 13.1 to 29.3 per 1000 children aged 8 years. Males were four times more likely than females to be identified with ASD. When data from all sites were combined, the estimated prevalence among white children (17.2 per 1000) was 7% greater than that among black children (16.0 per 1000) and 22% greater than that among Hispanic children (14.0 per 1000).

CRITICAL STAGES OF DEVELOPMENT

Given that the onset of both ASD and ADHD occur very early in childhood, logic dictates that there is some disruption in the normal development of the fetal/infant brain. The development of the central nervous system occurs in very structured steps. Though the sequence is essentially identical in humans and experimental animals, the pace and timing differ related to gestational period and lifespans of the studied species. The earliest stages of in utero development characterized by cell proliferation, migration, and differentiation are likely affected by environmental exposures. Toxic exposures during this period of development are characterized by malformations such as neural tube defects.[11,12] Pesticides have been implicated in some animal and human studies of neural tube defects, though the evidence is not exceptionally strong. Later in utero and early life exposures are expected to alter processes such as synaptogenesis, gliogenesis, myelination, and apoptosis. Pesticide exposures during this period would be predicted to result in functional and behavioral alterations which would likely be permanent. These injuries would not be characterized by gross structural changes in the central nervous system. Exposures later in life may alter neurotransmitter function but would be less likely to be long term or permanent. All of these processes of central nervous system development have been considered critical periods of vulnerability to toxic exposures including pesticides.[13,14] Injuries to these processes are biologically plausible as a mechanism for disorders such as ADHD and ASD.[15]

ROLE OF GENETICS AND THE ENVIRONMENT AND GENETICS IN NEUROCOGNITIVE DISORDERS

Genetics plays a role in the causation of ASD and ADHD. A number of genes are identified as a factor for ASD, and a strong family history has been documented with ASD and ADHD.[16,17] Twin studies of ASD have indicated that the heritability may be as high as 64–91%.[18] A study of North American and Italian families evaluated for the presence of genetic susceptibility for deactivating OP insecticides. American children, but not Italian children, were found to have the presence of the PON1 L55/R192 gene variant, coding for a form of the enzyme less active in vitro on diazinon.[19]

Environmental chemicals have biological plausibility in the causation of neurocognitive disorders. Multiple studies have demonstrated that lead is associated with ADHD.[20–23] Methylmercury and polychlorinated biphenyl compounds (PCBs) have also been widely studied and found to be associated with neurocognitive effects.[7,24–27] Several powerful epidemiologic and laboratory studies have implicated pesticides in a variety of neurologic effects. Three prospective childhood cohorts have been established and followed children through the middle elementary school period. Two cohorts are urban populations in New York City. Another is a rural California cohort. These very important cohort studies have generated numerous publications, including findings related to other relevant neurodevelopmental effects such as measures of intellectual or cognitive development.[28–33] Papers relevant to the outcomes of ADHD and ASD are discussed below.

ASSOCIATIONS BETWEEN PESTICIDES AND ADHD OR ASD

ADHD: laboratory evidence

There is a scientific basis for the biologic plausibility of an association with ADHD and pesticide exposure in humans, based on in vitro and animal studies. Studies have been conducted with multiple pesticides, including chlorpyrifos (an OP insecticide), deltamethrin and cypermethrin (pyrethroid insecticides), endosulfan (an organochlorine insecticide), and carbaryl (carbamate insecticide) in multiple animals.[34–38] In addition to the mammalian models, studies have been conducted in fruit flies and zebrafish.[34,35] It is difficult to reconcile these studies due to the complex differences between these non-mammalian species and the mammalian nervous system.

A recent publication examined prenatal and postnatal exposure in mice to the pyrethroid insecticide deltamethrin at dosages below the developmental no observable adverse effect level defined by pesticide registrants based on required risk assessment studies approved by the USEPA. Exposed mice exhibited behavior mimicking ADHD in children with impulsive behavior, hyperactivity, and poor attention. These mice had elevated dopamine transporter levels, decreased synaptic domaine levels, and increased dopamine receptor levels.[37] A similar study was conducted in ten-day-old mice with a single low-dose exposure to either endosulfan or cypermethrin. The authors describe this as a critical period of brain development. Both insecticides altered levels of brain proteins and resulted in increased spontaneous activity in adult mice. This effect persisted for months suggesting a permanent injury from this single exposure.[36] Another observation in rats suggested that maternal exposure to chlorpyrifos at sub-toxic doses prior to pregnancy resulted in increased motor activity and agitation compared to controls. Offspring of rats exposed to a single dose of chlorpyrifos at 6 days of gestation showed neurologic effects that differed from offspring of rats exposed prenatally. While the mechanism for these observations is unclear, it appears an epigenetic mechanism for these effects may be present.[38] Studies of this nature are highly suggestive but also point out that further investigation of the biologic mechanisms for these observations is necessary.

Imidacloprid is a relatively newer insecticide of the neonicotinoid class, often used in agriculture and for flea control on domestic pets. There are only limited data on animal and human exposures to this newer agent. Experiments on fruit flies (*Drosophila*) demonstrated abnormal social interactions, increased flight speed and distance, and mobility compared to control flies, all indicative of behavioral changes in the fly that would be similar to behavioral changes in children with ADHD.[39] While differing from the complex nervous system of the mammal, the entire genome of *Drosophila* is fully sequenced, and researchers have been able to demonstrate numerous behaviors also found in humans.[40] The mechanism of action of imidacloprid is similar to OPs (they act on nicotinic acetylcholinesterase receptors), although their affinity for the insect receptor is much higher than for mammalian receptors.[41] The similar mode of action suggests that similar adverse effects found with OPs upon further study may be observed with neonicotinoids.

A study of neonatal rat cultured cerebellar neurons from neonatal rats attempted to observe the effects of neonicotinoids (imidacloprid and acetamiprid) on these neurons. The authors noted that compared to nicotine, a well-documented neurodevelopmental toxin, neonicotinoids caused similar though quantitatively different effects.[42] Another study evaluated the effect of two neonicotinoids on human nicotinic receptors. The observations in this study suggest that neonicotinoids may have stronger human side effects than was previously thought.[43] Conversely, a review by several industry-affiliated scientists presented the view that there was no conclusive evidence of a specific neurodevelopmental effect of neonicotinoids.[5]

ADHD: epidemiological science

Table 1 provides a summary of the epidemiological studies evaluating pesticide exposure and ADHD. The National Health and Nutrition Examination Survey (NHANES) conducted by the CDC has been widely used to explore relationships between ADHD and pesticide exposure. A portion of the children who participated in NHANES submitted a urine sample to measure levels of numerous toxicants, including metabolites of pyrethroid and OP insecticides. These studies examined a distribution of the metabolites and designated detectable levels compared with non-detectable levels. Participants in NHANES were not chosen based on the degree of pesticide exposure that would cause acute toxicity. The diagnosis of ADHD was initially assessed through parental/patient self-report. Beginning with the 2001 data the diagnosis was assessed by the National Institute of Mental Health Diagnostic Interview Schedule for Children Fourth Edition (DISC-IV). Although the limitations of cross-sectional studies include the inability to verify whether the exposure preceded the outcome, these studies, one using OP insecticide metabolites, and three using pyrethroid metabolites, provide insight to possible associations and generate hypotheses.[37,44–46]

NHANES data from 2000 to 2004 were used to assess a relationship between OP insecticides and ADHD in 8–15-year-old children. Children's urinary dimethyl alkyl phosphate (DMAP) metabolite levels were positively associated with a diagnosis of ADHD. Children with DMAP levels that were greater than the median were almost two times more likely to have ADHD compared to children without detectable DMAP levels.[44]

Three studies have used the NHANES data to examine relationships between pyrethroid exposure and ADHD and LD.[37,45,46] Data in one study used the NHANES data from 1999 to 2002 that included children aged 6–15 years of age and the measurement of urinary metabolites of pyrethroids. ADHD and LD were measured by parental report or report of the adolescents aged 12–15 years. The authors used multivariate logistic regression to determine associations between LD, ADHD, and combined LD and ADHD with detectable levels of three different pyrethroid metabolites and did not find any statistically significant associations.[45] A second study used the 1999–2002 NHANES data along with parent/self-report of ADHD. The study was enhanced by including medication use of common ADHD medications. Children aged 6–15 years with detectable pyrethroid metabolites were more than two times likely to have ADHD than those without detectable metabolites.[37] The third study that evaluated the 2001–2001 NHANES data[46] also found a positive association between pyrethroid exposure and 8–15-year-old children with ADHD, specifically hyperactivity/impulsivity. The diagnosis of ADHD was determined by DISC-IV as well as parental report of ADHD. The presence of hyperactive/impulsive symptoms on the DISC-IV was higher with every 10× increase in metabolite levels.[46] The fact that these diagnoses are observed with older children and concurrent exposure suggests either that later-life exposure contributes to these diagnoses or that concurrent exposure simply implies earlier exposure.

A cross-sectional study in Costa Rica evaluated children living near banana plantations and used urinary metabolites to chlorpyrifos, mancozeb (fungicide), and pyrethroids to determine pesticide exposure. Elevated chlorpyrifos metabolites were associated with poor working memory in boys and poor visual motor coordination. Findings on the Conners' Parent Rating Scale-Revised Short Version scale included an increase in cognitive problems, inattention, and oppositional defiance. One outcome measure was positively associated with each of the other two exposures—poor verbal learning outcomes with exposure to mancozeb, and poor processing speeds with pyrethroid exposure.[47]

In a case–control study, 97 children aged 4–15 years with ADHD were compared to 110 control children without ADHD. Pesticide

Table 1. Epidemiological studies evaluating pesticide exposure and ADHD

Relevant papers	Name of study	Type of study	Brief summary findings
Bouchard et al.[44]	National Health and Nutrition Examination Survey (NHANES)	Cross-sectional	Children's DMAP metabolite levels were positively associated with a diagnosis of ADHD
Quiros- Alcala et al.[45]	National Health and Nutrition Examination Survey (NHANES)	Cross-sectional	No statistically significant association was found between pyrethroid metabolites and ADHD or learning disability
Richardson et al.[37]	National Health and Nutrition Examination Survey (NHANES)	Cross-sectional	Children with detectable pyrethroid metabolites were more likely to have ADHD than those without detectable pyrethroid metabolites
Wagner-Shuman et al.[46]	National Health and Nutrition Examination Survey (NHANES)	Cross-sectional	Results showed that children with urinary pyrethroid metabolite levels above the detectable limit were more likely to have ADHD compared to those with levels below the detectable limit
van Wendel de Joode et al.[47]		Cross-sectional	Living near a banana plantation and being exposed to pesticides may affect neurodevelopment. Relevant findings included poorer verbal leaning and poor processing speed scores, as documented by Conner's scales. Differences were noted between boys and girls
Yu et al.[48]		Case–control	The level of dimethyl phosphate was significantly higher in children with ADHD. There is no guarantee that the concurrent pesticide levels are similar to pesticide levels during important periods of a child's development
Saez et al.[49]		Population-based retrospective cohort	Air pollutants due to traffic and environmental factors associated with exposure to pesticides could be associated with ADHD
Marks et al.[50]	The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)	Prospective cohort	Prenatal DAP levels were significantly related to measures of attention
Rauh et al.[30,51]	Columbia Center for Children's Environmental Health	Prospective cohort	Chlorpyrifos exposure was associated with attention problems, psychomotor delay, and mental delay at 3 years of age Prenatal pest control exposure is associated with hand tremors compared to children that are unexposed. Hand tremor can lead to bad handwriting, which is a well-known sign of ADHD
Ribas-Fito et al.[52]		Prospective cohort	Children with prenatal exposure to hexachlorobenzene are twice as likely to have ADHD as those that are unexposed Children with prenatal exposures to organophosphate
Sagiv et al.[53]		Cohort	Results show an association between low-level prenatal pesticide exposure and ADHD in children
Sioen et al.[54]	Flemish Mother–Newborn Cohort	Prospective cohort	Doubling of cord blood concentrations of environmental contaminants were associated with behavioral problems in children, no association was found with boys

exposure was assessed by urinary metabolites to OP insecticides, collected at the time of the visit. To attempt to control for effects of lead, a known contributor to ADHD, a whole blood lead level was also obtained. The level of dimethyl phosphate was significantly higher in the children with ADHD compared to controls.[48] Of note, this study measured concurrent pesticide levels, and while the design is case–control, there is no assurance that the concurrent pesticide levels are similar to pesticide levels during critical periods of development. A retrospective cohort study examined associations between ADHD and home residence near several environmental exposures. The authors found two clusters of cases, one of which corresponded to living within 100 m from an agricultural center, and another related to residence within 300 m from a high traffic density area and/or industrial site.[49] This hypothesis-generating study is not able to accurately link a pesticide exposure with ADHD.

A prospective cohort design allows for an assessment of exposure early in life (prenatal and/or early infancy) along with an extended period of follow-up for outcomes, thus providing the most powerful epidemiologic design. In each of the following cohort studies, the pesticide exposure occurred at levels not associated with acute toxicity.[50–52] The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is a prospective birth cohort in California that followed children of agricultural workers through elementary school. Women were recruited in the first half of their pregnancy and pesticide exposure was due to working in the agricultural setting and living with agricultural workers (i.e., take-home exposure), as opposed to acute OP poisoning. Pesticide exposure was measured by maternal concentrations of dialkyl phosphate (DAP) metabolites. Maternal report of attention problems in children was measured by the Child Behavioral Checklist and children were assessed by functional testing at 3½ and 5 years of age. Prenatal DAP levels were significantly related measures of attention, especially at age 5 years.[50]

Children in the Columbia Center for Children's Environmental Health in New York cohort study were prenatally exposed to chlorpyrifos in their households from pest control exposure. Early findings from this cohort included increased attention problems and developmental problems at 3 years of age.[30] At 11 years of age, exposed children were found to have a greater likelihood of hand tremor than those in the unexposed group.[51] The authors speculate that the tremor may be a factor in the poor handwriting that is a well-known sign of ADHD. However, it is unclear whether

poor handwriting is due to impaired motor function or inattentive/hyperactive behaviors. A cohort of children from Spain was exposed prenatally to hexachlorobenzene (HCB), an organochlorine fungicide. These children lived in a town of 5000 people near an electrochemical factory. Exposed children were defined as having serum HCB concentrations >1.5 ng/mL from cord blood samples. Children in this study underwent testing for ADHD at 4 years of age. Children in the exposed group were twice as likely to have a diagnosis of ADHD as unexposed children.[52] A cohort of 607 Massachusetts children exposed to *p,p′*-dichlorodiphenyl dichloroethylene (*p,p′*-DDE), a metabolite of the insecticide DDT, were evaluated for behavioral problems associated with ADHD. Children in the highest quartile of *p,p′*-DDE exposure were 1.8 times more likely to have elevated ADHD index scores compared to other exposed children.[53] A cohort from the Flemish Environment and Health study evaluated for associations between behavioral problems and a number of environmental toxicants, one of which was *p,p′*-DDE. They found that a doubling of cord blood concentrations of *p,p′*-DDE were associated with an almost five times likelihood of hyperactivity in girls.[54]

ASD: laboratory evidence

There are several in vitro studies that have evaluated the effects of pesticides to develop better evidence for a mechanistic understanding of the etiology of ASD. A study was conducted using DNA methylation (one mechanism of epigenetics influence) in human placenta as a biomarker of response to pesticides, including partially methylated domains of placenta tissue from children diagnosed with ASD at 3 years of age. Self-reported pesticide exposure that was professionally applied to the outdoor and lawns was predictive of DNA methylation.[55] Another study exposed mouse cortical neuron enriched cell cultures to multiple chemicals. Several pesticides were found to produce transcriptional changes similar those seen in brain samples from humans with ASD and certain neurodegenerative diseases. The authors suggest that these chemicals disrupt microtubules in neurons and stimulate free radical production.[56]

A gene enrichment analysis was conducted using a database of 206 autism susceptibility genes "to interrogate ~1 million chemical/gene interactions in the comparative toxicogenomics database".[18] The list of chemicals includes various persistent organic chemicals, metals, particulate matter, ozone, and pesticides. Pesticides that had the most significant "enrichment scores", a sign of gene–environment interaction, include diazinon, chlorpyrifos, DDE, and cypermethrin.[18]

There is increasing interest in animal studies to evaluate gene–environment interactions using rodent strains that have behaviors that mimic ASD in humans.[57] The BTBR T + tf/J mouse strain is one that displays behavioral traits thought to be similar to idiopathic autism and is considered an animal model relevant to studies of the etiology of childhood autism.[58] Prenatal exposure of this strain to chlorpyrifos has been found to result in developmental delays.[59] There are some studies relevant to an understanding of potential mechanisms for this observation. Prenatal exposure of mice to chlorpyrifos oxon resulted in alterations in reelin protein expression and morphology of neurons in the hippocampus and cerebellum.[60,61] Reelin is a glycoprotein that regulates neuronal migration and brain lamination.[62] Another study of prenatal exposure to chlorpyrifos in the reeler mouse (which lacks reelin) resulted in brain morphologic alterations. Exposure to chlorpyrifos in mice showed long-term adverse effects on behavior up to postnatal day 90. The authors noted defects in social behavior and exploration of novel objects. These defects are thought to be similar to problems observed with ASD in humans.[63]

ASD: epidemiological science

A comprehensive review of the epidemiological literature examined multiple chemical and other environmental exposures, including but not limited to pesticides, and their association with ASD. The chemicals in this review that were found to be associated with ASD include air pollution, some metals, and several pesticides.[6] Table 2 summarizes the studies evaluating pesticide exposure and outcomes related to ASD.

Maternal residence near agriculture application during gestation may be associated with ASD development. A case–control study in the central valley of California evaluated residential distance from fields sprayed with two organochlorine pesticides, dicofol and endosulfan. Children whose mother lived within 500 m during the time of gestation were six times more likely to have ASD compared to those living farther away.[64]

The Childhood Autism Risks from Genetics and the Environment (CHARGE) study is a population-based case–control study conducted at the University of California Davis. CHARGE compares children with ASD to those who have normal developmental progression and assesses pesticide exposure by linking home addresses with commercial pesticide application data. Proximity to OP exposure and/or pyrethroid exposure during third trimester of gestation was associated with an increased risk of the child having ASD.[65] Mothers of children with ASD were also more likely to be consistent users of imidacloprid than mothers with normally developing children; however, this study may be affected by exposure misclassification.[66] Preliminary evidence from CHARGE also suggests that folic acid (FA) may also be protective in efforts to decrease the risk of developing ASD. The odds of having a child with ASD were 2.5 times higher for mothers with pesticide exposure and low FA intake. This exceeded the risks of those with pesticide exposure and high FA intake. This study is among the first to suggest a possible preventive measure of FA supplementation.[67]

In another case–control study conducted in California, 545 children with ASD were compared with 418 control subjects. Organochlorine pesticide metabolites were not associated with an increased risk of ASD. PCBs, another group of persistent organochlorine compounds, were found to correlate with an elevated risk for ASD.[68] A study from Finland evaluated 778 matched case–control pairs and maternal prenatal serum for *p,p′*-DDE levels. Highest levels of maternal pesticide levels were associated with ASD, and even higher with ASD and intellectual disability.[69]

A prospective birth cohort measured multiple endocrine-disrupting chemicals including pesticides from the blood of 15 pregnant women in Cincinnati. Using an outcome measure of the Social Responsiveness Scale, a measure of autistic behaviors, children exposed to *trans*-nonachlor (a metabolite of chlordane, a persistent organochlorine pesticide that is no longer used in the United States) were four times more likely to have autistic behaviors, but the confidence intervals were wide (odds ratio (OR) = 4.1, 95% confidence interval (CI) 0.8, 7.3).[70] Data from the Mount Sinai Children's Environmental Health evaluated children exposed to OPs as measured by DAP and the relationship with social behavior, which may be a component of ASD. An increase in DAP concentrations was associated with poorer socially responsive scores in blacks and in boys. No association was found among white or Hispanics or for girls.[71]

In the CHAMACOS prospective birth cohort, early findings included higher scores for pervasive developmental delays in 24-month-old children in the higher exposed group of children to OPs.[72] A more recent CHAMACOS paper reported mixed findings. Children in the highly exposed groups had modest increases in parent-reported and teacher-reported problems with social behavior. However, there were no overt associations between proximity of prenatal OP use and ASD traits.[73]

The MARBLES (Markers of Autism Risk in Babies – Learning Early Signs) prospective birth cohort follows pregnant mothers and women planning pregnancy whose offspring are at a higher risk for ASD, due to having a first-degree relative with ASD. When

Table 2. Epidemiological studies evaluating pesticide exposure and ASD

Relevant papers	Name of the study	Type of study	Brief summary findings
Roberts et al.[64]		Case–control	Children who live near fields sprayed with pesticides are six times more likely to have ASD than those living further away from the fields
Shelton et al.[65]	Childhood Autism Risks from Genetics and the Environment (CHARGE)	Population-based case–control	Residential proximity to insecticide application at critical periods of development is associated with increased risk of ASD
Keil et al.[66]	Childhood Autism Risks from Genetics and the Environment (CHARGE)	Population-based case–control	Mothers of children with ASD were more likely to be users of imidacloprid than those of children with typical development
Schmidt et al.[67]	Childhood Autism Risks from Genetics and the Environment (CHARGE)	Population-based case–control	Associations were found between pesticide exposure and ASD among those with high FA intake and low FA intake during a mother's first month of pregnancy
Lyall et al.[68]		Case–control	No association between organochlorine pesticides and ASD
Brown et al.[69]	Finish Prenatal Study of Autism	Prospective cohort	Maternal exposure to organochlorine pesticides is associated with increased odds of having a child with autism
Braun et al.[70]	Health Outcomes and Measures of the Environment (HOME)	Prospective birth cohort	Children exposed to endocrine-disrupting chemicals were four times more likely to have autistic behaviors. Study may be affected by small sample size
Furlong et al.[71]	Mt. Sinai Children's Environmental Health Study	Prenatal cohort	Prenatal pesticide exposure is associated with deficits in social functioning among blacks and in boysNo association was found among whites, Hispanics, or girls
Eskenazi et al.[72]	The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)	Longitudinal birth cohort	Prenatal OP exposure is associated with delayed mental development and pervasive developmental delay in 2-year-old children
Sagiv et al.[73]		Longitudinal birth cohort	Prenatal OP exposure is associated with poorer social behavior
Phillippat et al.[74]	Markers of Autism Risk in Babies – Learning Early Signs (MARBLES)	Prospective cohort	OP metabolite concentrations are associated with increased risk of ASD in girls, but not for boys

stratified by sex, DAP metabolite concentrations were associated with an increased risk of ASD among girls, but not boys.[74] As was the case with the epidemiological studies that assessed ADHD, the pesticide exposure measurements in the above studies were also at levels that would be considered below the acutely toxic range.

CONCLUSIONS

A growing body of literature provides evidence that pesticides may have a role in the development of ASD and ADHD. The observed pesticide exposures in the epidemiologic studies are incidental and generally low level. They occur prior to conception, during gestation, and in early childhood at critical stages of neurologic development. It is likely for both ASD and ADHD that there are gene–environment interactions. This brief review provides a framework to understand evidence for pesticides in the etiology of ASD and ADHD. The laboratory science provides mechanistic data as well as confirmation of symptoms in controlled dosing experiments. The epidemiological data provide supporting evidence for human outcomes beginning in the earliest stages of childhood development and at exposures well below those resulting in acute toxicity. While the evidence cannot be considered conclusive, the existing data justifies further research.

ADDITIONAL INFORMATION

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