ehp ENVIRONMENTAL HEALTH PERSPECTIVES

ehponline.org

Tipping the Balance of Autism Risk: Potential Mechanisms Linking Pesticides and Autism

Janie F. Shelton, Irva Hertz-Picciotto, Isaac N. Pessah

http://dx.doi.org/10.1289/ehp.1104553

Online 25 April 2012

NIEHS
National Institute of Environmental Health Sciences

National Institutes of Health
U.S. Department of Health and Human Services

Tipping the Balance of Autism Risk: Potential Mechanisms Linking Pesticides and Autism

Janie F. Shelton[1], Irva Hertz-Picciotto[1,2], Isaac N. Pessah[2,3]

1. Graduate Group in Epidemiology, Department of Public Health Science, University of California, Davis, Davis, CA.

2. UC Davis MIND Institute, Sacramento, CA.

3. Molecular Biosciences, University of California, Davis, Davis, CA.

Corresponding Author:

Janie F. Shelton, MPH

MS1C, Rm 182 B

One Shields Avenue

Davis, CA

95616

Phone: (626) 419-1644

Email: jfshelton@ucdavis.edu

Keywords: autism spectrum disorders, carbamate, gene environment interaction, immune, mitochondria, neuroexcitation, organochlorine, organophosphate, oxidative stress, pesticide, pyrethroid

Running title: Potential mechanisms linking pesticides and autism

Competing financial interests: None

Abbreviations:

ACh	Acetylcholine
AChE	Acetylcholinesterase
ASD	Autism Spectrum Disorder
CB	Carbamate
GABA	Gamma-Aminobutyric acid
OC	Organochlorine
OP	Organophosphate

Abstract

Background: Autism spectrum disorders (ASDs) have been increasing in many parts of the world and a portion of cases are attributable to environmental exposures. So far, conclusive replicated findings have yet to appear on any specific exposure, but mounting evidence suggests gestational pesticides exposures are strong candidates. Because multiple developmental processes are implicated in ASDs during gestation and early life, biological plausibility is more likely if these agents can be shown to affect core pathophysiological features. Here we review shared mechanisms between autism pathophysiology and effects of pesticide exposures, focusing on neuroexcitability, oxidative stress, and immune functions.

Objectives: The objectives of this review are to outline the biological correlates between pesticide exposure and autism risk.

Methods: We review and discuss previous research related to autism risk, developmental effects of early pesticide exposure, and basic biological mechanisms by which pesticides may induce or exacerbate pathophysiological features of autism.

Discussion: Based on experimental and observational research, certain pesticides may be capable of inducing core features of autism but little is known about the timing, dose, or which of various mechanisms is sufficient to induce this condition.

Conclusions: In animal studies, we encourage more research on gene X environment interactions, as well as experimental exposure to mixtures of compounds. Similarly, epidemiologic studies in humans with exceptionally high exposures can identify which pesticide classes are of greatest concern, and studies focused on gene X environment are needed to determine if there are susceptible sub-populations at greater risk from pesticide exposures.

Introduction

Causes for the recent rise in autism diagnosis throughout the US remain largely unknown. In California, a 600% increased incidence in autism was observed among children up to age five for births from 1990 to 2001, yet only one third of the rise could be explained by identified factors such as changing diagnostic criteria and younger age at diagnosis (Hertz-Picciotto and Delwiche 2009). Across the U.S., autism spectrum disorders (ASD) are now estimated to affect 1 in 88 eight year olds, with much higher prevalence in boys (1 in 54) than girls (1 in 252) (Centers for Disease Control and Prevention 2012). Autism is a heterogeneous, behaviorally defined condition presenting prior to age three. Although each individual diagnosis must meet specific criteria related to deficits in social interaction, and language, and the presence of repetitive behaviors or restricted interests, autism phenotypes vary widely, even among concordant twins (Le Couteur et al. 1996).

Idiopathic autisms are diagnosed 4-5 times more often in boys than girls and frequently involve a wide range of genes that confer susceptibility as opposed to a singular heritable factor (Geschwind 2011). Genetic contributions to autism risks involve rare mutations, complex gene-gene interactions and copy number variants (CNVs), including deletions and duplications (Stankiewicz and Lupski 2010). In a recent series of papers, rare de novo point mutations were associated with autism in parent-child trios with sporadic ASD (Neale et al. 2012; O'Roak et al. 2012; Sanders et al. 2012), and those mutations were more frequently derived from fathers, increasing with paternal age (O'Roak et al. 2012). Twin studies have demonstrated evidence of heritability due to stronger concordance among monozygotic that dizygotic twins, yet in a recent study that parsed the contribution from genetics versus the environment, a larger component of

the risk of autism was attributable to environmental factors than genetics alone (Hallmayer et al. 2011). The genetic and twin studies of autism point to variability unexplained by heritable factors, and in recent years, associations between gestational pesticide exposures and ASD or behaviors that are characteristic of pervasive developmental disorders have been reported.

Using exposure estimates from a historical pesticide use database, a study of mothers living in the California central valley showed that children born to mothers exposed to agriculturally applied organochlorine (OC) insecticides within 500 meters of the home between days 26 and 81 post-fertilization (during neural tube closure) were 7.6 times more likely to be diagnosed with ASD than the children of mothers who lived in the lowest exposure quartile. Associations were also observed for the pyrethroid insecticide bifenthrin and for the organophosphate chemical class, comparing the cumulative exposure over the course of gestation among the highest vs. lowest quartile (Roberts et al. 2007). Although this study presents provocative preliminary data and higher odds at closer proximity (dose-response), unmeasured confounding could have occurred for other exposures such as prenatal vitamin intake or occupational exposures. Additionally, because cases were obtained from the Department of Developmental Services (DDS) and controls from the birth certificate registry, misclassification of cases and controls may have occurred as children who receive an early diagnosis of autism are sometimes reclassified at a later date, and controls may include children who are on spectrum but have not obtained a DDS diagnosis.

In a prospective cohort study also from the California central valley, a 230% increase in maternally reported symptoms of Pervasive Developmental Disorders (PDD) was observed per

10 nanomole/liter increase in prenatal maternal urinary levels of OP metabolites (Eskenazi et al. 2007). PDD is the greater diagnostic umbrella under which ASD falls, and includes Rett Syndrome, Childhood Disintegrative Disorder (CDD), and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). Although the prospective study design has the benefit of accuracy in exposure ascertainment from biospecimens collected during pregnancy, it is generally not feasible to obtain a cohort large enough to observe enough cases of full syndrome autism. Consequently the broader definition of borderline PDD increases the numbers but lacks specificity. Although these studies are by no means conclusive in establishing an autism and pesticide association, they do raise important questions regarding the health effects of these compounds on the developing fetus. In light of these findings and current theories of autism pathophysiology, we review here potential pathways by which gestational pesticide exposure might contribute to autism, linking what is known about the origins of autism with information on biological effects of pesticides to generate clearer hypotheses that can help guide future research in this area.

Pesticide exposure in the general population

Pregnant women are exposed to pesticides through a wide variety of sources, and while many of the mechanisms of action outlined here have been observed in association with higher exposures than are likely to occur in the general population, it is difficult to estimate the direct dosage to a pregnant woman who may be applying pesticides in or around her home or to her pets, consuming food with residues of pesticides and pesticide metabolites, and inhaling air from agricultural or urban spraying nearby her home and workplace. Moreover, urine and blood levels indicate exposure to pregnant women is widespread. In the 2003-2004 NHANES, which recruits

a representative sample of the U.S. adult population, 83% of pregnant women had detectable levels of dimethylthiophosphate, an OP metabolite in their urine (geometric mean 2.43 micrograms/ Liter of urine). DDE, the breakdown product of the persistent OC pesticide DDT, was detected in 100% of pregnant women with a GM of 140.4 nanogram/gram lipid (Woodruff et al. 2011). Trends in pesticide use in the United States since 1964 have shown steep increases in OP use which make up the vast majority of sales, and rapid decreases in OC use following the 1972 ban on DDT (Figure 1). More recently, as OP's have been banned for residential uses, pyrethroid sales have increased rapidly (Williams et al. 2008).

The quest for animals models of autism & environment

A variety of animal models have been developed that aid in the understanding of mechanisms that may induce one or several of the core features of autism (Ey et al. 2011; Hamilton et al. 2011; Tabuchi et al. 2007). In particular, transgenic and knock-in mouse lines with targeted anomalies in genes associated with autism and the development of a comprehensive set of rodent assays to assess social interaction, communication, and repetitive behaviors, have greatly enhanced our ability to test hypotheses about the causes of autism (Silverman et al. 2010). However, implementations of these tools towards understanding gene X environment interactions that promote impairments in the three key behavioral domains have lagged. The *Shank3* (Peca et al. 2011) and oxytocin knockout mice (Crawley et al. 2007) are examples of monogenetic insults that disrupt all three domains. However, because only a small proportion of autism cases result from complete loss of a single gene, knockout animal models may not be as useful as models that carry mutations which impart partial gain or loss of gene function.

Functional impairments as seen in the Reeler Mouse (RM) (Laviola et al. 2009) and Timothy syndrome (TS) mouse models (Bader et al. 2011) are more relevant to the multi-gene and environment model of autism risk. In a subsection of a paper describing the paradoxical effects of acetylcholinesterase in the Reeler mouse, Laviola and colleagues describe the complexity of a gene X environment model whereupon exposure to chlorpyrifos restored behaviors to near normal that were initially impaired in the homozygous RM, and partially impaired in the heterozygous RM (Laviola et al. 2006). It was shown that deficient cholinergic transmission in RM mice could be restored by chlorpyrifos-mediated acetylcholinesterase inhibition. In subsequent studies, it was found that perinatal estradiol levels influence the number of Purkinje cells, and were regulated by reelin levels (Biamonte et al. 2009; Sigala et al. 2007). This sex by gene by environment interaction model serves more readily as a clue for further epidemiologic follow-up to understand autism etiology in humans (Halladay et al. 2009).

Several autism associated genes are involved in Ca^{2+} signaling and regulation (Halladay et al. 2009; Pessah and Lein 2008). The TS mouse model of autism involves a single nucleotide mutation essential for proper voltage dependent inactivation of the pore-forming subunit of the L-type calcium channel $Ca_v1.2$ (Splawski et al. 2004). $Ca_v1.2$ has been proposed to play direct roles in the development of synaptic plasticity (Morgan and Teyler 1999) and in gene translation and transcription (Dolmetsch 2003; Lenz and Avruch 2005; West et al. 2002).

Ca^{2+} signaling can be disrupted by polychlorinated biphenyls (PCBs) (Pessah et al. 2010), the organochlorine pesticides lindane and dieldrin (Heusinkveld and Westerink 2012), and several types of pyrethroid pesticides (Soderlund 2012). In a study comparing physiological effects of

eleven pyrethroid compounds in rats, the type II pyrethroids strongly induced increased Ca^{2+} channel influx into the cell, whereas the type I pyrethroids did not (Breckenridge et al. 2009). It should be noted that these three exposure types induced calcium perturbations at levels below those described as having a toxic effect based on primary mechanisms of action.

One could argue that mouse, rat, or zebrafish models may not demonstrate the core deficit that sets autism apart from other developmental disorders, a lack of social reciprocity. Recently, the prairie vole has been cited as a better model of autism due to their high degree of socialized behavior. For example, male prairie voles demonstrated social withdrawal after 10-days of dietary exposure to mercury, indicating a sex-specific effect of the exposure which induced a unique attribute of autism, social avoidance (Curtis et al. 2010).

Excitation/ Inhibition dysregulation of neuronal development

Rubenstein and Merzenich elegantly describe a model of autism whereby the cortical networks that govern language and social behavior are skewed towards increased excitation or away from inhibition resulting in an overall hyper-excitable state. Their hypothesis addresses both genomic and environmental factors influencing glutamate and GABA mediated neurotransmission, resulting in more noise in neural networks (Rubenstein and Merzenich 2003).

By poundage applied, the majority of pesticides inhibit acetylcholinesterase (AChE), the enzyme responsible for hydrolyzing the neurotransmitter acetylcholine (ACh). Examples include two of the most frequently used pesticides worldwide, organophosphates chlorpyrifos and diazinon, as well as the monomethyl carbamates (CB) including propoxur and methomyl. Insecticides that

target voltage-gated sodium channels (e.g., pyrethroids and DDT), the nicotinic ACh receptors themselves (e.g., imidacloprid), and $GABA_A$ receptors [e.g., organochlorines (OC), and fipronil] are ranked next highest in use in overall pounds applied (Casida 2009). The levels of ACh and GABA mediated neurotransmission and the activity of voltage dependent sodium channels are critical throughout prenatal and postnatal development, defining the ratio of excitatory and inhibitory neurotransmission in the brain, but also promoting and refining neural networks in the developing and adult brain (Belmonte and Bourgeron 2006).

GABA signaling pathways

GABA (gamma-Aminobutyric acid) is critical for normal development and regulation of neurotransmission (Campbell 1996). GABA activates two major families of receptors expressed in the mammalian brain: (1) $GABA_A$ and $GABA_C$ receptors that promote chloride fluxes and (2) $GABA_B$ receptors that are coupled to G-protein signaling. In adults, $GABA_A$ receptor activation promotes chloride influx and hyperpolarization of the membrane and decreases neuronal excitability. However, during fetal development, the chloride gradients across the membrane are reversed and therefore activation of $GABA_A$ receptors in the hippocampus and neocortex causes net chloride efflux and enhanced excitation (Watanabe et al. 2002). Thus, the temporal expression and spatial localization of GABA receptors within the brain can determine the patterns and activity of neural circuits. Numerous subunit isoforms for the $GABA_A$ receptor are developmentally regulated during the perinatal period and have distinct biophysical and pharmacological properties that contribute to their physiological (Cossart et al. 2005) and pathophysiological (Stafstrom CE 2010) functions. GABA is known to regulate many aspects of neural stem cell proliferation, differentiation, migration, and elongation (Varju et al. 2001). Due

to observed deficits in social and exploratory behavior, the $GABA_A$ receptor β3-gene deficient mouse has been suggested as an animal model of autism spectrum disorder (DeLorey et al. 2008).

Disruptions in the GABA system have been reported to be associated with autism in studies of receptor density from brain tissue (Blatt et al. 2001) as well as genetic association studies (Buxbaum et al. 2002; Cook et al. 1998; McCauley et al. 2004). In postmortem cerebellar tissue samples from brains of adults with autism, $GABA_A$ receptors were reduced comparing 4 cases to 8 controls and $GABA_B$ expression was altered in 5 cases as compared to 7 controls (Fatemi et al. 2009a; Fatemi et al. 2009b). Decreased expression of $GABA_A$ receptor β3 was shown to be associated with *MECP2* impairment in brain tissue samples from cases of autism, Angelman syndrome, and Rett syndrome (Samaco et al. 2005). In a family-based study, single nucleotide polymorphisms were examined in 470 families with at least one case of autism (266 multiplex, 204 triads) for GABA subunits on 14 alleles. Findings showed significant associations for $GABA_A$ receptor polymorphisms, in particular the A4 sub-unit and gene-gene interaction between receptor subunits (Ma et al. 2005).

In rats, prenatal exposure to the OC pesticides dieldren and lindane reduced $GABA_A$ receptor binding capabilities in the brainstem (Brannen et al. 1998). In another rat study, prenatal dieldren exposure was found to alter mRNA expression and subunit composition of $GABA_A$ receptors (Liu et al. 1998). Results from in vitro cortical neuronal cultures have shown endosulfan and related OC pesticides were shown to increase Akt phosphorylation, an effect mediated by the activation of ERβ, and to activate ERK1/2 through a mechanism involving $GABA_A$ and

glutamate receptors (Briz et al. 2011). In humans, a diminished ability to bind GABA contributes to poor muscle tone, which is observed in over half of persons with autism (Ming et al. 2007), and induces hyper excitable states as seen in epilepsy, a co-morbidity in approximately 20% of autistic cases (Bolton et al. 2011; Tuchman and Cuccaro 2011).

Polychlorinated biphenyls (PCBs) are organochlorines that had broad industrial uses, including adjuvants in paints and pesticide formulations (United States Environmental Protection Agency 2011). Although banned ~40 years ago, PCB exposures still remain a concern to human health due to their persistence in the environment. Developmental and in vitro studies in rodents and non-human primates have demonstrated the ability of non-coplanar PCBs to cause imbalances in excitatory and inhibitory neurotransmission within critical regions for language development (Kenet et al. 2007), social cognition (Nakagami et al. 2010), and for seizures (Kim et al. 2009; Kim and Pessah 2011). A substantial epidemiologic literature has provided evidence that cognitive deficits are associated with elevated PCB exposures, and more recently, elevated prenatal exposures to mono-ortho PCBs predicted lower scores on both the Mental Development Index (MDI) and the Psychomotor Development Index (PDI) of the Bayley Scales of Infant Development (Park et al. 2010). Furthermore, an analysis of seven hydroxylated metabolites of PCBs in cord blood revealed that the metabolite from mono-ortho substituted PCBs were the only ones associated with reduced MDI and PDI scores (Park et al. 2009). These findings underscore the complexity of toxicities within a compound class, and by the same principle, the critical need to characterize differences, e.g., among organophosphates or among pyrethroids.

Acetylcholine Signaling Pathways

ACh mediated neurotransmission is widely involved in the development of both peripheral and central nervous systems, and continues to play a critical role in regulation of muscle movement, learning, attention, cognition, and memory throughout adulthood. ACh regulates aspects of nerve excitation and inhibition that influence brain plasticity, arousal, and reward. ACh increases excitation both directly and indirectly, and works through both nicotinic and muscarinic receptors to stimulate inhibitory interneurons, thereby modulating the activity of downstream effectors in a complex manner (Brown 2010; Scharf 2003).

Several cholinergic abnormalities have been reported in autism (Bauman and Kemper 2005; Perry et al. 2001), as reviewed by Deutsch et al. (2010). In brief, studies of post-mortem brain tissue have reported reduced nicotinic acetylcholine (ACh) receptor binding in the frontal and parietal cortices (comparing 7 cases and 10 controls), reduced M1-muscarinic receptor binding in the parietal cortex (comparing 5 cases and 5 controls), and increased concentration of brain-derived neurotrophic factor (BDNF) (comparing 5 cases and 5 controls), which is involved in the development and function of cholinergic neurons (Deutsch et al. 2010). Although these studies involved small sample sizes, they suggest cholinergic abnormalities may be present in persons with autism.

OP insecticides irreversibly inhibit the active site of acetylcholinesterase (AChE), and while the severity of neurodevelopmental effects in animal studies correlate with AChE inhibition, additional neurotoxic effects have been observed at concentrations below the level sufficient to induce enzyme inhibition (Eddins et al. 2010; Levin et al. 2003; Slotkin et al. 2008). These

effects include altered cell packing density, decreases in serotonin receptors and nicotinic cholinergic receptor levels (Levin et al.), altered Ca^{2+} and K^{+} ion concentrations (Harrison et al. 2002; Murgia 2004), and oxidative stress (Aluigi et al. 2005). Metabolism of OP's is mediated by the paraoxonase1 enzyme (PON1), whereby fast metabolizers suffer less AChE inhibition than slow metabolizers in response to the same level of exposure (Costa et al. 2005).

Pertinent to the male predominance observed in autism, sex selective developmental effects have been seen in animal models exposed to OPs. Chlorpyrifos exposure (1mg/kg/day) in rats during postnatal days 1-4 decreased the number of errors in working and reference memory made by females, but increased errors made by males. These effects persisted into adolescence and adulthood, indicating a long-term consequence of exposure (Levin et al. 2001). Another study in rats showed that developmental exposures to low doses of parathion (an OP) induced greater developmental deficits in spatial navigation and working memory among males than females (Levin et al. 2009). Although these behaviors are not core features of autism, these findings provide evidence of different effects of early exposures between the sexes. In addition, parathion administration on postnatal days 1 – 4 at levels that barely inhibited cholinesterase was associated with deficits at 14-19 months; these deficits worsened with age (Levin et al. 2009).

The ability of OP's to inhibit AChE varies dramatically by chemical structure, which also determines reversibility. Aluigi et al. conducted a study examining the ChE mediated developmental effects of OP exposure on chick embryos, and discovered that 10^{-6} M chlorpyrifos was sufficient to inhibit head development (Aluigi et al. 2005). Even lower concentrations of chlorpyrifos-oxon disrupt axonal growth of rat dorsal root ganglia neurons (Yang et al. 2008),

and zebrafish sensory neurons (Yang et al. 2011), indicating that exposure to very low levels of this OP has the potential to adversely influence development of neural networks (Yang et al. 2011). Persistent neurobehavioral consequences of chlorpyrifos exposure in zebrafish have also been demonstrated (Eddins et al. 2010; Levin et al. 2003). Although chlorpyrifos is still used worldwide in residential settings, residential use has been banned in the U.S. due to neurotoxicity. However, no restrictions have been placed on agricultural use.

Oxidative stress and Mitochondrial Dysfunction

Cellular energy production through the degradation of adenosine tri-phosphate (ATP) by mitochondria is necessary for muscle development and brain function. Mitochondrial dysfunction has three major consequences: 1) decreased ATP production, 2) increased production of reactive oxygen species (ROS) and oxidative damage, and 3) induction of apoptosis (Rossignol and Frye 2012). These biochemical changes have been implicated in autism and can also be induced by exposure to OP, OC, and carbamate (CB) pesticides (Franco et al. 2009; Karami-Mohajeri and Abdollahi 2011; Rohlman et al. 2010). Although multiple modes of action have been described for specific organohalogens and halogenated insecticides, many induce dysregulation of Ca^{2+}-mediated signaling and production of mitochondrial ROS (Mariussen and Fonnum 2006). A thorough mechanistic hypothesis of autism via genetic risk and oxidative stress has been described by Deth et al. (Deth et al. 2008).

Nearly all insecticides discussed in this review induce oxidative stress. Permethrin, a pyrethroid used in agriculture and in topical creams for lice and scabies induces oxidative stress and apoptosis in the nervous system of zebrafish (Shi et al. 2011). Malathion, an OP commonly used

in aerial spraying throughout the 1980's for the Mediterranean fruit fly, and more recently to control mosquito vectors of West Nile Virus, induced mitochondrial dysfunction in liver cells at low concentrations and cytotoxicity at higher concentrations (Moore et al. 2010). The OC insecticide methoxychlor has been shown in mice to inhibit brain mitochondrial respiration (Schuh et al. 2005), and cause mitochondrial dysfunction and oxidative damage in the mouse ovary (Gupta et al. 2006). More recently, methoxychlor-mediated mitochondrial dysfunction was found to cause oxidative damage and dysfunction of the dopamine system in brains of mice (Schuh et al. 2009). Another study examining the effect of the OP dichlorvos on rat brain mitochondria found that chronic, low-level exposure can cause mitochondrial disruption and apoptosis of neuronal cells due to the release of cytochrome c and activation of caspase 3 following in vitro exposure (Kaur et al. 2007). Developmental exposure to the OP chlorpyrifos permanently decreased dopamine levels in zebrafish into adulthood (Eddins et al. 2010), important in the context of an already disrupted dopamine system in autism (Muhle et al. 2004).

Several recent studies have shown that toxicity of pyrethroid insecticides, many of which are organohalogen derivatives, is mediated by both dysregulation of cytoplasmic Ca^{2+} signaling and induction of oxidative stress (Cao et al. 2010; Kale et al. 1999; Soderlund 2011; Yan et al. 2011; Zhang et al. 2010). After the ban on residential uses of chlorpyrifos, household OP insecticides have been replaced with the other insecticides, namely pyrethroids and fipronil, a phenylpyrazole insecticide. A comparative toxicity study was conducted on rat PC12 cells to evaluate the hypothesis that fipronil is less toxic than chlorpyrifos, but fipronil was found to induce higher oxidative stress than chlopyrifos, an effect that was not mediated by the $GABA_A$ pathway (Lassiter et al. 2009).

Although the role of mitochondrial function in the autistic phenotype is not fully understood, approximately 8% of ASD cases experience mitochondrial dysfunction, compared with 0.05% of the general population (as reviewed by Haas, 2010). Mitochondrial dysfunction and increased mtDNA over-replication and mtDNA deletions were reported more frequently in lymphocytes from 10 children with autism as compared with lymphocytes from 10 typically developing controls (Giulivi et al. 2010).

Immune Toxicity

Prenatal disruption of immune development can result in atopy, allergy, deficits in immune-competence, and auto-immunity in early childhood (Hertz-Picciotto et al. 2008). Recent studies on intestinal flora have shown the immune system is highly involved and inextricably linked with neurodevelopment and subsequent behavior (Diamond et al. 2011; Heijtz et al. 2011). In turn, the immune response can also be strongly influenced by neurochemistry (Diamond et al. 2011). Children with autism experience a wide array of immune abnormalities. Recent reviews on this topic report altered cytokine profiles, altered cellular immunity, low levels of lymphocytes and t-cell mitogen responses, neuro-inflammation, and auto-antibodies directed at nuclear proteins (Ashwood et al. 2006; Goines and Van de Water 2010). Reduced levels of IgG and IgM have also been reported, which were correlated with higher prevalence of aberrant behavioral symptoms in a study of 271 children with autism, or developmental delay, or who were typically functioning (Heuer et al. 2008). In a comparison of plasma cytokine levels from children with autism (N=97) and typically developed controls (N=87), cases had higher levels of pro-inflammatory cytokines compared with neurotypical children, and the concentrations of cytokines corresponded with impaired behavioral outcomes in a dose-response fashion (Ashwood et al. 2011).

Exposure to several types of pesticides may result in decreased immune-competence, immune-enhancement, and/or auto-immunity (Corsini et al. 2008). OP's are particularly immunotoxic (Galloway and Handy 2003), and have been shown to suppress natural killer cells, lymphokine activated killer cells, and cytotoxic t-lymphocytes by inhibiting granzymes, impairing the FasL/Fas pathways, and inducing apoptosis of immune cells (Li 2007). Pyrethroids have also been shown to be immunotoxic in animal models. Rats treated sub-chronically with permethrin showed large increases of superoxide anion production and hydrogen peroxide-myeloperoxidase activity in polymorphonuclear neutrophils (Gabbianelli et al. 2009). These effects were demonstrated not only for permethrin, but also for its major metabolites.

Insecticide exposures can induce inflammatory or suppressive immunological effects depending on the compound and the immunological outcome in question. Gestational exposure of rats to atrazine, an endocrine disrupting triazine herbicide, induced immunosuppressive effects (specifically, decreased delayed type hypersensitivity and antibody production) in male offspring only (Rooney et al. 2003). In a study of both male and female mice, gestational exposure to atrazine at non-toxic, environmentally relevant doses administered from gestation day 14 to postnatal day 21,was associated with decreased socialization behaviors and changes in exploratory behavior, with males displaying feminized behavioral profiles (Belloni et al. 2011).

Neuro-inflammation has been observed in post-mortem brain tissue of people with autism across several age ranges (Li et al. 2009; Morgan et al. 2010; Vargas et al. 2005). Chlorpyrifos, an organophosphate banned for residential use in 2002, and cyfluthrin, a type II pyrethroid used to replace chlorpyrifos, were compared for toxicological and toxicogenomic effects to primary

human fetal astrocytes. Cyfluthrin had equivalent or more toxic effects in most assays, and up-regulated several insulin related genes and pro-inflammatory genes on the IFN-γ pathway, including *IL6R* and *GFAP*. Additionally, both compounds were found to promote inflammatory activation of astrocytes. The authors suggested that the combination of increased insulin production and inflammation could lead to a state of chronic brain inflammation that might significantly alter brain development (Mense et al. 2006).

Taken together, these studies indicate that gestational exposure to pesticides can induce immunological abnormalities as well as behavioral abnormalities. It is possible that the neurodevelopmental and the immune abnormalities observed in autism are downstream manifestations of the same underlying process given the tightly regulated interconnection between the developing systems in utero. The role of the immune phenomena as a cause, effect, or side effect of autism was recently reviewed and was postulated to be in part causal (Onore et al. 2012). In addition to autism, schizophrenia and major depressive disorders have also been noted to be accompanied by perturbations of the immune system, recently reviewed in an extensive monograph (Patterson 2011).

Parental thyroid hormone levels and brain development

Adequate levels of in utero thyroid hormones are critical for brain development. Maternal thyroid impairment has been suggested as an underlying mechanism for developmental impairments resulting from exposures to environmental chemicals such as polychlorinated biphenyls (PCBs), utilized in a wide variety of industrial uses, and polybrominated diphenyl ethers (PBDEs) used as flame retardants (Winneke 2011). Pesticides have been found to interfere

with thyroid function by preventing iodine uptake (mancozeb, thiocyanates, 2,4-D) and peroxidation (aminotriazole, endosulfan, malathion), and by preventing the conversion of thyroxine(T4) to triiodothyronine (T3) (aminotriazole, dimethoate, fenvalerate) (Colborn 2004). In a review of the effects of mild to moderate iodine deficiency in humans, diminished maternal T4 was associated with disorders of mental and/or psychomotor development (Zimmermann 2007) .

Roman hypothesized that even transient intrauterine deficits in thyroid hormones (3 days) at critical points in gestation could alter the cortical architecture interfering with neuronal migration and Purkinje cell growth (Roman 2007), both of which have been observed in autopsy studies of autism (Fatemi et al. 2002; Wegiel et al. 2010). Because the human fetus does not start producing sufficient thyroid hormones until gestational week 18 (Burrow et al. 1994), adequate maternal thyroid hormones are critical to neurodevelopment in early fetal life, particularly for reelin regulated neuronal migration (Pathak et al. 2011). Additionally, sex mediated effects have been observed following exposure to chlorpyrifos on gestational days 17-20, inducing increased levels of free T4 in female but not male mice (Haviland et al. 2009).

Vulnerable genetic sub-populations

The primary neurological targets of commonly used insecticides (Scharf 2003) can be paired with vulnerable genetic subpopulations that may be at increased risk for autism (Table 1). Because of both the large number of genetic alterations and gene-gene interactions that have been implicated in autism, and the phenotypic heterogeneity in cases, the notion that a single environmental exposure will be to blame for the majority of cases is unrealistic. Also, because

the dosage of pesticides to non-occupationally exposed women is likely to be lower than that required to induce mechanisms of injury observed in many animal models, genetic susceptibility becomes a critical factor in this discussion.

In 2001, the reelin gene was implicated in autism risk when repeats (11+) in the 5' untranslated region were associated with 72% transmission to affected siblings and only 32% transmission to unaffected siblings (Persico et al. 2001). The proteolytic activity of reelin on extracellular matrix proteins that control neuronal migration is significantly inhibited by OP pesticides (Sinagra et al. 2008), and OP metabolism efficiency is regulated by paraoxonase 1 (*PON1*) (Mackness et al. 1997). Interestingly, an association between less active forms of the *PON1* gene and autism was observed in Caucasian families in North America, but not in Italian families, leading authors to hypothesize that the slow metabolizing polymorphism confers risk in areas with high levels of organophosphates but may not affect autism risk otherwise (D'Amelio et al. 2005).

Conclusions

Here we have reviewed several mechanisms by which pesticides may increase the risk of autism, summarized in Table 2. Pesticides may or may not, however, have played a role in the trend of increasing autism prevalence, which itself is likely due to a confluence of multiple phenomena, including changes in diagnostic practices, physician and lay awareness, the availability of treatments, and the prevalence of a variety of environmental chemical, medical, and food-related exposures. While pesticide use patterns have changed, home and ambient environments also include other exposures that have changed over time due to regulatory and economic factors (e.g., flame retardants, plasticizers, solvents, stabilizers, and anti-microbials).

Pesticides are composed of a parent product, inert ingredients, and in some cases agonists that enhance the functionality of the parent compound, and all of these ingredients may be degraded to metabolites that also distribute throughout the body. Consequently, pesticide formulations represent a mixture of compounds that might contribute to observed effects. Difficulties in distinguishing the effects of metabolites vs. parent compounds may have confounded associations observed in many studies of urinary metabolites and neurodevelopment, and very few studies have examined main effects of effect modification of exposure to agonists such as piperonyl butoxide, which slows the metabolism of several types of pesticides by inhibiting cytochrome P450 enzymes.

Although pesticides are a biologically plausible contributor to autism, research in several critical areas is needed to understand cognitive and behavioral consequences of gestational exposure in humans. First, animal studies suggest critical windows of exposure, yet in humans the window or windows of biologic susceptibility remain unknown, and would be expected to vary by mechanism. Second, studies of non-toxic, environmentally relevant doses are needed to understand effects of developmental neurotoxicity in the context of a background of genetic susceptibilities. Third, the vast majority of exposures occur in combination with exposures to other ubiquitous and/or persistent compounds such as flame retardants, plasticizers, and other pesticides. More research on combinations of exposures may reveal interactions between environmental exposures, such as effect modification by chemical additives to pesticide compounds. In light of the recently revised prevalence estimates of autism (1 in 88), large birth cohorts, such as the National Children's Study (NCS), which aim to enroll women at pregnancy

and follow the children over time, are well positioned to obtain enough cases and to examine prenatal exposures prospectively. Pending accurate and reliable exposure estimates in critical time windows, and enrollment of approximately 100,000 children resulting in 1,000 or more cases of autism, NCS can contribute greatly to our understanding of these associations. Finally, more case-control studies with large populations of participants with confirmed diagnoses of autism that examine environmental exposures in relation to severity of the core domains of language impairment, social avoidance, and repetitive behaviors or insistence on sameness may shed light on possible exposure-related endophenotypes.

Although we have described several possible avenues by which pesticide exposure may influence autism, the dearth of studies on large occupational and pregnancy cohorts with adequate exposure assessment impedes our understanding of 1) whether pesticides are consistently associated with autism risk and 2) if so, which pesticide compounds and which components of those compounds might actually contribute to autism risk. Grandjean and Landrigan hypothesize that our exposure to chemicals that have not been adequately tested for developmental neurotoxicity has led to a silent pandemic (Grandjean and Landrigan 2006). Further research is warranted to provide the evidence base that can ultimately lead to reduction or elimination of these potentially damaging exposures through changes to regulatory policy, consumer behavior, or dietary choices.

References

Aluigi MG, Angelini C, Falugi C, Fossa R, Genever P, Gallus L, et al. 2005. Interaction between organophosphate compounds and cholinergic functions during development. Chem Biol Interact 157-158:305-316.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. 2011. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 25(1):40-45.

Ashwood P, Wills S, Van de Water J. 2006. The immune response in autism: a new frontier for autism research. J Leukoc Biol 80(1):1-15.

Bader PL, Faizi M, Kim LH, Owen SF, Tadross MR, Alfa RW, et al. 2011. Mouse model of Timothy syndrome recapitulates triad of autistic traits. Proc Natl Acad Sci U S A 108(37):15432-15437.

Bauman ML, Kemper TL. 2005. Neuroanatomic observations of the brain in autism: a review and future directions. Int J Dev Neurosci 23(2-3):183-187.

Belloni V, Dessi-Fulgheri F, Zaccaroni M, Di Consiglio E, De Angelis G, Testai E, et al. 2011. Early exposure to low doses of atrazine affects behavior in juvenile and adult CD1 mice. Toxicology 279(1-3):19-26.

Belmonte MK, Bourgeron T. 2006. Fragile X syndrome and autism at the intersection of genetic and neural networks. Nat Neurosci 9(10):1221-1225.

Biamonte F, Assenza G, Marino R, D'Amelio M, Panteri R, Caruso D, et al. 2009. Interactions between neuroactive steroids and reelin haploinsufficiency in Purkinje cell survival. Neurobiology of disease 36(1):103-115.

Blatt GJ, Fitzgerald CM, Guptill JT, Booker AB, Kemper TL, Bauman ML. 2001. Density and distribution of hippocampal neurotransmitter receptors in autism: an autoradiographic study. J Autism Dev Disord 31(6):537-543.

Bolton PF, Carcani-Rathwell I, Hutton J, Goode S, Howlin P, Rutter M. 2011. Epilepsy in autism: features and correlates. The British journal of psychiatry : the journal of mental science 198(4):289-294.

Brannen KC, Devaud LL, Liu J, Lauder JM. 1998. Prenatal exposure to neurotoxicants dieldrin or lindane alters tert-butylbicyclophosphorothionate binding to GABA(A) receptors in fetal rat brainstem. Dev Neurosci 20(1):34-41.

Breckenridge CB, Holden L, Sturgess N, Weiner M, Sheets L, Sargent D, et al. 2009. Evidence for a separate mechanism of toxicity for the Type I and the Type II pyrethroid insecticides. Neurotoxicology 30 Suppl 1:S17-31.

Briz V, Molina-Molina JM, Sanchez-Redondo S, Fernandez MF, Grimalt JO, Olea N, et al. 2011. Differential estrogenic effects of the persistent organochlorine pesticides dieldrin, endosulfan, and lindane in primary neuronal cultures. Toxicol Sci 120(2):413-427.

Brown DA. 2010. Muscarinic acetylcholine receptors (mAChRs) in the nervous system: some functions and mechanisms. Journal of molecular neuroscience : MN 41(3):340-346.

Burrow GN, Fisher DA, Larsen PR. 1994. Maternal and fetal thyroid function. N Engl J Med 331(16):1072-1078.

Buxbaum JD, Silverman JM, Smith CJ, Greenberg DA, Kilifarski M, Reichert J, et al. 2002. Association between a GABRB3 polymorphism and autism. Mol Psychiatry 7(3):311-316.

Campbell NA. 1996. Biology. 4th ed. Menlo Park, CA Benjamin/Cummings Publishing Company.

Cao Z, Shafer TJ, Murray TF. 2010. Mechanisms of pyrethroid insecticide-induced stimulation of calcium influx in neocortical neurons. J Pharmacol Exp Ther 336(1):197-205.

Casida JE. 2009. Pest toxicology: the primary mechanisms of pesticide action. Chem Res Toxicol 22(4):609-619.

Centers for Disease Control and Prevention. 2012. Prevalence of autism spectrum disorders - autism and developmental disabilities monitoring network, 14 sites, United States, 2008. In: MMWR Surveill Summ, 1-19.

Colborn T. 2004. Neurodevelopment and endocrine disruption. Environ Health Perspect 112(9):944-949.

Cook EH, Jr., Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ, et al. 1998. Linkage-disequilibrium mapping of autistic disorder, with 15q11-13 markers. Am J Hum Genet 62(5):1077-1083.

Corsini E, Liesivuori J, Vergieva T, Van Loveren H, Colosio C. 2008. Effects of pesticide exposure on the human immune system. Hum Exp Toxicol 27(9):671-680.

Cossart R, Bernard C, Ben-Ari Y. 2005. Multiple facets of GABAergic neurons and synapses: multiple fates of GABA signalling in epilepsies. Trends Neurosci 28(2):108-115.

Costa LG, Cole TB, Vitalone A, Furlong CE. 2005. Measurement of paraoxonase (PON1) status as a potential biomarker of susceptibility to organophosphate toxicity. Clin Chim Acta 352(1-2):37-47.

Crawley JN, Chen T, Puri A, Washburn R, Sullivan TL, Hill JM, et al. 2007. Social approach behaviors in oxytocin knockout mice: comparison of two independent lines tested in different laboratory environments. Neuropeptides 41(3):145-163.

Curtis JT, Hood AN, Chen Y, Cobb GP, Wallace DR. 2010. Chronic metals ingestion by prairie voles produces sex-specific deficits in social behavior: an animal model of autism. Behav Brain Res 213(1):42-49.

D'Amelio M, Ricci I, Sacco R, Liu X, D'Agruma L, Muscarella LA, et al. 2005. Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene-environment interactions. Mol Psychiatry 10(11):1006-1016.

DeLorey TM, Sahbaie P, Hashemi E, Homanics GE, Clark JD. 2008. Gabrb3 gene deficient mice exhibit impaired social and exploratory behaviors, deficits in non-selective attention and hypoplasia of cerebellar vermal lobules: a potential model of autism spectrum disorder. Behav Brain Res 187(2):207-220.

Deth R, Muratore C, Benzecry J, Power-Charnitsky VA, Waly M. 2008. How environmental and genetic factors combine to cause autism: A redox/methylation hypothesis. Neurotoxicology 29(1):190-201.

Deutsch SI, Urbano MR, Neumann SA, Burket JA, Katz E. 2010. Cholinergic abnormalities in autism: is there a rationale for selective nicotinic agonist interventions? Clin Neuropharmacol 33(3):114-120.

Diamond B, Huerta PT, Tracey K, Volpe BT. 2011. It takes guts to grow a brain: Increasing evidence of the important role of the intestinal microflora in neuro- and immune-modulatory functions during development and adulthood. Bioessays 33(8):588-591.

Dolmetsch R. 2003. Excitation-transcription coupling: signaling by ion channels to the nucleus. Science's STKE : signal transduction knowledge environment 2003(166):PE4.

Eddins D, Cerutti D, Williams P, Linney E, Levin ED. 2010. Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. Neurotoxicol Teratol 32(1):99-108.

Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, et al. 2007. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect 115(5):792-798.

Ey E, Leblond CS, Bourgeron T. 2011. Behavioral profiles of mouse models for autism spectrum disorders. Autism Res 4(1):5-16.

Fatemi SH, Folsom TD, Reutiman TJ, Thuras PD. 2009a. Expression of GABA(B) receptors is altered in brains of subjects with autism. Cerebellum 8(1):64-69.

Fatemi SH, Halt AR, Realmuto G, Earle J, Kist DA, Thuras P, et al. 2002. Purkinje cell size is reduced in cerebellum of patients with autism. Cellular and molecular neurobiology 22(2):171-175.

Fatemi SH, Reutiman TJ, Folsom TD, Thuras PD. 2009b. GABA(A) receptor downregulation in brains of subjects with autism. J Autism Dev Disord 39(2):223-230.

Franco R, Sanchez-Olea R, Reyes-Reyes EM, Panayiotidis MI. 2009. Environmental toxicity, oxidative stress and apoptosis: menage a trois. Mutat Res 674(1-2):3-22.

Gabbianelli R, Falcioni ML, Nasuti C, Cantalamessa F, Imada I, Inoue M. 2009. Effect of permethrin insecticide on rat polymorphonuclear neutrophils. Chem Biol Interact 182(2-3):245-252.

Galloway T, Handy R. 2003. Immunotoxicity of organophosphorous pesticides. Ecotoxicology 12(1-4):345-363.

Geschwind DH. 2011. Genetics of autism spectrum disorders. Trends Cogn Sci 15(9):409-416.

Giulivi C, Zhang YF, Omanska-Klusek A, Ross-Inta C, Wong S, Hertz-Picciotto I, et al. 2010. Mitochondrial dysfunction in autism. JAMA 304(21):2389-2396.

Goines P, Van de Water J. 2010. The immune system's role in the biology of autism. Curr Opin Neurol 23(2):111-117.

Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368(9553):2167-2178.

Gupta RK, Schuh RA, Fiskum G, Flaws JA. 2006. Methoxychlor causes mitochondrial dysfunction and oxidative damage in the mouse ovary. Toxicol Appl Pharmacol 216(3):436-445.

Halladay AK, Amaral D, Aschner M, Bolivar VJ, Bowman A, DiCicco-Bloom E, et al. 2009. Animal models of autism spectrum disorders: information for neurotoxicologists. Neurotoxicology 30(5):811-821.

Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, et al. 2011. Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry 68(11):1095-1102.

Hamilton SM, Spencer CM, Harrison WR, Yuva-Paylor LA, Graham DF, Daza RA, et al. 2011. Multiple autism-like behaviors in a novel transgenic mouse model. Behav Brain Res 218(1):29-41.

Harrison PK, Falugi C, Angelini C, Whitaker MJ. 2002. Muscarinic signalling affects intracellular calcium concentration during the first cell cycle of sea urchin embryos. Cell Calcium 31(6):289-297.

Haviland JA, Butz DE, Porter WP. 2009. Long-term sex selective hormonal and behavior alterations in mice exposed to low doses of chlorpyrifos in utero. Reprod Toxicol 29(1):74-79.

Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, et al. 2011. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 108(7):3047-3052.

Hertz-Picciotto I, Delwiche L. 2009. The rise in autism and the role of age at diagnosis. Epidemiology 20(1):84-90.

Hertz-Picciotto I, Park HY, Dostal M, Kocan A, Trnovec T, Sram R. 2008. Prenatal exposures to persistent and non-persistent organic compounds and effects on immune system development. Basic Clin Pharmacol Toxicol 102(2):146-154.

Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, Hertz-Picciotto I, et al. 2008. Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. Autism Res 1(5):275-283.

Heusinkveld HJ, Westerink RH. 2012. Organochlorine insecticides lindane and dieldrin and their binary mixture disturb calcium homeostasis in dopaminergic PC12 cells. Environ Sci Technol 46(3):1842-1848.

Kale M, Rathore N, John S, Bhatnagar D. 1999. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. Toxicol Lett 105(3):197-205.

Karami-Mohajeri S, Abdollahi M. 2011. Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: a systematic review. Hum Exp Toxicol 30(9):1119-1140.

Kaur P, Radotra B, Minz RW, Gill KD. 2007. Impaired mitochondrial energy metabolism and neuronal apoptotic cell death after chronic dichlorvos (OP) exposure in rat brain. Neurotoxicology 28(6):1208-1219.

Kenet T, Froemke RC, Schreiner CE, Pessah IN, Merzenich MM. 2007. Perinatal exposure to a noncoplanar polychlorinated biphenyl alters tonotopy, receptive fields, and plasticity in rat primary auditory cortex. Proc Natl Acad Sci U S A 104(18):7646-7651.

Kim KH, Inan SY, Berman RF, Pessah IN. 2009. Excitatory and inhibitory synaptic transmission is differentially influenced by two ortho-substituted polychlorinated biphenyls in the hippocampal slice preparation. Toxicol Appl Pharmacol 237(2):168-177.

Kim KH, Pessah IN. 2011. Perinatal exposure to environmental polychlorinated biphenyls sensitizes hippocampus to excitotoxicity ex vivo. Neurotoxicology 32(6):981-985.

Lassiter TL, MacKillop EA, Ryde IT, Seidler FJ, Slotkin TA. 2009. Is fipronil safer than chlorpyrifos? Comparative developmental neurotoxicity modeled in PC12 cells. Brain Res Bull 78(6):313-322.

Laviola G, Adriani W, Gaudino C, Marino R, Keller F. 2006. Paradoxical effects of prenatal acetylcholinesterase blockade on neuro-behavioral development and drug-induced stereotypies in reeler mutant mice. Psychopharmacology 187(3):331-344.

Laviola G, Ognibene E, Romano E, Adriani W, Keller F. 2009. Gene-environment interaction during early development in the heterozygous reeler mouse: clues for modelling of major neurobehavioral syndromes. Neurosci Biobehav Rev 33(4):560-572.

Le Couteur A, Bailey A, Goode S, Pickles A, Robertson S, Gottesman I, et al. 1996. A broader phenotype of autism: the clinical spectrum in twins. Journal of child psychology and psychiatry, and allied disciplines 37(7):785-801.

Lenz G, Avruch J. 2005. Glutamatergic regulation of the p70S6 kinase in primary mouse neurons. J Biol Chem 280(46):38121-38124.

Levin ED, Addy N, Nakajima A, Christopher NC, Seidler FJ, Slotkin TA. 2001. Persistent behavioral consequences of neonatal chlorpyrifos exposure in rats. Brain research Developmental brain research 130(1):83-89.

Levin ED, Chrysanthis E, Yacisin K, Linney E. 2003. Chlorpyrifos exposure of developing zebrafish: effects on survival and long-term effects on response latency and spatial discrimination. Neurotoxicol Teratol 25(1):51-57.

Levin ED, Timofeeva OA, Yang L, Petro A, Ryde IT, Wrench N, et al. Early postnatal parathion exposure in rats causes sex-selective cognitive impairment and neurotransmitter defects which emerge in aging. Behav Brain Res 208(2):319-327.

Levin ED, Timofeeva OA, Yang L, Petro A, Ryde IT, Wrench N, et al. 2009. Early postnatal parathion exposure in rats causes sex-selective cognitive impairment and neurotransmitter defects which emerge in aging. Behav Brain Res 208(2):319-327.

Li Q. 2007. New mechanism of organophosphorus pesticide-induced immunotoxicity. J Nippon Med Sch 74(2):92-105.

Li X, Chauhan A, Sheikh AM, Patil S, Chauhan V, Li XM, et al. 2009. Elevated immune response in the brain of autistic patients. J Neuroimmunol 207(1-2):111-116.

Liu J, Brannen KC, Grayson DR, Morrow AL, Devaud LL, Lauder JM. 1998. Prenatal exposure to the pesticide dieldrin or the GABA(A) receptor antagonist bicuculline differentially alters expression of GABA(A) receptor subunit mRNAs in fetal rat brainstem. Dev Neurosci 20(1):83-92.

Ma DQ, Whitehead PL, Menold MM, Martin ER, Ashley-Koch AE, Mei H, et al. 2005. Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. Am J Hum Genet 77(3):377-388.

Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. 1997. Effect of the molecular polymorphisms of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon. British journal of pharmacology 122(2):265-268.

Mariussen E, Fonnum F. 2006. Neurochemical targets and behavioral effects of organohalogen compounds: an update. Crit Rev Toxicol 36(3):253-289.

McCauley JL, Olson LM, Delahanty R, Amin T, Nurmi EL, Organ EL, et al. 2004. A linkage disequilibrium map of the 1-Mb 15q12 GABA(A) receptor subunit cluster and association to autism. Am J Med Genet B Neuropsychiatr Genet 131B(1):51-59.

Mense SM, Sengupta A, Lan C, Zhou M, Bentsman G, Volsky DJ, et al. 2006. The common insecticides cyfluthrin and chlorpyrifos alter the expression of a subset of genes with diverse functions in primary human astrocytes. Toxicol Sci 93(1):125-135.

Ming X, Brimacombe M, Wagner GC. 2007. Prevalence of motor impairment in autism spectrum disorders. Brain Dev 29(9):565-570.

Moore PD, Yedjou CG, Tchounwou PB. 2010. Malathion-induced oxidative stress, cytotoxicity, and genotoxicity in human liver carcinoma (HepG2) cells. Environmental toxicology 25(3):221-226.

Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, et al. 2010. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. Biol Psychiatry 68(4):368-376.

Morgan SL, Teyler TJ. 1999. VDCCs and NMDARs underlie two forms of LTP in CA1 hippocampus in vivo. J Neurophysiol 82(2):736-740.

Muhle R, Trentacoste SV, Rapin I. 2004. The genetics of autism. Pediatrics 113(5):e472-486.

Murgia AZ, I.; Basso, M.; Deplano, S.; Falugi, C.; Prestipino, G. Electrophysyological and immunohistochemical studies of a organophospate pesticide on neuron K+ channels modulated by muscarinic receptor. In: Proceedings of the Proceedings of the Meeting Società Italiana Biofisica applicata (SIBPA), 2004. Pisa, Italy.

Nakagami A, Koyama T, Kawasaki K, Negishi T, Ihara T, Kuroda Y, et al. 2010. Maternal plasma polychlorinated biphenyl levels in cynomolgus monkeys (Macaca fascicularis) affect infant social skills in mother-infant interaction. Dev Psychobiol 53(1):79-88.

Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, et al. 2012. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature.

O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, et al. 2012. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 000(00):1-7.

Onore C, Careaga M, Ashwood P. 2012. The role of immune dysfunction in the pathophysiology of autism. Brain Behav Immun 26(3):383-392.

Park HY, Hertz-Picciotto I, Sovcikova E, Kocan A, Drobna B, Trnovec T. 2010. Neurodevelopmental toxicity of prenatal polychlorinated biphenyls (PCBs) by chemical structure and activity: a birth cohort study. Environ Health 9:51.

Park HY, Park JS, Sovcikova E, Kocan A, Linderholm L, Bergman A, et al. 2009. Exposure to hydroxylated polychlorinated biphenyls (OH-PCBs) in the prenatal period and subsequent neurodevelopment in eastern Slovakia. Environ Health Perspect 117(10):1600-1606.

Pathak A, Sinha RA, Mohan V, Mitra K, Godbole MM. 2011. Maternal thyroid hormone before the onset of fetal thyroid function regulates reelin and downstream signaling cascade affecting neocortical neuronal migration. Cereb Cortex 21(1):11-21.

Patterson PH. 2011. Infectious Behavior Brain-Immune Connections in Autism, Schizophrenia, and Depression In Infectious Behavior: MIT Press.

Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, et al. 2011. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. Nature 472(7344):437-442.

Perry EK, Lee ML, Martin-Ruiz CM, Court JA, Volsen SG, Merrit J, et al. 2001. Cholinergic activity in autism: abnormalities in the cerebral cortex and basal forebrain. Am J Psychiatry 158(7):1058-1066.

Persico AM, D'Agruma L, Maiorano N, Totaro A, Militerni R, Bravaccio C, et al. 2001. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. Mol Psychiatry 6(2):150-159.

Pessah IN, Cherednichenko G, Lein PJ. 2010. Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity. Pharmacology & therapeutics 125(2):260-285.

Pessah IN, Lein P. 2008. Evidence for Environmental Susceptibility in Autism. What we know What We Need to Know About Gene x Environment Interactions. In: Autism: Current Theories and Evidence, (Zimmerman AW, ed):Humana Press.

Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. 2007. Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. Environ Health Perspect 115(10):1482-1489.

Rohlman DS, Anger WK, Lein PJ. 2010. Correlating neurobehavioral performance with biomarkers of organophosphorous pesticide exposure. Neurotoxicology 32(2):268-276.

Roman GC. 2007. Autism: transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. J Neurol Sci 262(1-2):15-26.

Rooney AA, Matulka RA, Luebke RW. 2003. Developmental atrazine exposure suppresses immune function in male, but not female Sprague-Dawley rats. Toxicol Sci 76(2):366-375.

Rossignol DA, Frye RE. 2012. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. Mol Psychiatry 17(3):290-314.

Rubenstein JL, Merzenich MM. 2003. Model of autism: increased ratio of excitation/inhibition in key neural systems. Genes Brain Behav 2(5):255-267.

Samaco RC, Hogart A, LaSalle JM. 2005. Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. Hum Mol Genet 14(4):483-492.

Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, et al. 2012. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature.

Scharf ME. 2003. Neurological effects of insecticides. In: Encyclopedia of Pest Management: Marcel Dekker, Inc.

Schuh RA, Kristian T, Gupta RK, Flaws JA, Fiskum G. 2005. Methoxychlor inhibits brain mitochondrial respiration and increases hydrogen peroxide production and CREB phosphorylation. Toxicol Sci 88(2):495-504.

Schuh RA, Richardson JR, Gupta RK, Flaws JA, Fiskum G. 2009. Effects of the organochlorine pesticide methoxychlor on dopamine metabolites and transporters in the mouse brain. Neurotoxicology 30(2):274-280.

Shi X, Gu A, Ji G, Li Y, Di J, Jin J, et al. 2011. Developmental toxicity of cypermethrin in embryo-larval stages of zebrafish. Chemosphere 85(6):1010-1016.

Sigala S, Zoli M, Palazzolo F, Faccoli S, Zanardi A, Mercuri NB, et al. 2007. Selective disarrangement of the rostral telencephalic cholinergic system in heterozygous reeler mice. Neuroscience 144(3):834-844.

Silverman JL, Yang M, Lord C, Crawley JN. 2010. Behavioural phenotyping assays for mouse models of autism. Nature reviews Neuroscience 11(7):490-502.

Sinagra M, Gonzalez Campo C, Verrier D, Moustie O, Manzoni OJ, Chavis P. 2008. Glutamatergic cerebellar granule neurons synthesize and secrete reelin in vitro. Neuron glia biology 4(3):189-196.

Slotkin TA, Bodwell BE, Levin ED, Seidler FJ. 2008. Neonatal exposure to low doses of diazinon: long-term effects on neural cell development and acetylcholine systems. Environ Health Perspect 116(3):340-348.

Soderlund DM. 2011. Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. Arch Toxicol.

Soderlund DM. 2012. Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances. Arch Toxicol 86(2):165-181.

Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. 2004. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell 119(1):19-31.

Stafstrom CE HP, Pessah IN. 2010. Epilepsy in autism spectrum disorders. In: Jasper's Basic Mechanisms of the Epilepsies in Epilepsia, Vol. 51, (Noebels JL AM, Rogawski MA, Olsen RW, Delgado-Escueta AV, ed):Oxford University Press, 78.

Stankiewicz P, Lupski JR. 2010. Structural variation in the human genome and its role in disease. Annu Rev Med 61:437-455.

Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, et al. 2007. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 318(5847):71-76.

Tuchman R, Cuccaro M. 2011. Epilepsy and autism: neurodevelopmental perspective. Current neurology and neuroscience reports 11(4):428-434.

United States Department of Agriculture. 2006. Pest Management Practices. Available: http://www.ers.usda.gov/publications/arei/eib16/Chapter4/4.3/ [accessed January 17, 2012].

United States Environmental Protection Agency. 2011. Basic Information about Polychlorinated Biphenyls (PCBs) in Drinking Water. Available: http://water.epa.gov/drink/contaminants/basicinformation/polychlorinated-biphenyls.cfm [accessed 03 January 2012].

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57(1):67-81.

Varju P, Katarova Z, Madarasz E, Szabo G. 2001. GABA signalling during development: new data and old questions. Cell Tissue Res 305(2):239-246.

Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki H. 2002. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol 213:1-47.

Wegiel J, Kuchna I, Nowicki K, Imaki H, Marchi E, Ma SY, et al. 2010. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta neuropathologica 119(6):755-770.

West AE, Griffith EC, Greenberg ME. 2002. Regulation of transcription factors by neuronal activity. Nature reviews Neuroscience 3(12):921-931.

Williams MK, Rundle A, Holmes D, Reyes M, Hoepner LA, Barr DB, et al. 2008. Changes in pest infestation levels, self-reported pesticide use, and permethrin exposure during pregnancy after the 2000-2001 U.S. Environmental Protection Agency restriction of organophosphates. Environ Health Perspect 116(12):1681-1688.

Winneke G. 2011. Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls. J Neurol Sci 308(1-2):9-15.

Woodruff TJ, Zota AR, Schwartz JM. 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect 119(6):878-885.

Yan Y, Yang Y, You J, Yang G, Xu Y, Huang N, et al. 2011. Permethrin modulates cholinergic mini-synaptic currents by partially blocking the calcium channel. Toxicol Lett 201(3):258-263.

Yang D, Howard A, Bruun D, Ajua-Alemanj M, Pickart C, Lein PJ. 2008. Chlorpyrifos and chlorpyrifos-oxon inhibit axonal growth by interfering with the morphogenic activity of acetylcholinesterase. Toxicol Appl Pharmacol 228(1):32-41.

Yang D, Lauridsen H, Buels K, Chi LH, La Du J, Bruun DA, et al. 2011. Chlorpyrifos-oxon disrupts zebrafish axonal growth and motor behavior. Toxicol Sci 121(1):146-159.

Zhang X, Daugherty SL, de Groat WC. 2010. Activation of CaMKII and ERK1/2 contributes to the time-dependent potentiation of Ca2+ response elicited by repeated application of capsaicin in rat DRG neurons. Am J Physiol Regul Integr Comp Physiol 300(3):R644-654.

Zimmermann MB. 2007. The adverse effects of mild-to-moderate iodine deficiency during pregnancy and childhood: a review. Thyroid 17(9):829-835.

Table 1. Insecticide compounds with a generalized excitatory neurological effect. Adapted from Michael E. Scharf's *Neurological Effects of Insecticides*, published in the Encyclopedia of Pest Management, 2003 (Scharf 2003).

Primary neurological target	**Insecticide class**	**Mode of action**	**Vulnerable subpopulations**
Acetylcholinesterase	Organophosphate	Inhibition	PON1 polymorphisms
	Carbamate	Inhibition	
Voltage-gated sodium channel	Organochlorine	Modified gating kinetics	SCN1A, SCN1B
	Pyrethrin/pyrethroid	Modified gating kinetics	HCE1 (CES1) HCE2 (CES2)
GABA-gated chloride channel	Cyclodienes, a form of organochlorines	Antagonism	GABA receptor polymorphisms
	Phenylpyrazole	Antagonism	
Nicotinic acetylcholine receptor[a]	Neonicotinoid	Agonism	Haploinsufficiency of α7 nAChR

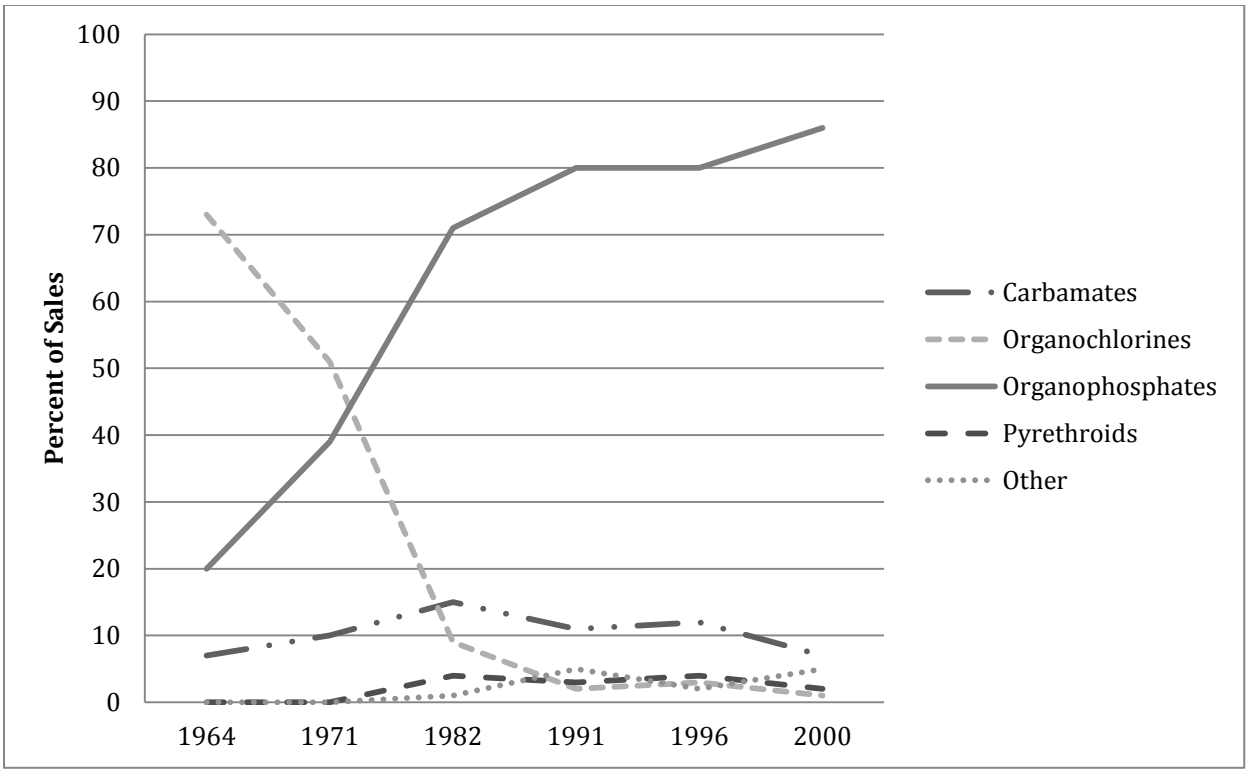
[A] Neonicotinoids first induce excitation, which is followed by inhibition

Table 2. Mechanisms by which gestational exposure to certain classes of pesticides may induce observed pathophysiologic symptoms of autism.

Mechanism of Action	Route to Autism Pathophysiology	Observed effects	Specific pesticides	Class of pesticide	Reference
Developmental neurotoxicity	Alteration of excitation/ inhibition mechanisms	Decrease in GABA receptors	Dieldrin (prenatal exposure in rats)	Organochlorines	(Brannen et al. 1998; Liu et al. 1998)
		Inhibition of GABA	General function of organochlorine, pyrethroid pesticides	Organochlorine, pyrethroid	
		Inhibition of AChE	General function of organophosphate, carbamate pesticides	Organophosphates, carbamates	
Mitochondrial dysfunction	Oxidative stress	Apoptosis of neuronal cells	Dichlorvos (rat brain)	Organophosphates	
		Inhibition of mitochondrial respiration	Methoxychlor (mice brain)	Organochlorines	(Schuh et al. 2005; Kaur et al. 2007)
Immune toxicity	Immunosuppression	Decreased delayed type hypersensitivity and antibody production	Atrazine (gestational exposure to rats)	Triazine	(Rooney et al. 2003)
	Neuro-inflammation	Activation of human fetal astrocytes, increased expression of pro-inflammatory cytokines	Cyfluthrin, chlorpyrifos (primary human fetal astrocytes)	Pyrethroid, organophosphate	(Mense et al. 2006)
Maternal hypothyroxinemia	Insufficient gestational thyroid hormones	Decreased T4, inhibition of T4 de-iodination to T3, prevention of iodine uptake	Acetechlor, alachlor, mancozeb, thiocyanates, 2,4-D, aminotraizole, endosulfan, malathion (multiple animal studies)	Organochlorines, thiocyanates, organophosphates	(Colborn 2004; Goldner et al. 2010; Rathore et al. 2002; Cheek et al. 1999)

Figure Legend

Figure 1. Agricultural pesticide trends in the US by percent of sales, 1964-2000 (United States Department of Agriculture 2006).


100
90
80
70
60
50
40
30
20
10
0
Percent of Sales
1964
1971
1982
1991
1996
2000
Carbamates
Organochlorines
Organophosphates
Pyrethroids
Other