

## Environmental Risk Factors for Autism: The Case for Organophosphorus Pesticides (OPs)

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## What is the evidence that environmental factors contribute to ASD risk?

### Rise in Autism Prevalence v. Other Major Chronic Conditions in US

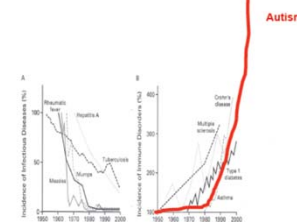


Figure 1. Incidence of Infectious Diseases (A) and the Incidence of Autism (B) from 1980 to 2000. In Panel A, data on the incidence of infectious diseases are derived from reports of the Centers for Disease Control and Prevention, except for the data on hepatitis A, which are derived from Jansen et al. (7). In Panel B, data on autism incidence are derived from Lord et al. (8), Tager et al. (9), Tager et al. (10), and Tager et al. (11).

## What is the evidence that environmental factors contribute to ASD risk? *continued*

How much of the increased prevalence of ASD  
represents an actual growth in numbers?

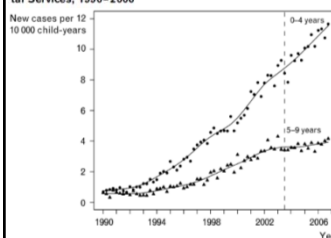
Increased awareness, improved detection and  
broadening of diagnostic criteria for ASD likely  
contribute to increased prevalence

e.g., Diagnostic substitution – labeling people  
autistic who previously would have been diagnosed  
with something else

However, Hertz-Picciotto and Delwiche (2009) Epidemiology 20: 84-90:

600% increase in cases:  
24% due to earlier diagnosis  
56% due to inclusion of milder cases  
120% due to changes in diagnostic criteria

Figure 2. Annual incidence rates of autism based on the administrative database of the California Department of Developmental Services, 1990–2006.



400% of increased cases  
cannot be attributed to  
diagnostic distribution

## What is the evidence that environmental factors contribute to ASD risk? *continued*

1. Rapid increase in ASD prevalence
2. Genetic studies
  - a. Incomplete monozygotic concordance
  - b. Most genes associated with ASD are not major effect genes but rather create modest vulnerabilities
  - c. In some cases, genes create major vulnerabilities but even in genetic syndromes highly associated with ASD, a significant percentage of carriers do NOT have ASD
  - d. De novo gene mutations
  - e. Some gene variants confer altered vulnerability to environmental stressors and environmental exposures
    - i. Redox or methylation
    - ii. Heavy metal metabolism
    - iii. Metabolism of organophosphorus pesticides (OPs)

## What is the evidence that environmental factors contribute to ASD risk? *continued*

1. Rapid increase in ASD prevalence
2. Genetic studies
3. Clinical heterogeneity of ASD

## What is the evidence that environmental factors contribute to ASD risk? *continued*

1. Rapid increase in ASD prevalence
2. Genetic studies
3. Clinical heterogeneity of ASD
4. Systemic and CNS pathophysiology

- Oxidative stress
- Immune dysfunction (including neuroinflammation)
- Mitochondrial dysfunction

These pathophysiological outcomes known to be exacerbated by environmental factors

air pollution, organophosphorus pesticides, heavy metals

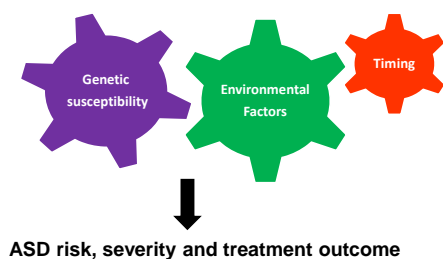
## Environmental risk factors for ASD

- Rubella infection during the first trimester of pregnancy
- *In utero* exposure to thalidomide or valproic acid
- Paternal age
- Environmental chemicals (?)

*However, efforts to identify specific environmental risk factors for ASD have produced a number of candidates but few definitive hits*

- Heavy metals (lead, methylmercury)
- Pesticides
  - Organophosphorus pesticides (OPs), e.g., chlorpyrifos, diazinon
  - Organochlorine pesticides (OCs), e.g., DDT, dieldrin, lindane
- Persistent organic pollutants (POPs)
  - Polychlorinated biphenyls (PCBs)
  - Polybrominated diphenyl ethers (PBDEs)
  - Polycyclic aromatic hydrocarbons (PAHs)

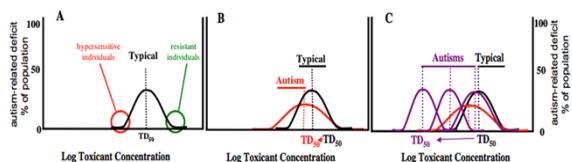
## How might environmental factors influence ASD risk?



## The Challenge of Identifying Environmental Risk Factors for ASD, *continued*

A significant challenge, particularly for epidemiological studies:

*The complexity of heritable factors contributing to ASD susceptibility creates a range of sensitivities to environmental factors*

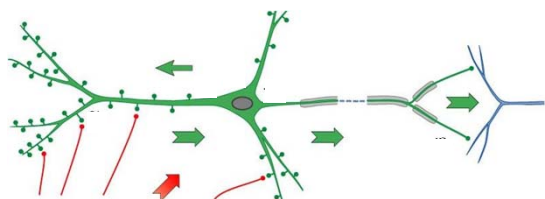


Pessah and Lein (2008) In: *Autism: Current Theories and Evidence* (Zimmerman A, ed) Humana Press, pp. 409-428

## ASD Pathology

*Autism reflects altered patterns of neuronal connectivity within the developing brain*

*Autism may also involve altered neuronal connectivity of the autonomic and sensory nervous system*



## Neurodevelopmental processes that determine neuronal connectivity

*and are thus likely to be altered in ASD:*

- Neuronal migration
- Interneuron development
- Neuronal programmed cell death
- Axonal growth and branching
- Dendritic growth and plasticity
- Synaptogenesis and synaptic plasticity

## The Case for Organophosphorus Pesticides (OPs) as ASD risk factors



## OPs as ASD risk factors: Epidemiological evidence

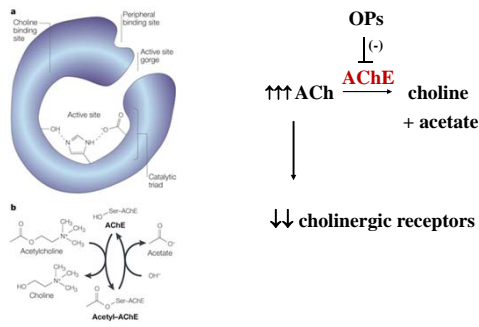
- Human studies have reported behavioral and cognitive problems in school-aged children following chronic exposure to low-level OPs

– One of these studies linked perinatal OP exposures to ASD (Eskenazi et al., 2007, EHP)

– Other studies showed that susceptibility to ASD is influenced by polymorphisms in PON-1, a key enzyme in OP detoxification (D'Amelio et al., 2005, Mol Psych; Pasca et al., 2006; Life Sci)

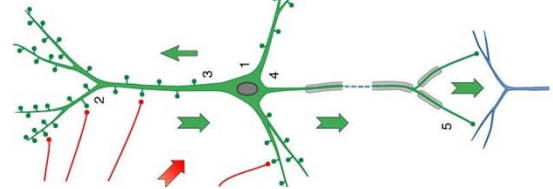


## Organophosphorus pesticides (OPs): Mechanism of acute toxicity

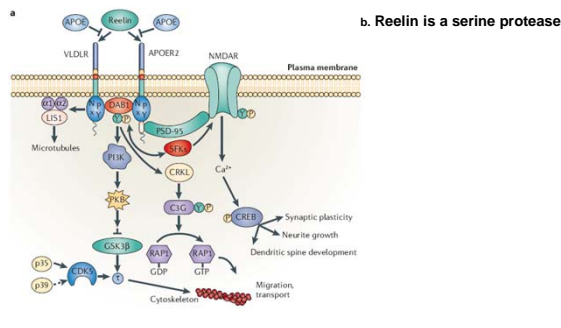


## Most mothers and children are NOT exposed to OPs at levels that cause acute toxicity

Does exposure to OPs at lower “environmental” doses interfere with neuronal connectivity in the developing brain?



## How might OPs influence ASD risk? OP interactions with Reelin



## Reelin gene (*RELN*) mutations linked to ASD

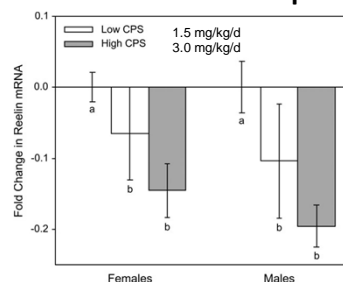
- Linkage between chromosome 7q and ASD one of most replicated genetic findings in ASD
  - RELN found within the 7q22 region
- Reeler mice are devoid of reelin due to spontaneous deletion of gene

Brain regions	Autistic patients	Reln	Reeler mice	Reln
Cerebral cortex	Increased cell density	[171]	Invasion of cortical lamination	[121,122]
	Smaller cortical minicolumns	[171]	Neuronal disorganization	[121,125]
	Ectopic neurons	[171]	Altered intracortical courses of efferent fibers, with quantitatively normal thalamocortical and callosal connections	[124,126]
	Neuronal disorganization	[171]		
Cerebellar cortex	Area of increased cortical thickness	[171]	Decreased cell counts and dysplasia in lateral nucleus (dentate nucleus in humans)	[126]
	Poor lamination in the anterior cingulate cortex	[171]	Checkered fibers innervate more than one Purkinje cell	
Striop cerebral nuclei	Decreased Purkinje cell number	[17,116]	Decreased Purkinje cell number	[126]
	Marked decrease in granule cell counts	[171]	Purkinje cells are disorganized	
Inferior olivary nucleus	Increased cell size before age 12 and decreased cell counts after age 22	[171]	Ectopic subnuclear Purkinje cells	
	Dysplasia in the dentate nucleus	[171]	Checkered fibers innervate more than one Purkinje cell	
Entorhinal cortex	Subcortical ectopic gray matter	[171]	Decreased cell counts and dysplasia in lateral nucleus (dentate nucleus in humans)	[126]
	Increased cell size before age 12 and decreased cell size after age 22	[171]	Subcortical ectopic gray matter	
Facial nucleus	Olivary dysplasia	[17,116]	Olivary dysplasia	[124,127]
	Increased cell density and reduced neuronal size	[171]	Cytoarchitectonic disturbances	[126]
Hypothalamus (CA4 and subthalami)	Cell density decreased by 36%	[20]	Heterotopic neurons	[127,128,130]
	Increased cell density and reduced neuronal size	[171]	Less distinct boundaries	
Amygdala (central, medial, cortical nuclei)	Altered fiber input from entorhinal cortex	[171]	Altered fiber input from entorhinal cortex	[131]
	Decreased dendritic branching	[171]	Cytoarchitectonic disturbances	[132,133]
	Increased cell density and reduced neuronal size	[171]	Cytoarchitectonic disturbances	[126]

### Reelin gene (*RELN*) mutations linked to ASD

- Reduced levels of *reelin* in both brain and serum of autistic patients
- $\geq 12$  GGC repeats in 5'-UTR of *RELN* blunt *RELN* gene expression by 25-50%
- “Long” *RELN* variants associated with ASD in an initial study and in three independent samples but not in four others
  - Non-replication due to genetic heterogeneity
  - Alternative hypothesis: manifestation of genetic mutation influenced by exposure to OPs

### Developmental OP exposures decrease reelin expression

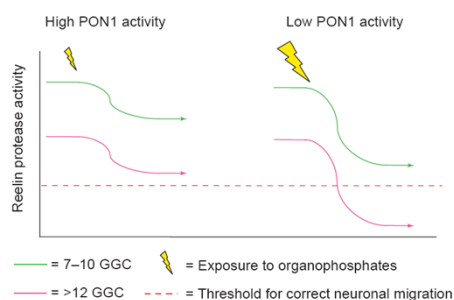


Chlorpyrifos (CPS) administered orally from PN1 to PN6

Betancourt et al., 2006, Toxicol Sci

DFP (another OP) inhibits proteolytic activity of Reelin

### Potential gene-environment interactions: Developmental OP exposure X *RELN* mutations X PON1 polymorphisms



Persico and Bougeron, 2006

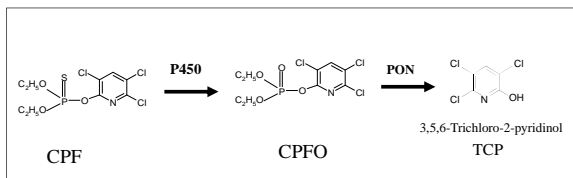
### Alternative hypothesis for how OPs might influence ASD risk

Acetylcholinesterase (AChE) is a morphogen that promotes axon outgrowth

Hypothesis: OPs disrupt axonal growth by interfering with the morphogenic activity of AChE

### Chlorpyrifos (CPF)

A phosphorothionate pesticide used for crop protection and in flea dips and roach control sprays



### Chlorpyrifos is a known developmental neurotoxicant

Wide-spread exposure of children in agricultural, suburban and urban populations

Multiple studies indicating that urine samples from a significant percentage (40%-100%) of children in the US contain CPF metabolites

NHANES II report indicates that levels of CPF metabolites in children are twice those found in adults



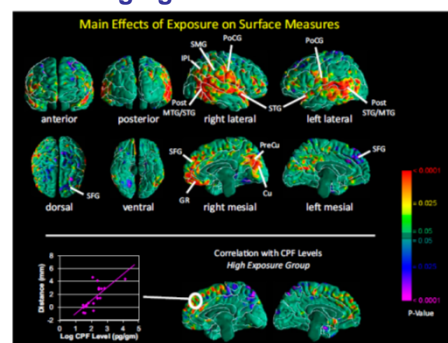
### Intriguing possibility

OP exposures during critical periods of development  
amplify effects of ASD-related gene mutations in  
neuregulin on synapse formation



Altered patterns of neuronal connectivity  
associated with ASD

### OPs as ASD risk factors: Imaging data from children



Rauh et al., 2012, PNAS 109: 7871-7876

### What do these findings mean to parents and clinicians?

- Chemical exposure both pre- and postnatal can influence clinical outcome (types and severity of behaviors, co-morbidities)
- Chemical exposures are more readily controlled than genetic factors to prevent or mitigate the expression of ASD-related traits



Herbert (2010) Current Opinion in Neurology 23: 103-110

### What do these findings mean to parents and clinicians?

- Minimizing or preventing exposure to chemical contaminants during pregnancy or early childhood may improve clinical outcome
  - Do not use OPs in the home/yard
  - Consume organically grown produce
  - Work with local agencies to minimize use of OPs in public places and/or increase notice to the public of OP spray schedules/locations
  - Keep dust levels as low as possible; wash stuffed toys routinely

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