Environmental Toxins and Autism Spectrum Disorders (ASD)

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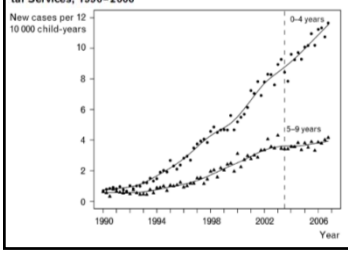
However, Hertz-Picciotto and Delwiche (2009) Epidemiology 20: 84-90:

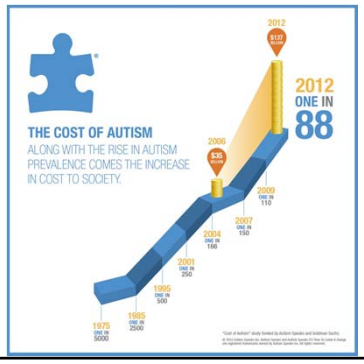
600% increase in cases:
24% due to earlier diagnosis
56% due to inclusion of milder cases
120% due to changes in diagnostic criteria

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 substitution

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



2014 (USA)
1 in 68 children
1 in 42 boys

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*

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

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
   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?

Epidemiological Data Linking Environmental Chemicals to Increased Risk of ASD

- Data recently critically reviewed
  - Kalkbrenner et al., 2014, Current Problems in Pediatric and Adolescent Health Care 44:277-318.
- Of 58 articles identified in the peer-reviewed literature published prior to March 1, 2014, 32 met inclusion criteria
  - Individual-level data on autism diagnosis
  - Exposure measures during pregnancy or 1st year of life
  - Valid comparison groups
  - Controls for confounding variables
  - Adequate sample size

Why focus on Environmental Chemicals as Risk Factors for ASD?

- In contrast to genetic risks, which are currently irreversible, environmental factors are modifiable risk factors
  - Therefore, identifying specific environmental factors that increase risk for neurodevelopmental disorders may provide rational approaches for the primary prevention of the symptoms associated with these disorders.

Summary of Kalkbrenner et al., 2014 Review of Published Epidemiological Data

- Environmental chemicals studied in the 32 articles reviewed by Kalkbrenner et al., 2014
  - Tobacco and alcohol
  - Air pollutants, volatile organic compounds and solvents
  - Metals, PCBs, PBDEs
  - Pesticides, BPA and phthalates
- The most strongly and consistently associated with increased ASD risk
  - Traffic-related air pollutants
  - Some metals
  - OP and OC pesticides
- Environmental chemicals NOT associated with increased ASD risk
  - Tobacco and alcohol

Environmental Chemicals Postulated to Confer Risk for ASD

- Legacy chemicals known to interfere with normal neurodevelopment
  - Lead
  - Methyl mercury
  - Polychlorinated biphenyls (PCBs)
- Contemporary contaminants
  - Pesticides
    - Organophosphorus (OP), organochlorine (OC), pyrethroids
  - Flame retardants
    - Polybrominated diphenyl ethers (PBDEs)
  - Plasticizers
    - Phthalates, bisphenol A (BPA)
- Complex environmental mixtures
  - Air pollution

Major Conclusion in Critical Analyses of Epi Data by Kalkbrenner et al., 2014

The relevant publications that are currently available, with the possible exception of studies of tobacco and alcohol, are too limited in scope to either infer causality or to rule out the possibility that these or additional environmental chemicals confer risk for ASD.

The Challenge of Identifying Environmental Risk Factors for ASD

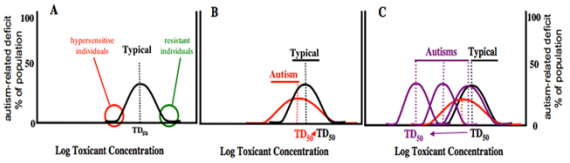
How do environmental chemicals interact with genetic mechanisms to increase ASD risk?

- **Heritable deficits in xenobiotic metabolism**
  - Decreased ability to detoxify environmental chemicals might effectively increase the neurotoxic potential of an environmental chemical
- **Endocrine disruption**
  - ASD occurs predominantly in boys and many hormones are required for normal neurodevelopment (sex steroids and thyroid hormones) or have significant effects on neurodevelopment (glucocorticoids)
- **Disruption of the gut microbiome**
  - Emerging evidence indicates that the gut microbiome regulates host response to pathogenic microbial or xenobiotic exposures, and the gut microbiota in children with autism differs from that of neurotypical children

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

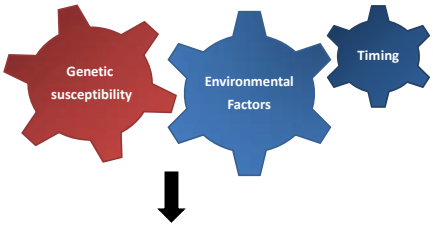


Pessah and Lein (2008) In: *Autism: Current Theories and Evidence* (Zimmerman A, ed) Humana Press, pp. 409-428

How do environmental chemicals interact with genetic mechanisms to increase ASD risk?

- **Epigenetic**
  - Environmental chemicals have been demonstrated to alter DNA methylation, histone acetylation and miRNA expression profiles, and these parameters are altered in at least some children with ASD
- **Immune Dysregulation**
  - Crosstalk between the nervous and immune systems is essential for normal neurodevelopment, environmental chemicals can alter immune function, and there is significant clinical evidence of immune dysregulation in ASD
- **Convergence of environmental and genetic factors on common signaling pathways critical in neurodevelopment**
  - Heritable genetic vulnerabilities amplify adverse effects triggered by environmental exposures if genes and environment converge to dysregulate the same signaling system at critical times of neural development

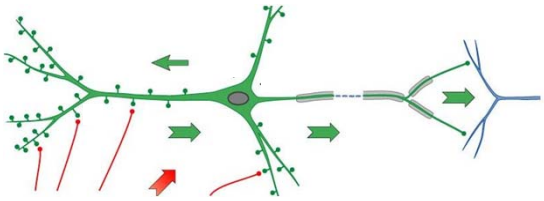
The Challenge of Identifying Environmental Risk Factors for ASD, *continued*



ASD risk, severity and treatment outcome

ASD Pathology

Autism reflects altered patterns of neuronal connectivity within the developing brain



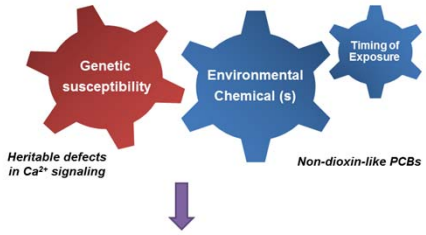
Genes associated with ASD susceptibility:

Neuronal connectivity

Genes	Chr	Function	Evidence	Disorder	Observation	Refs
Chromatin remodeling and gene regulation						
MECP2	Xq28	Methyl-binding protein	M	MR, Rett, ASD	Girls with autistic features, one male with ASD	[14]
FMRP	Xq28	RNA-binding protein	M	MR, FXS, ASD	20–40% of boys with FXS have ASD	[15,16,18]
EN2	7q36	Transcription factor	L, A	ASD		[21–23]
HOXA1	7p15	Transcription factor	A	ASD		[25–27]
WNT2	7q31	Transcription factor	L, A	ASD		[24]
Actin cytoskeleton dynamics						
TSC1/TSC2	9q34/16p13	Inactivation of GTPase	M	TCS	ASD in 43–86% of TS patients	[6]
NF1	17q11	Inactivation of GTPase	M	NF1	Learning disabilities in 30–45% of NF1 patients	[30]
cAMP-GEF	2q31	Activation of GTPase	L, A	ASD	Rare variants observed in ASD	[31]
Synaptic scaffolding proteins						
SHANK3	22q13	Dendrite induction	CR	MR, ASD	Binding partner of NLGN	[32]
Receptors and transporters						
GRIN2A	16p13	NMDA receptor subunit	L, A	ASD	Highly significant association	[46]
GRIK2	6q16–21	Kainate receptor subunit	L, A	ASD	Two independent studies	[47]
GABAR	15q12	GABA receptor subunit	CR	ASD	Duplication of 15q is the major CR in ASD	[45]
SLC6A4	17p11	Serotonin transporter	L, A, M	ASD	Evidence for allelic heterogeneity in ASD	[41]
SLC25A13	2q31	Aspartate–glutamate carrier	L, A	ASD	Two positive and one negative association	[48]
OXTR	3p25–26	Oxytocin receptor	L, A	ASD		[49]
AVPR1	12q14	Vasopressin receptor	L, A	ASD		[50]
Second-messenger systems						
PRKCB1	16p11.2	Protein kinase	L, A	ASD		[52]
CACNA1C	12p13.3	Ca^{2+} channel	M	TS, ASD	Multiorgan dysfunction	[55]
NBEA	13q13	PKA anchor protein	L, CR	ASD		[51]
Cell adhesion molecules						
NLGN4	Xp22.3	Synapse formation	L, CR, M	MR, ASD	Typical autism, Asp	[61–65]
NLGN3	Xq13.1	Synapse formation	L, M	MR, ASD	Typical autism, Asp	[61–65]
NrCAM	7q31	Neuronal migration	L, A	ASD		[70]
Secreted proteins						
RELN	7q22	Neuronal migration	L, A	ASD		[77]
LAMB1	7q31	Cell migration	L, A	ASD		[70]

Persico and Bourgeron, 2006, TINS

Mechanistic Models: The Case for PCBs as Environmental Risk Factors for ASD



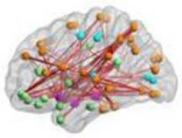
Increased risk and/or severity of adverse neurodevelopmental outcomes

Altered patterns of synaptic connectivity

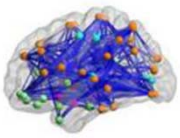
Imaging studies of autistic brains:

Neuronal connectivity

Typically developing children



Children with autism



Hyper-connected neurons in autism

increased connectivity in local circuits of the visual cortex

Keown et al. 2013, Cell Reports 5: 567-72

PCB Developmental Neurotoxicity

- Human epidemiological data indicate a negative association between developmental exposure to environmental PCBs and cognitive function in infancy or childhood
  - Decreased IQ, impaired learning and memory, attentional deficits, lowered reading comprehension, psychomotor problems
- Comparable cognitive and behavioral deficits observed in primate and rodent models following developmental PCB exposures
  - Developmental neurotoxic effects of PCBs have been observed at relatively low exposure levels corresponding to between 1 and 10x the background levels observed in humans

Neurodevelopmental processes that determine neuronal connectivity

and are thus likely to be altered in ASD:

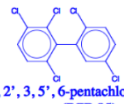
- Neuronal migration
- Interneuron development
- Neuronal programmed cell death
- Axonal growth and branching
- Dendritic growth and plasticity
- Synaptogenesis and synaptic plasticity

PCBs: A current public health concern

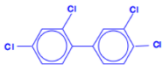
- Exposure from legacy sources as well as contemporary unintentional sources of PCBs, most notably commercial paint pigments
- PCB levels in the indoor air of elementary schools in the United States exceed the EPA's 2009 public health guidelines
- Latest NHANES study confirmed widespread exposure to PCBs among U.S. women of childbearing age
- Levels of NDL PCBs are NOT decreasing rapidly in the environment and human tissues
  - PCB153 levels in plasma of at risk MARBLES mothers are 7 to 20-fold higher than those reported in the 2007-2008 NHANES report

Non-dioxin-like *vs*. dioxin-like polychlorinated biphenyls (PCBs)

NDL predominate in environmental samples and human tissues



2, 2', 3, 5', 6-pentachlorobiphenyl (PCB 95)

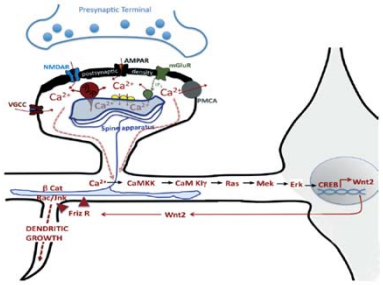


2, 3', 4, 4'-tetrachlorobiphenyl (PCB 66)

	Non-dioxin-like congeners	Dioxin-like congeners
Arylhydrocarbon Receptor (AhR)	Low to no affinity	High affinity
Cancer	+/-	+++
Developmental Neurotoxicity	+++	+/-

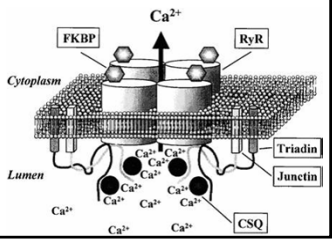
Overarching Hypothesis:

Non-dioxin-like PCBs disrupt neuronal connectivity via RyR-mediated mechanisms that modulate Ca^{2+}-dependent signaling pathways linked to activity-dependent dendritic growth and plasticity.

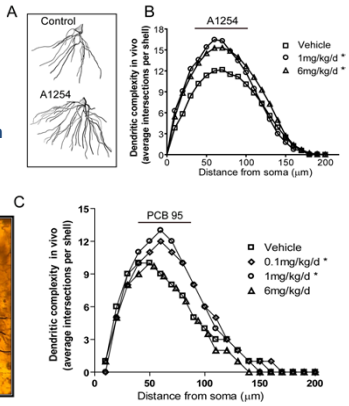


Postulated molecular mechanism(s) of PCB developmental neurotoxicity

- Decreased dopamine content
- Interference with thyroid hormone signaling
- Increased levels of intracellular calcium Ca^{2+}
  - Sensitization of the ryanodine receptor (RyR)

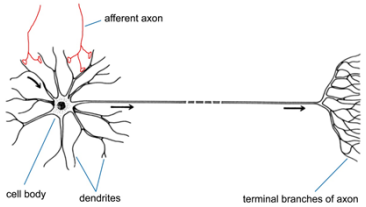


Golgi analyses of the hippocampus of weanling rats exposed developmentally to PCBs in the maternal diet



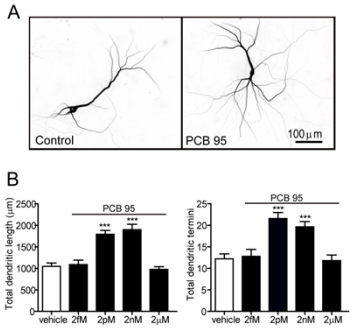
Wayman et al. (2012) *Environmental Health Perspectives* 120:997-1002.

Ca^{2+}-dependent signaling regulates dendritic growth in the developing brain



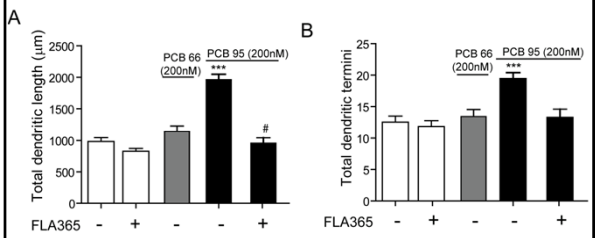
- Dendritic branching patterns influence the number, types and distribution of synaptic inputs
- Structural plasticity of dendrites is thought to be the cellular substrate of learning and memory
- Perturbations of normal patterns of dendritic growth and plasticity are associated with functional deficits

PCB 95 alters dendritic growth in primary cultures of hippocampal neurons



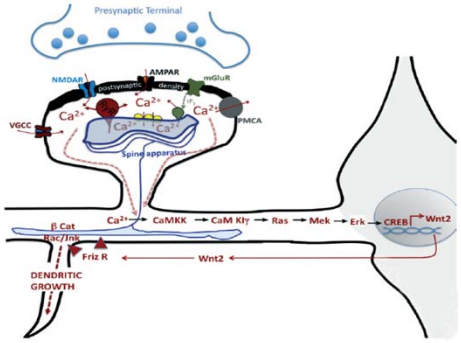
Wayman et al. (2012) *Environmental Health Perspectives* 120:997-1002.

The dendrite-promoting activity of PCBs is RyR-dependent

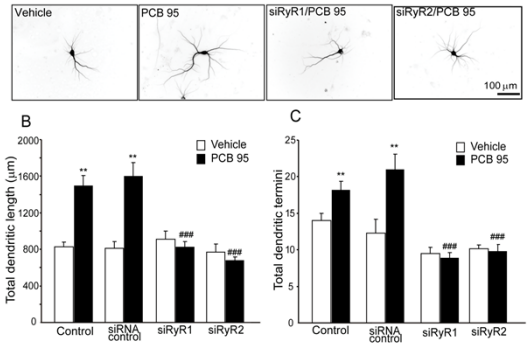


Wayman et al. (2012) *Environmental Health Perspectives* 120:997-1002.

Non-dioxin-like PCBs "high-jack" Ca^{2+}-dependent signaling pathways that control dendritic growth

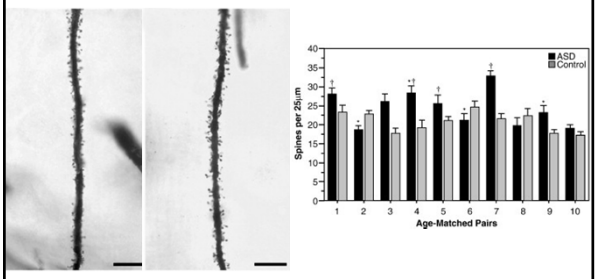


PCB 95 enhances dendritic growth in cultured hippocampal neurons via RyR-dependent mechanisms



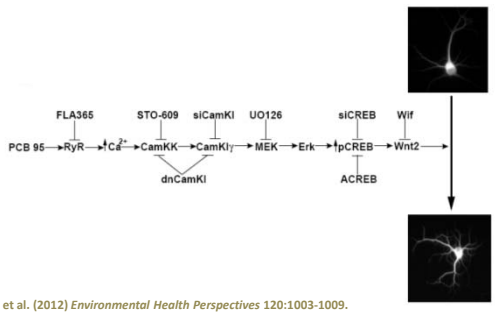
Wayman et al. (2012) *Environmental Health Perspectives* 120:997-1002.

Increased Dendritic Spine Density in ASD



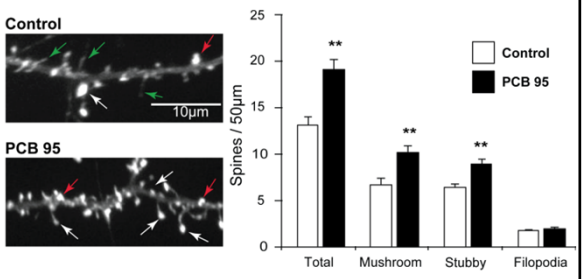
Hutsler and Zhang (2010) Brain Res 1309:83-94.

Experimental approaches for investigating Ca^{2+}-dependent signaling pathways in PCB-induced dendritic growth



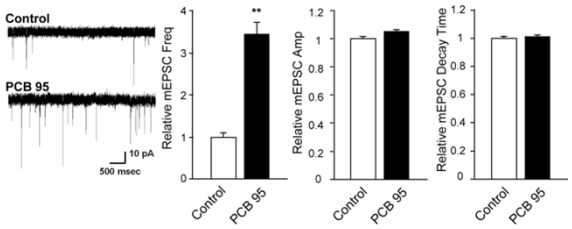
Wayman et al. (2012) *Environmental Health Perspectives* 120:1003-1009.

PCB 95 Triggers Dendritic Spine Formation in Cultured Hippocampal Neurons



Lesiak et al., 2014, J. Neurosci. 34(3): 717-25.

PCB-induced spine formation coincides with increased frequency of mini-excitatory postsynaptic currents (mEPSC)



Lesiak et al., 2014, J. Neurosci. 34(3): 717-25.

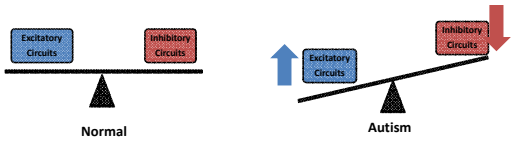
Relevance of these findings to ASD?

- Animal studies
  - Perinatal exposure to a mixture of the non-dioxin-like PCB 47 and dioxin-like PCB 77 shown to alter social behaviors in rats

    [Jolous-Jamshidi et al. (2010) Toxicology Letters 199:136-143.]
- Human exposure studies
  - PCB 95 found in significantly higher levels in postmortem brains of children with a syndromic form of autism (maternal 15q11-q13 duplication or Dup15q), but not idiopathic autism as compared to neurotypical controls

    [Mitchell et al. (2012) *Environmental and Molecular Mutagenesis* 58:589-98]

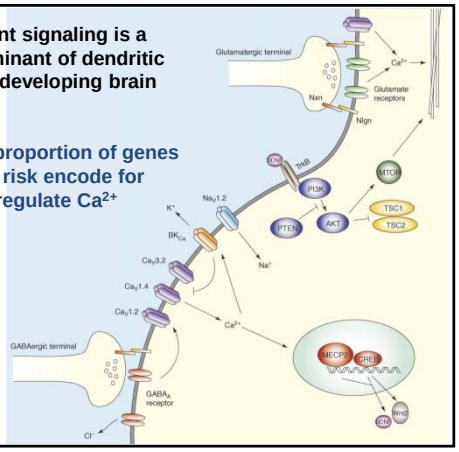
ASD Pathology



At least some forms of autism result from an imbalance in the ratio of excitatory and inhibitory circuits within the developing brain

Ca^{2+}-dependent signaling is a critical determinant of dendritic growth in the developing brain

A significant proportion of genes linked to ASD risk encode for proteins that regulate Ca^{2+}



Developmental exposure to PCB 95 in the maternal diet interferes with the topographic organization of the auditory cortex in rats

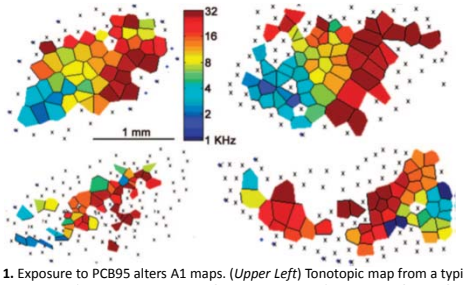


Fig. 1. Exposure to PCB95 alters A1 maps. (*Upper Left*) Tonotopic map from a typical control rat pup. (*Upper Right*, *Lower Left*, and *Lower Right*) Examples of maps from PCB95-exposed rat pups. ✖ indicates an unresponsive site. Color bar, CF (kilohertz).

Kenet et al. (2007) PNAS 104: 7646-7651

PCBs: Environmental Risk Factors for ASD

Environmental exposures *X* **Genetic susceptibility** *X* **Timing**

(non-dioxin-like PCBs) (heritable defects in Ca^{2+} signaling)

↓

Altered dendritic arborization

↓

Altered Neuronal Connectivity

↓

ASD risk, severity and treatment outcome

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 brilliantly colored paints in the home
  - Work with local agencies to determine levels of PCBs in public buildings
  - Limit dietary consumption of fatty fish, red meats
  - Remove skin, fat from fish and meats

Acknowledgements

Collaborators

Isaac Pessah, UC Davis

Gary Wayman, Washington State University

Adam Lesiak

Lein Laboratory

Christopher Barnhart

Donald Bruun

Hao Chen

Dongren Yang

Funding Sources

NIEHS

USEPA

Jane Johnson Foundation