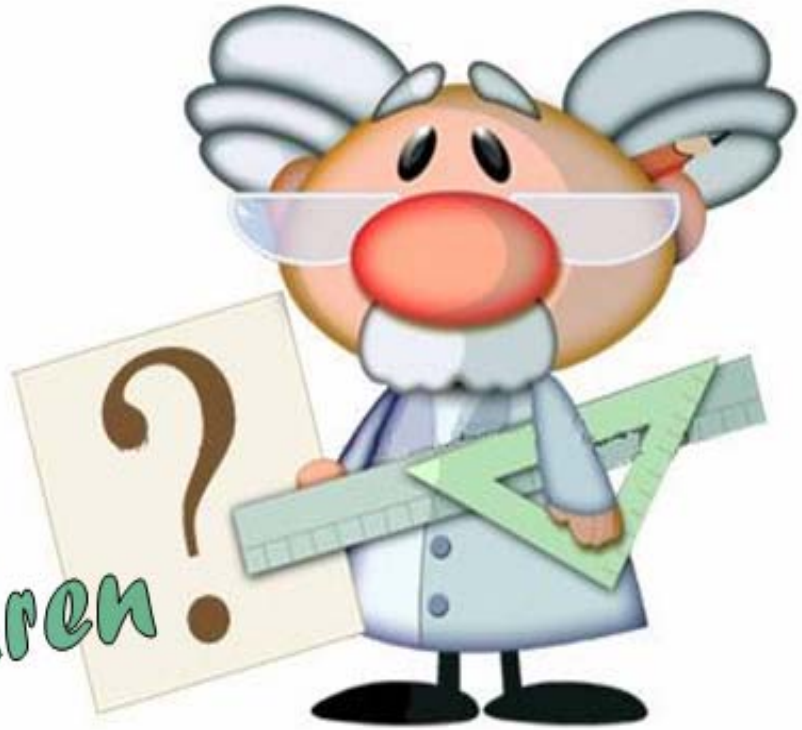


Biomarkers in Children?

Biomarkers, environment and children's health

Nina Holland, PhD

GETA Symposium, June 24, 2009

Children are not “little adults”

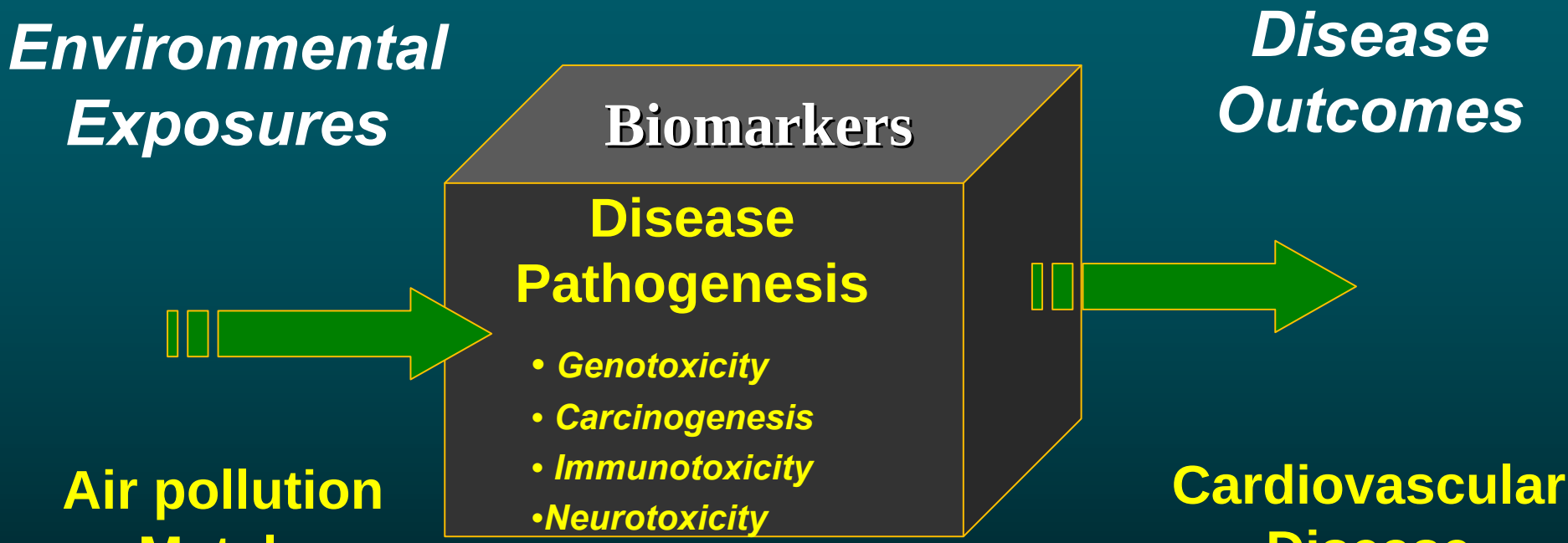
Early Life Exposure to Pollutants in:

Air

Water

Soil and Dust

Breast Milk and Food



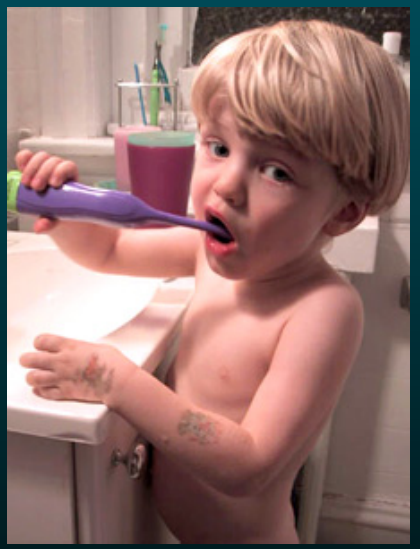
Biomarkers help link environmental exposures to disease outcomes

Types of Biomarkers

- Exposure
- Effect
- Susceptibility

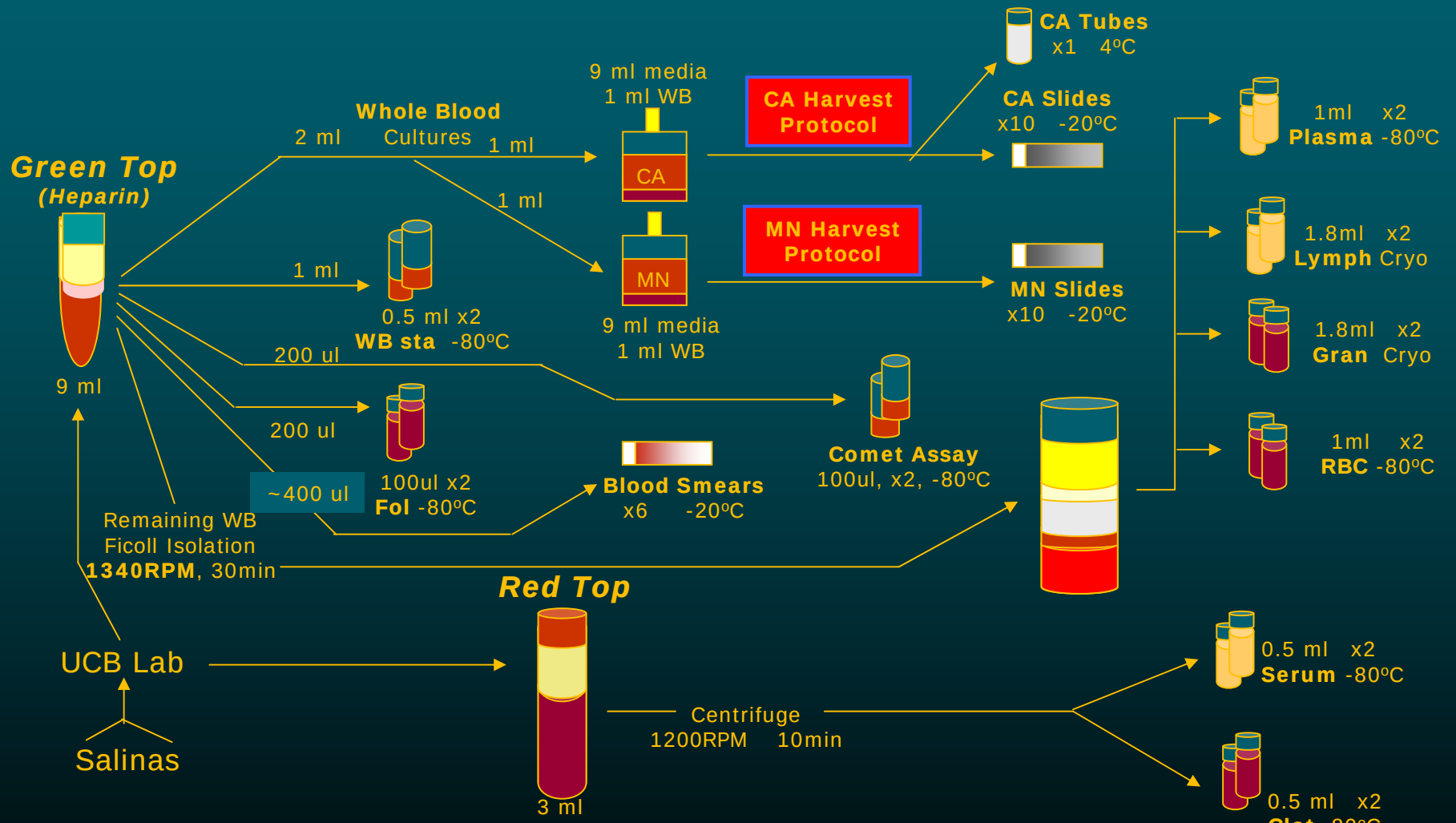
Challenges of Biomarker Studies in Children

- Specific needs of study design
- Non-invasive sampling
- **Biomarker validation for children**
- Ethics beyond consent
- Translation of biomarker results into intervention strategy



•Neri et al, 2005. Mut Research, 612: 1-13, 14-39

Specimens need to be collected and processed in a variety of ways for biomarker studies:



Holland, 2003; 2005

Biomarkers of exposure

Case study: Lead

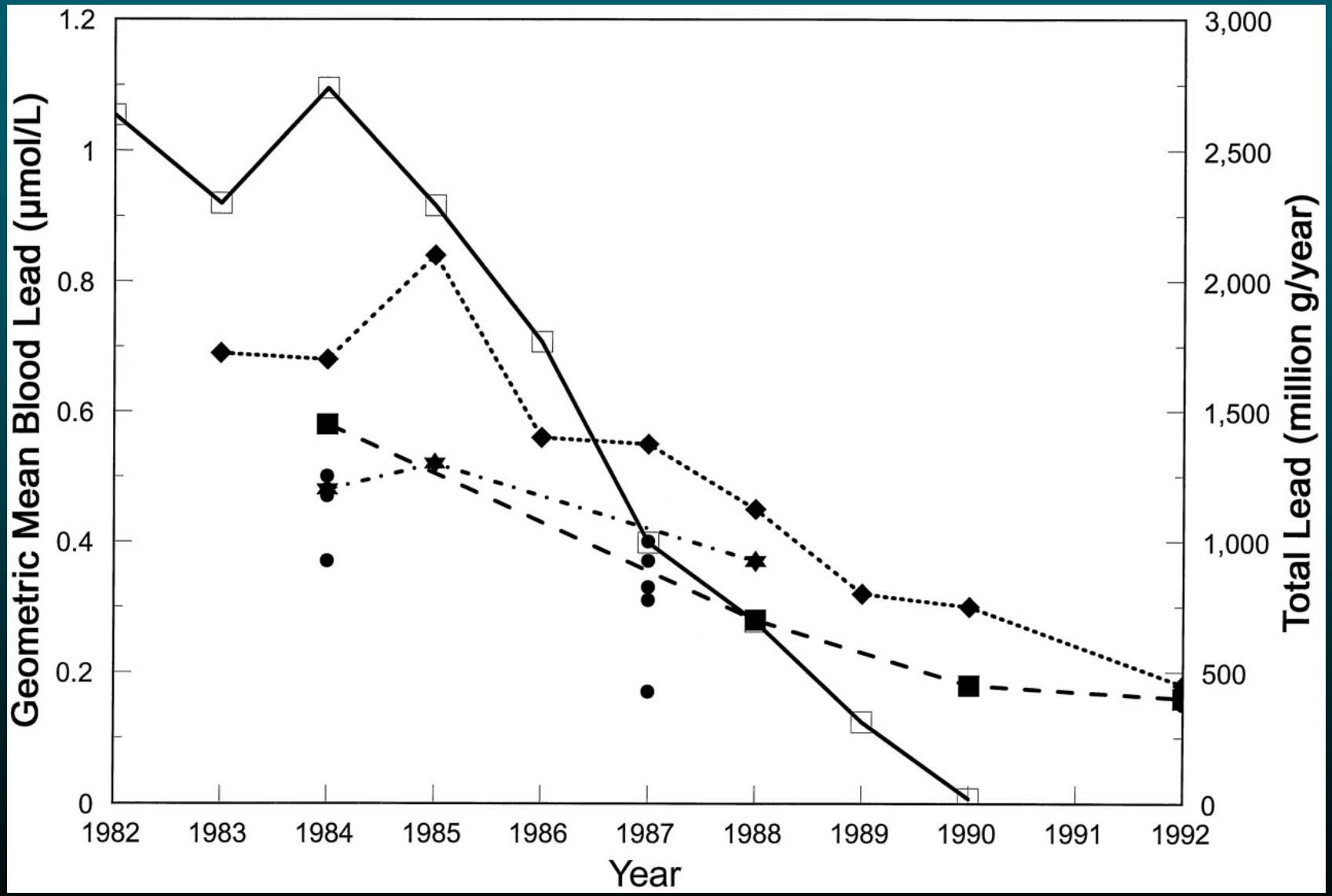
Effects of lead poisoning in children (>10 μg/dl):

- learning disability
- lowered IQ
- mental retardation
- behavior problems
- heart disease

Symptoms:

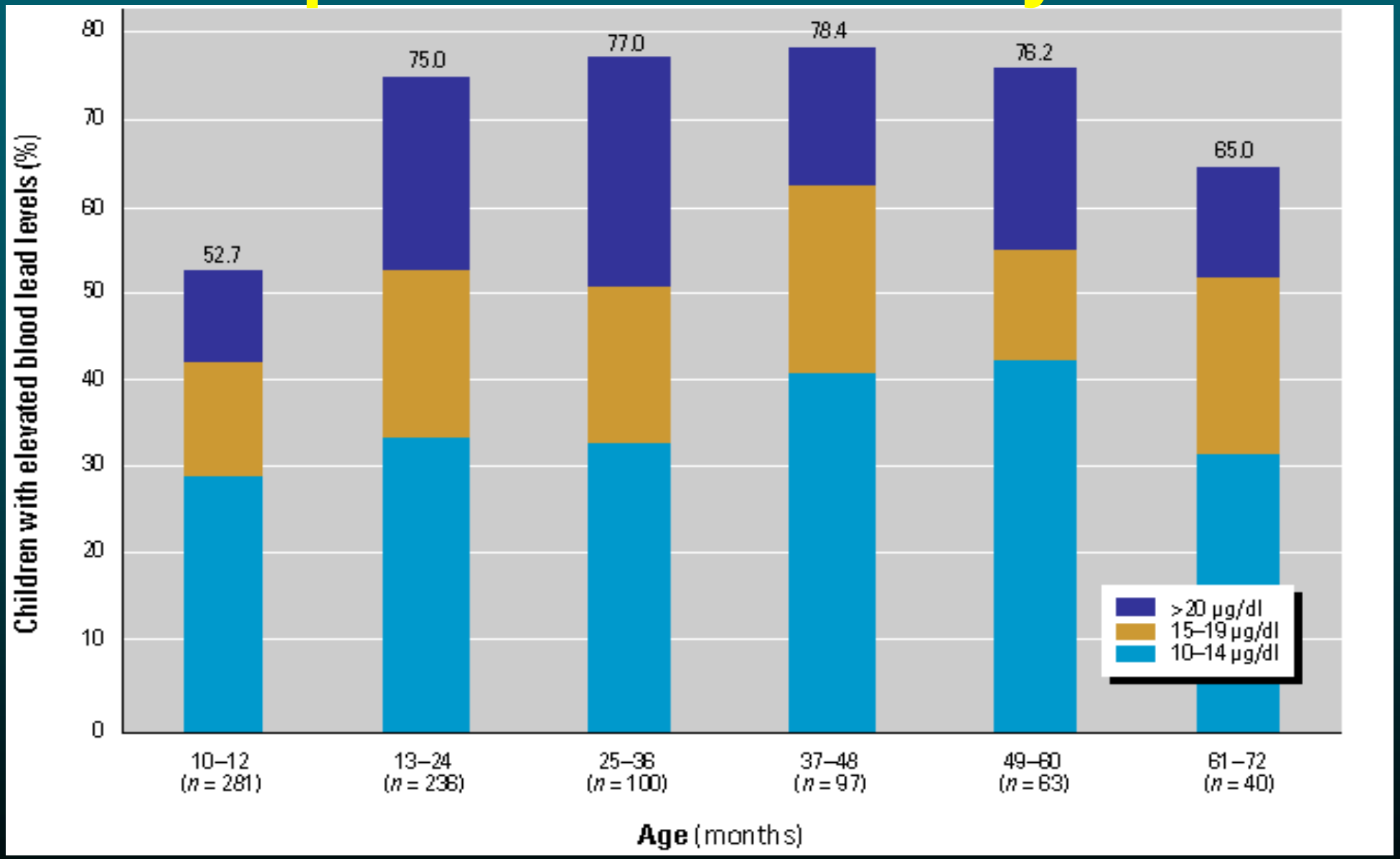
- hyperactivity
- low attention span
- headaches
- irritability
- anemia

Decline in blood lead in Ontario children correlated with decreasing consumption of leaded gasoline, 1983-1992



Wang ST, Pizzolato S, Demshar HP, Smith LF. Clin Chem. 1997 Jul;43(7):1251-2

Lead exposure in inner-city children



Melman et al, 1998 Env. Health Persp. 106; 655-657

Lead around the world

1. THAILAND		
Bangkok	5.6 ± 2.3 μg/dl (newborns)	*Ruanphanchanasetr, Suepiantham, 2002*
	9.0 ± 3.6 μg/dl (secondary school)	
2. PHILIPPINES		
Manilla	11.8 – 49.9 μg/dl	*Suplido, Ong 2000*
	9.9 μg/dl (unexposed)	
3. RUSSIA		
Volgograd Ekaterinburg Krasnouralsk	7.2 μg/dl	*Rubinet et. al. 2002*
4. USA		
National reference	2.2 μg/dl	*CDC, 2002*
California(Latinos)	0.92 μg/dl	*Eskenazi et.al. 2003*

Case Study:Pesticides

Does pesticide exposure

affect children's health?

What are Pesticides?

- **substances used for *preventing, destroying, mitigating, or repelling* any pest.**
- **Includes:**
  - **Insecticides**
  - **Herbicides**
  - **Rodenticides**
  - **Fungicides**

Environmental Protection Agency. http://www.epa.gov/pesticides/about/index.htm

Where are pesticides found?

Agricultural Fields
Pesticide Drift

Fruits & Vegetables

Household Products

Public Housing

Contaminated Water

Schools

Sanborn et al. Identifying and Managing Adverse Environmental Health Effects: 4 Pesticides. CMAJ May 28, 2002: 166 (11): 1431-1436

Children Often have Higher Pesticide Exposures

- Exploring environment
- Hand-to-mouth behavior
- Playing near the ground
- Children eat, drink, and breathe more
- Developing organs are more sensitive
- Children are smaller
- Children may be less able to detoxify pesticides

½ of lifetime pesticide exposure occurs in first 5 years of life

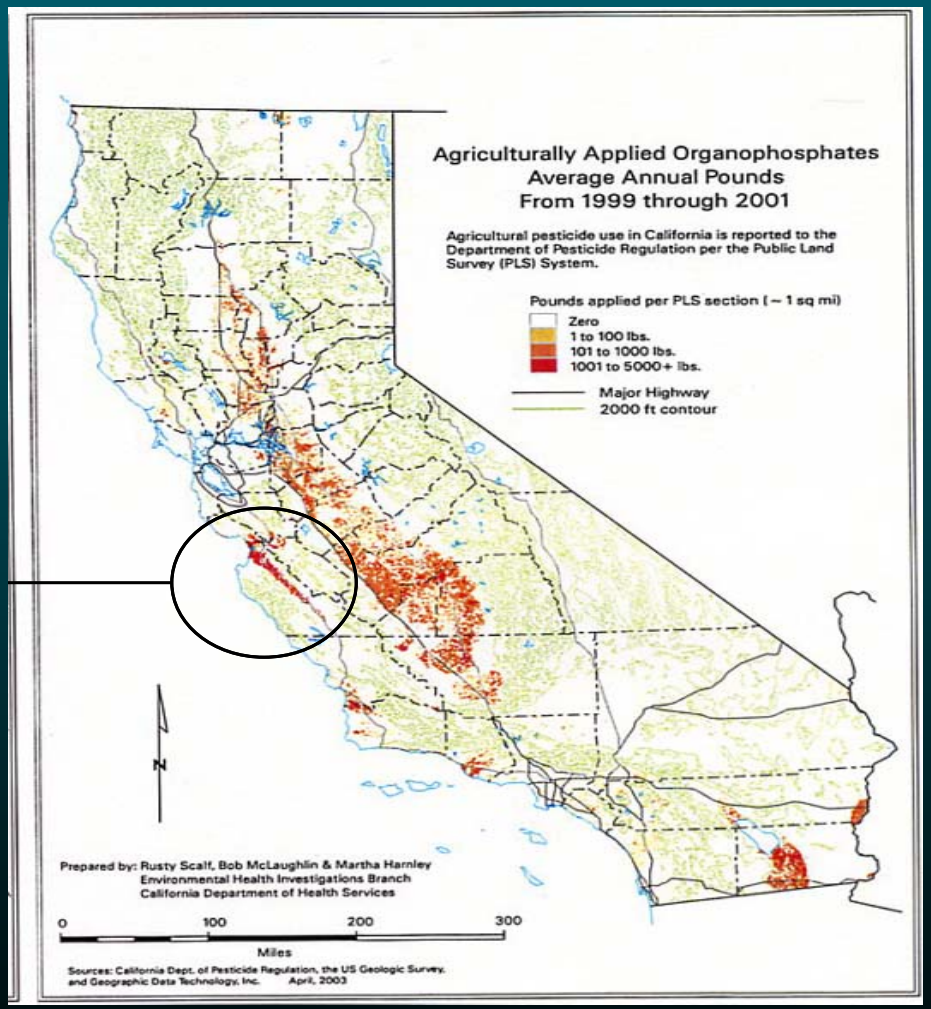
Weiss, Amler, Amler Pesticides Pediatrics 2004: 113:1030-1036

CHAMACOS Cohort (N=601)

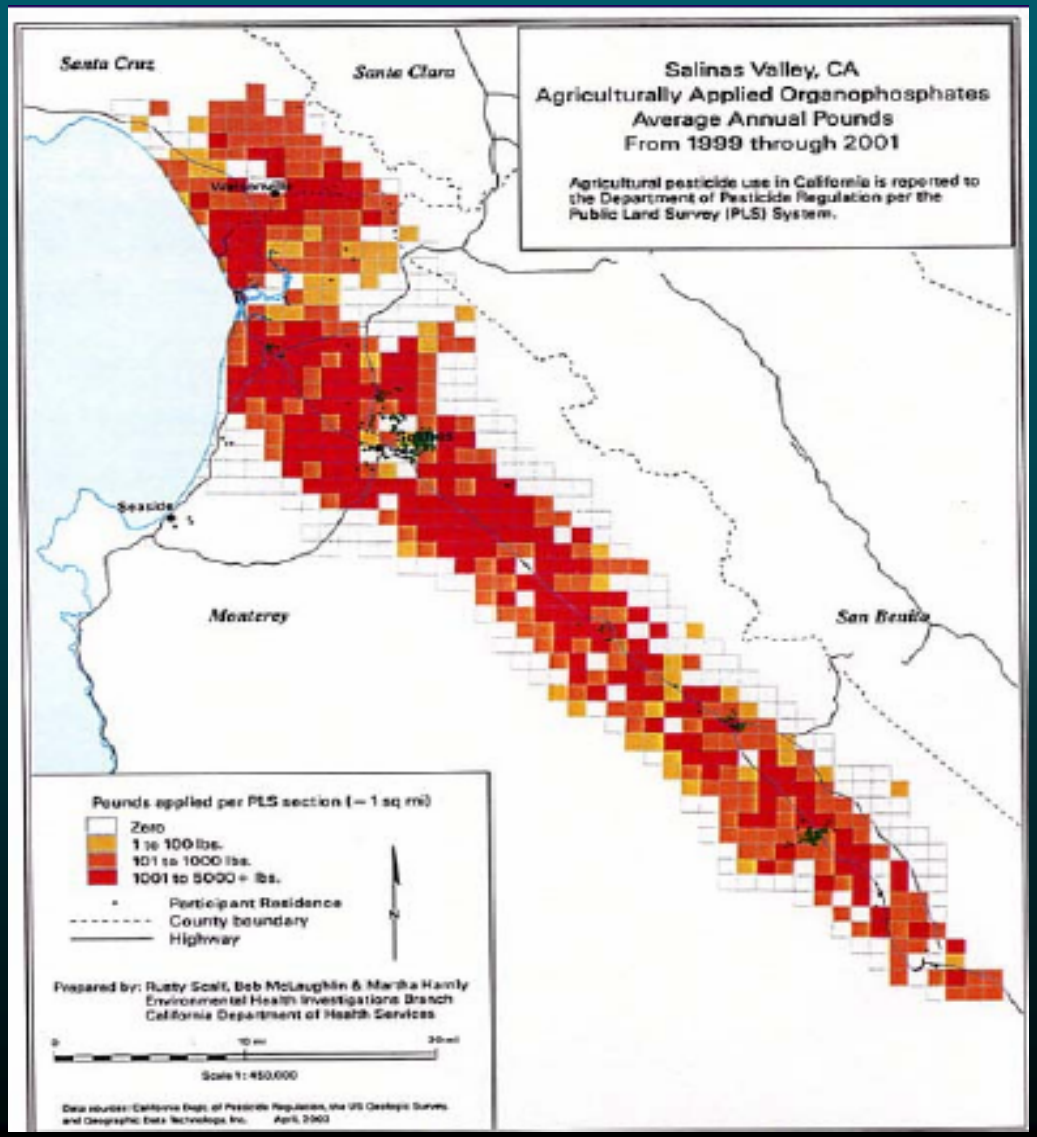
- 96% within 200% poverty
- 96% Latina
- 44% worked in agriculture during pregnancy
- 84% have farmworker in household
- Children are followed from birth to age 9

http://ehs.sph.berkeley.edu/chamacos/

CHAMACOS Study Area



Organophosphate Pesticide (OP) use in the Salinas Valley



500,000+ lbs used annually:

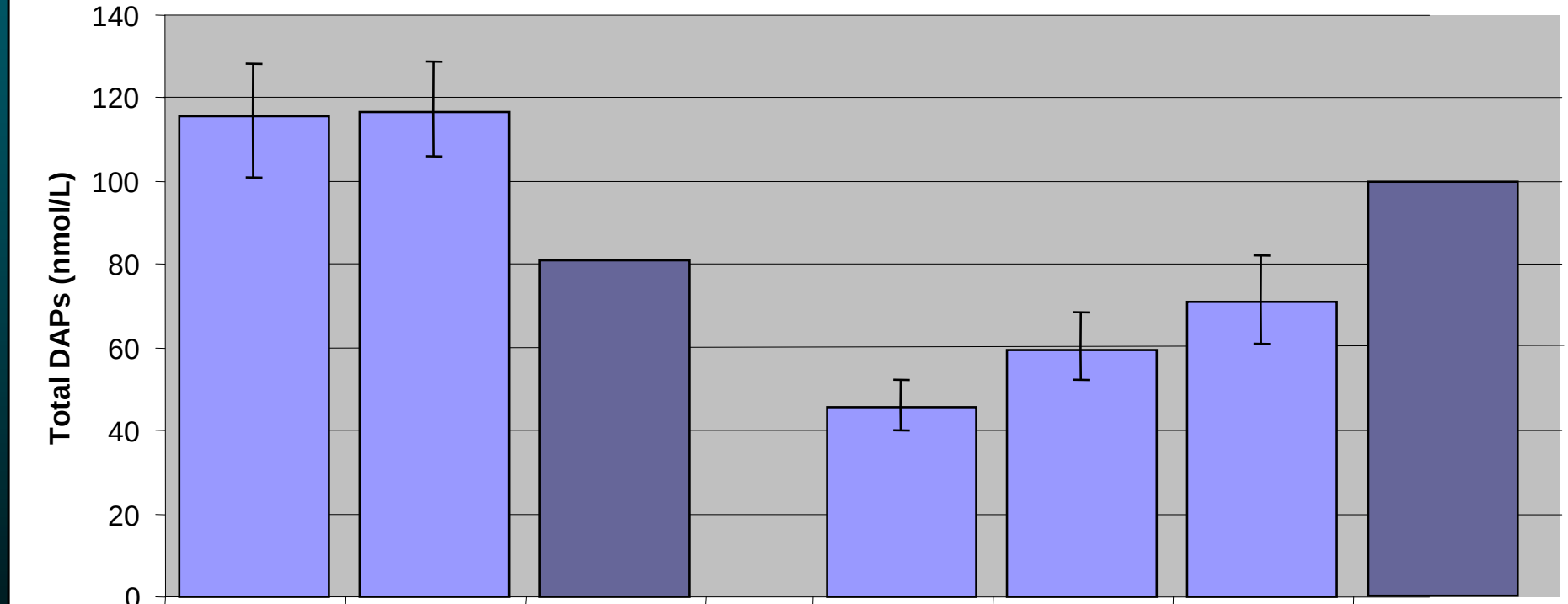
- Dimethyl (DM) phosphates ~220,000 lbs. (42%)
- Diethyl (DE) phosphates ~199,000 lbs. (38%)
- Other ~104,000 lbs. (20%)

Map Prepared by Bob McLaughlin, Rusty Scalf, Martha Harnly, Ca DHS

Organophosphates (OP)

- Widely used in agriculture
- Degrade quickly (hrs-days)
- Acute neurotoxins
- Cholinesterase inhibition is the main mechanism of OP toxicity

Prenatal and Child OP Metabolites in CHAMACOS and National Reference*



***National Health and Nutrition Examination Survey**

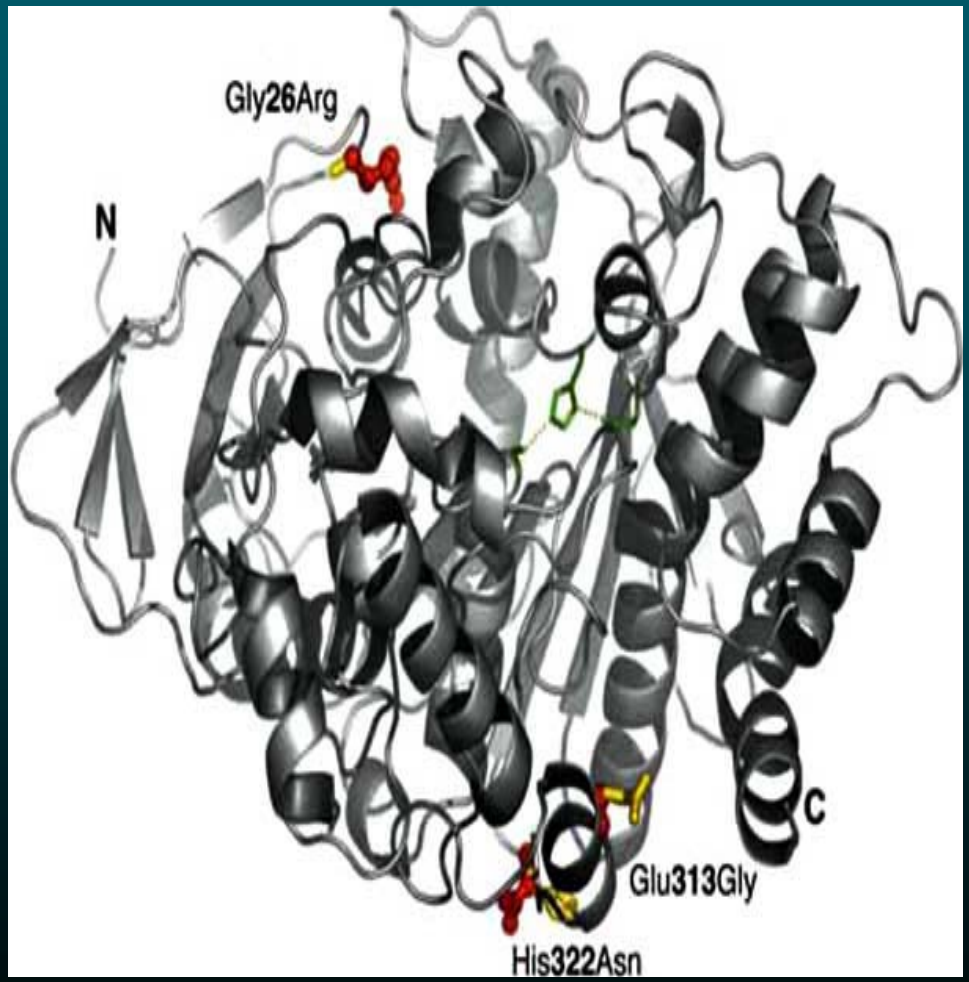
CHAMACOS studies have shown:

- Substantially higher levels of pesticide metabolites in nearly all urine samples collected from low-income woman in Salinas Valley, CA contained compared to national averages (*Bradman et al.*, 2005, 2007)
- Decrease in gestational duration were associated with pesticide exposures (Eskenazi *et al.*, 2004)
- Abnormal reflexes in infants (after first 3 days of life) were associated with prenatal organophosphate exposure (Young *et al.*, 2005)
- Pervasive developmental disorder< autism-like complex of behavioral characteristics> (Eskenazi, 2009)

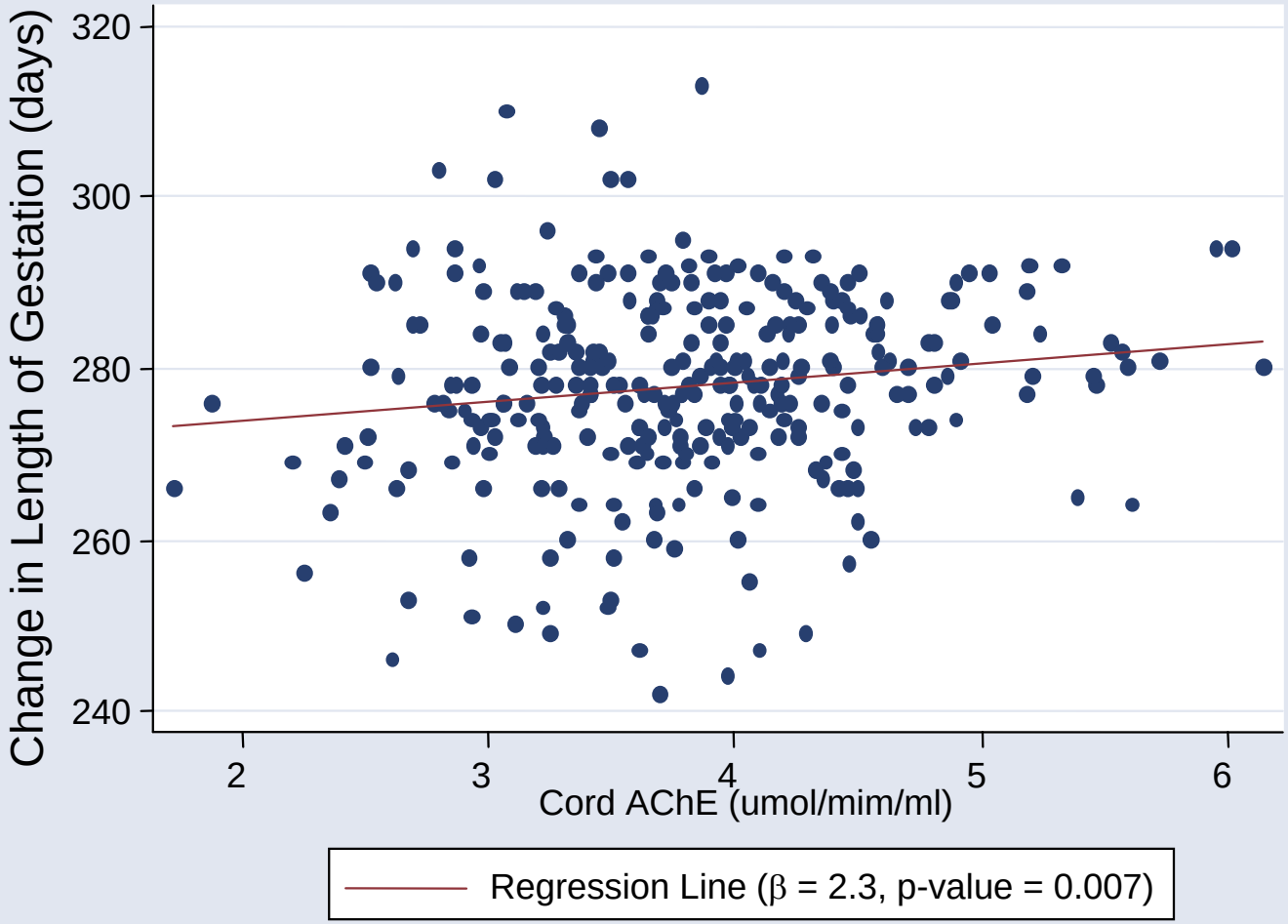
Biomarkers of effect

CHOLINESTERASE

- Acetylcholinesterase (AChE): AChE catalyses the rapid hydrolysis of acetylcholine (ACh) to acetate and choline
- Inhibited by OPs AChE may results in acute neurotoxicity and death
- Widely used to monitor OP exposure in agricultural workers and other exposed groups
- Broad inter-individual variability



Association of Cord Blood Acetyl Cholinesterase and Length of Gestation

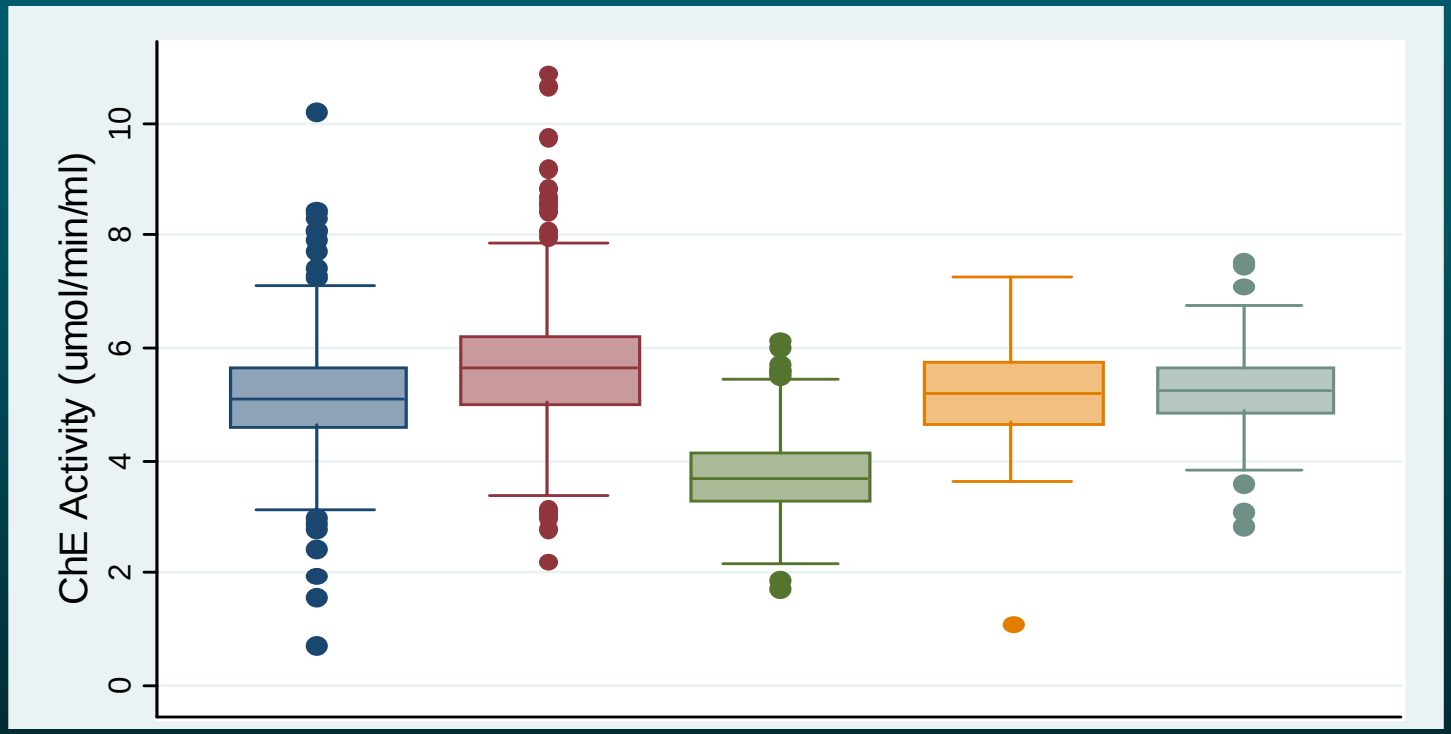


Low ChE correlates with:

- Low birthweight *(p=0.02)*
- Premature birth *(p=0.007)*

Eskenazi et al, 2004; EHP, 112, 10, 1116-1124

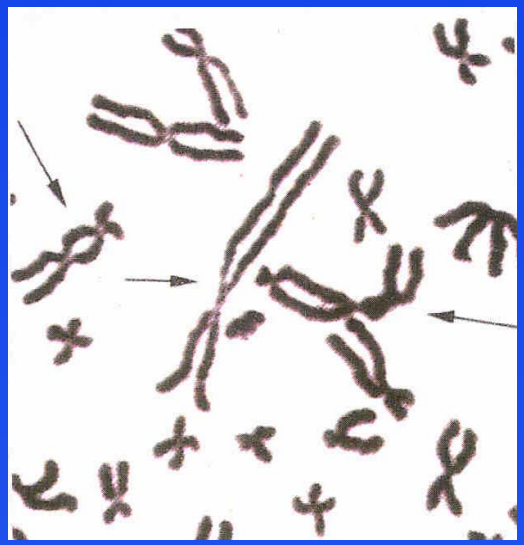
ChE Activity Profile of CHAMACOS Mothers and Children



- **ChE levels in cord blood were significantly lower than levels in older children and levels in mothers (p<.001)**
- **Small but significant differences in mothers levels at 26 wk versus delivery (p<.001)**
- **No difference in ChE activity between children at 24 month and 60 month of age**
- **In progress: nanosensors and ChE genomics**

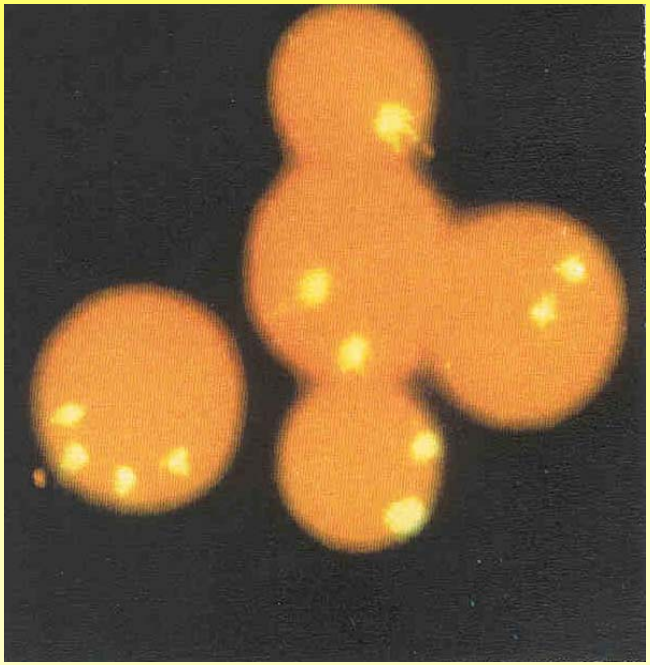
Case study: Cytogenetic damage

Cytogenetic Biomarkers:

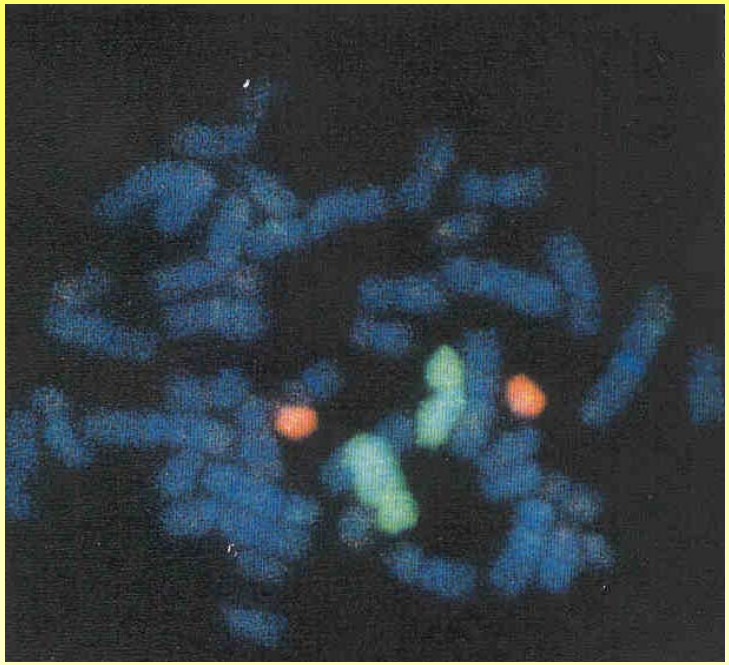
	Chromosome Aberrations	Sister Chromatid Exchanges	Micronuclei
			
Background Levels in Humans	**1% of cells with CA** **100 cells scored**	**5-8 SCE per cell** **25-50 cells scored**	**5-8 MN per 1000 cells** **1000 binucleated cells scored**

Cytogenetic Biomarkers: Second Generation

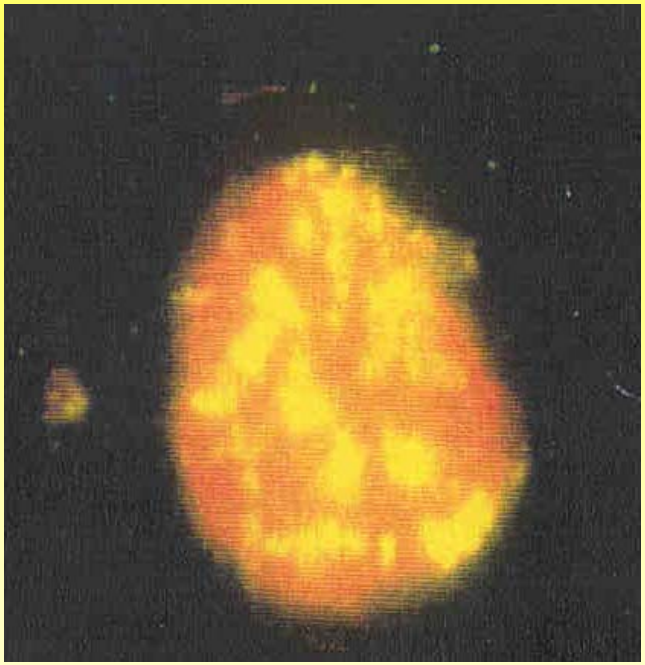
Interphase FISH



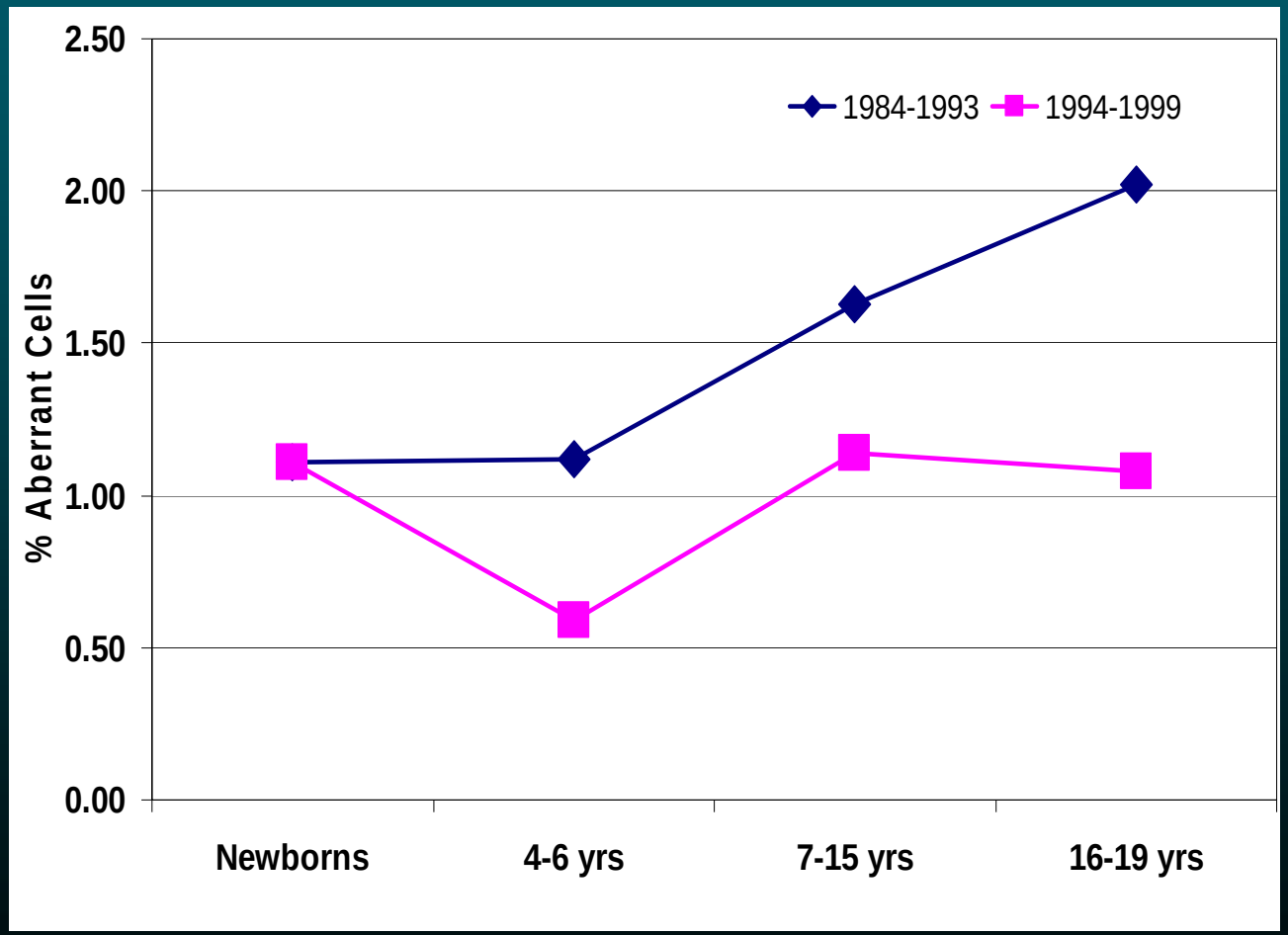
Metaphase FISH

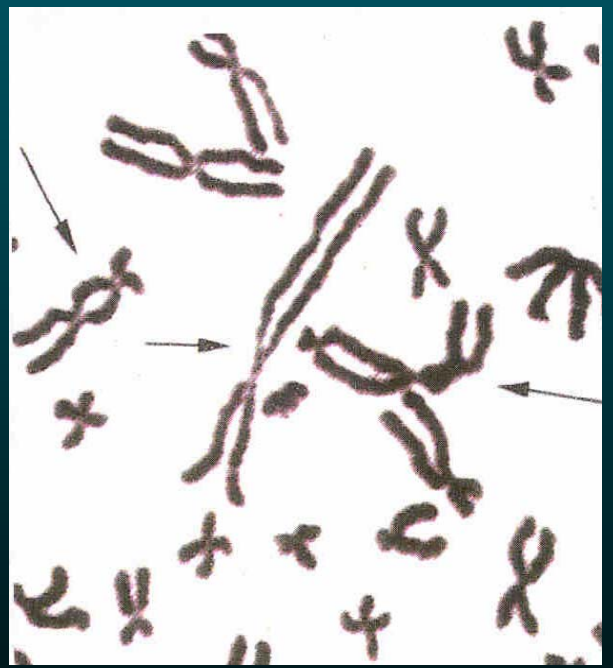


Novel MN Analyses



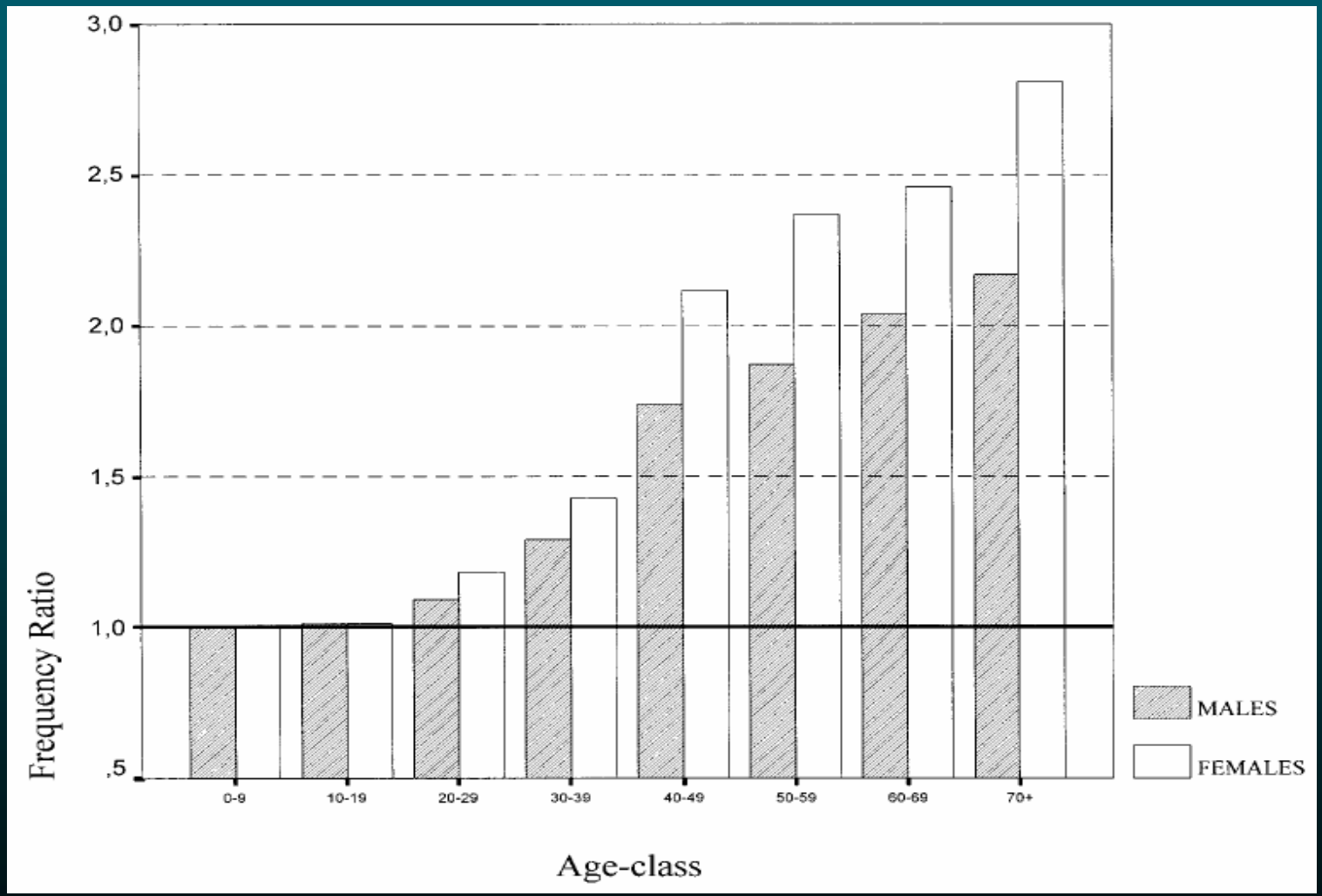
Biomarkers of Effect: Chromosome Aberrations

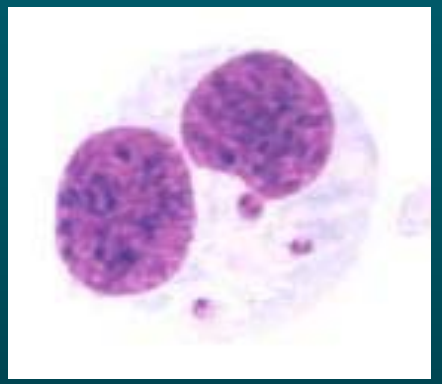




From: Rossner 2002

Effect of Age on MN frequency in Lymphocytes

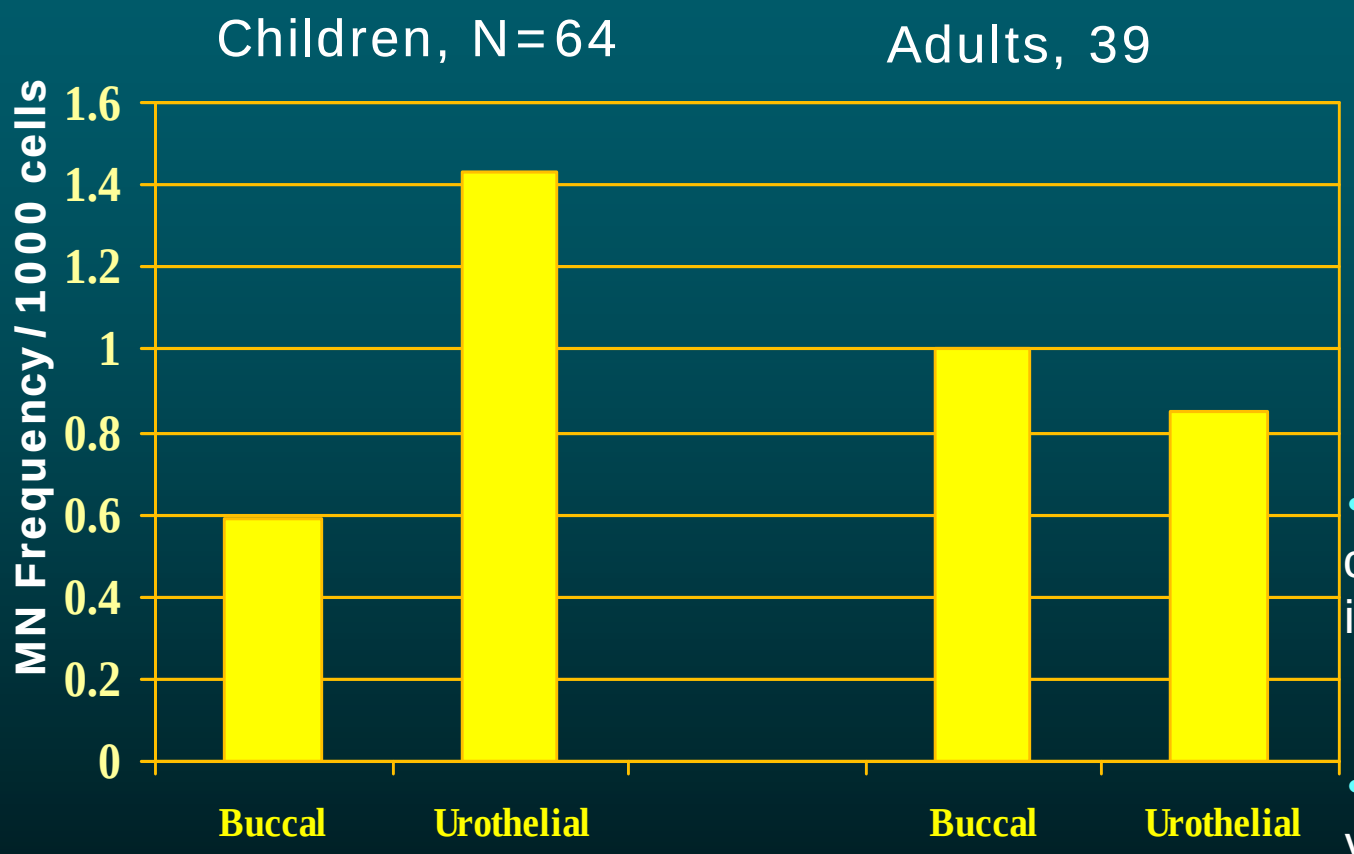




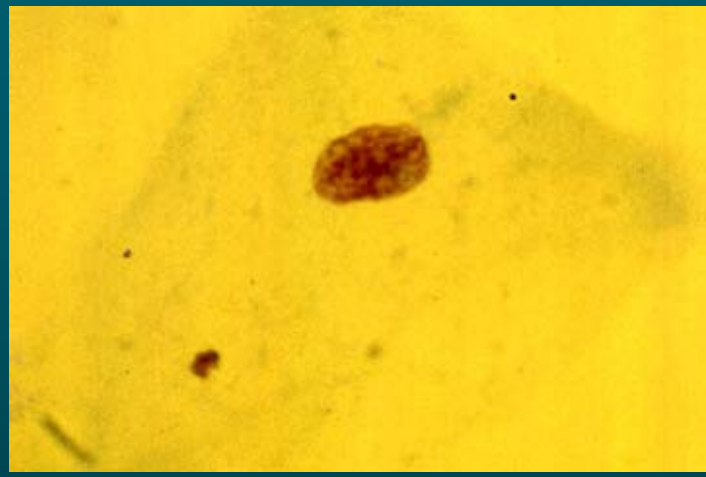
- Strong age effect in adults
- Effect in children unclear

Bonassi et al. EMM. 37: 31-45 (2001)

MN Frequency in Exfoliated Cells

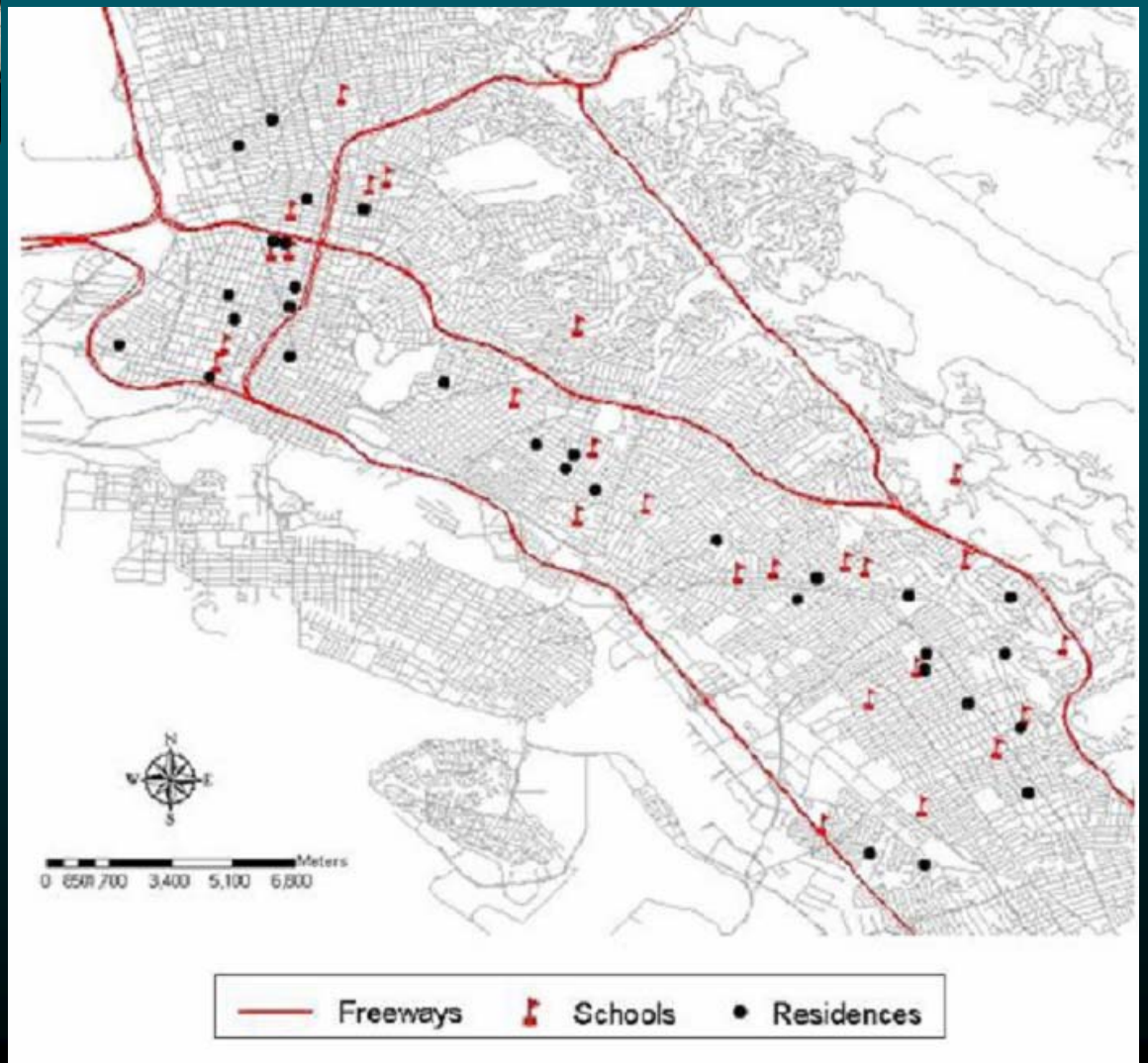


From: Huen et al, 2006



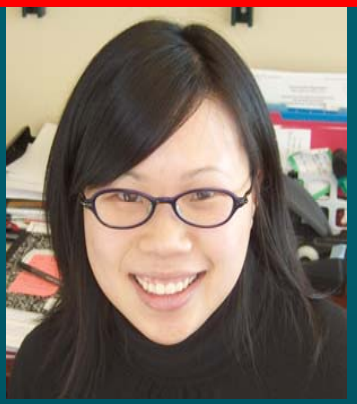
- MN levels are similar in children and adults of inner city (p=0.4)
- Broad inter-individual variability, ~3-fold for children, and ~2-fold for adults

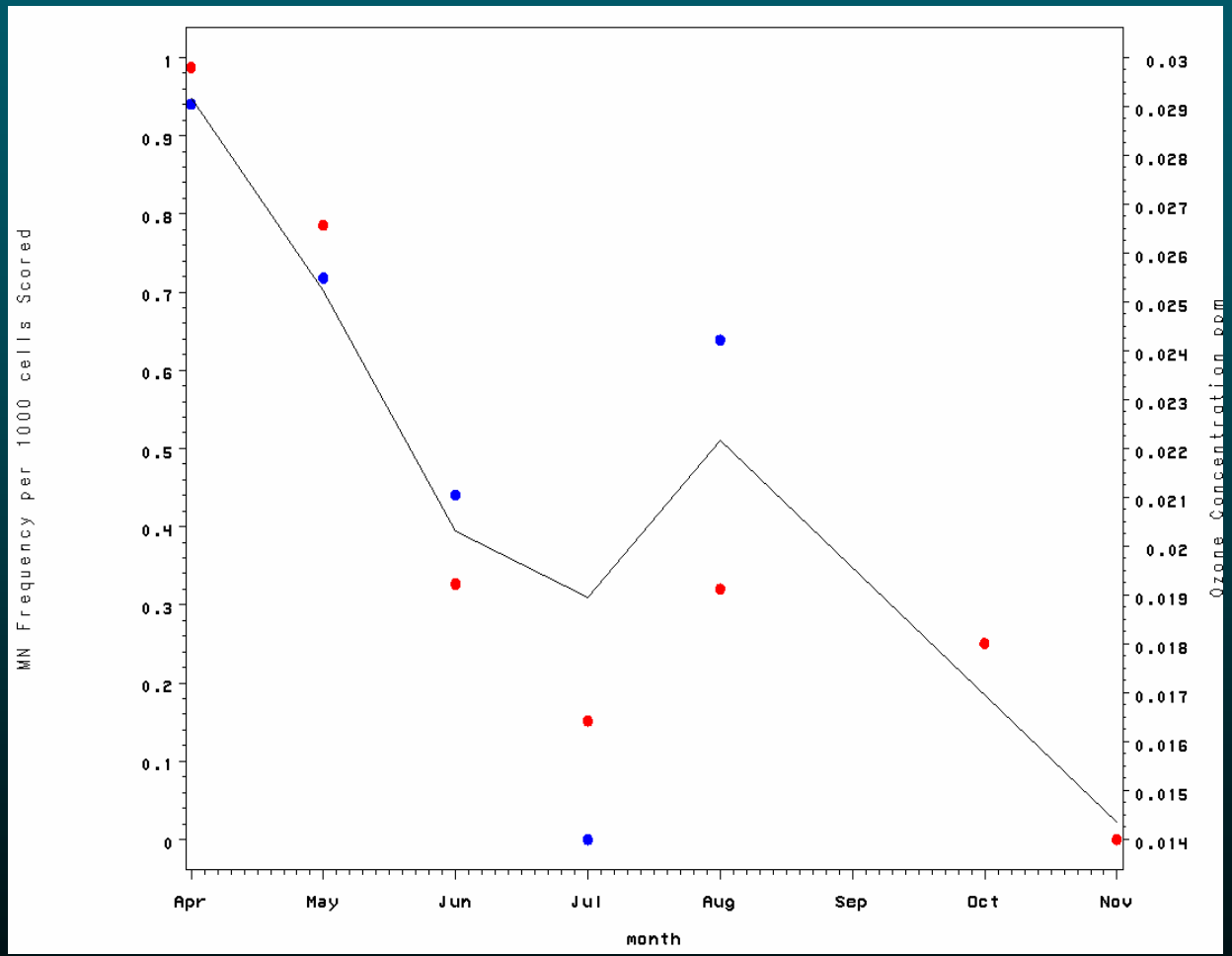
Inner City Oakland Mothers and Children: Effects of air Pollution



Huen et al. 2006. EMM.

Inner City Oakland Mothers and Children





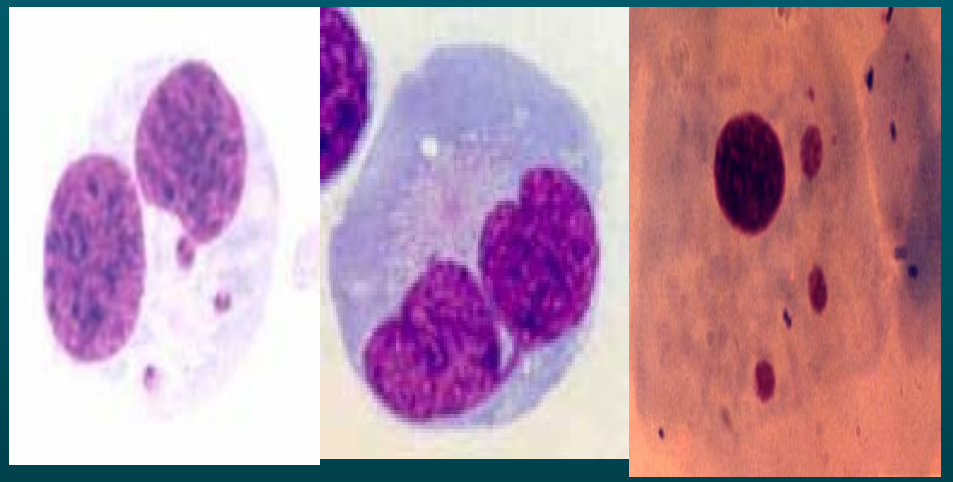
- Regional O_3 levels were highly correlated with season by month (r^2=0.84, P=0.02)
- O_3 levels were associated with MN frequency (FR=3.37) in both exfoliated and lymphocyte cells in children and adults.

Huen et al. 2006. EMM.

Cytogenetic Damage

Increase

- **Ozone levels**
  - **Mothers: FR=3.37 (p=0.01)**
  - **Children: FR=13.50 (p=0.04)**
- **Traffic-related air pollution in children: FR= 3.33 (p=0.05)**
- **Smoking in the household**
  - **Mothers: FR= 1.05 (p=0.08)**
  - **Children: FR= 1.09 (p=0.05)**



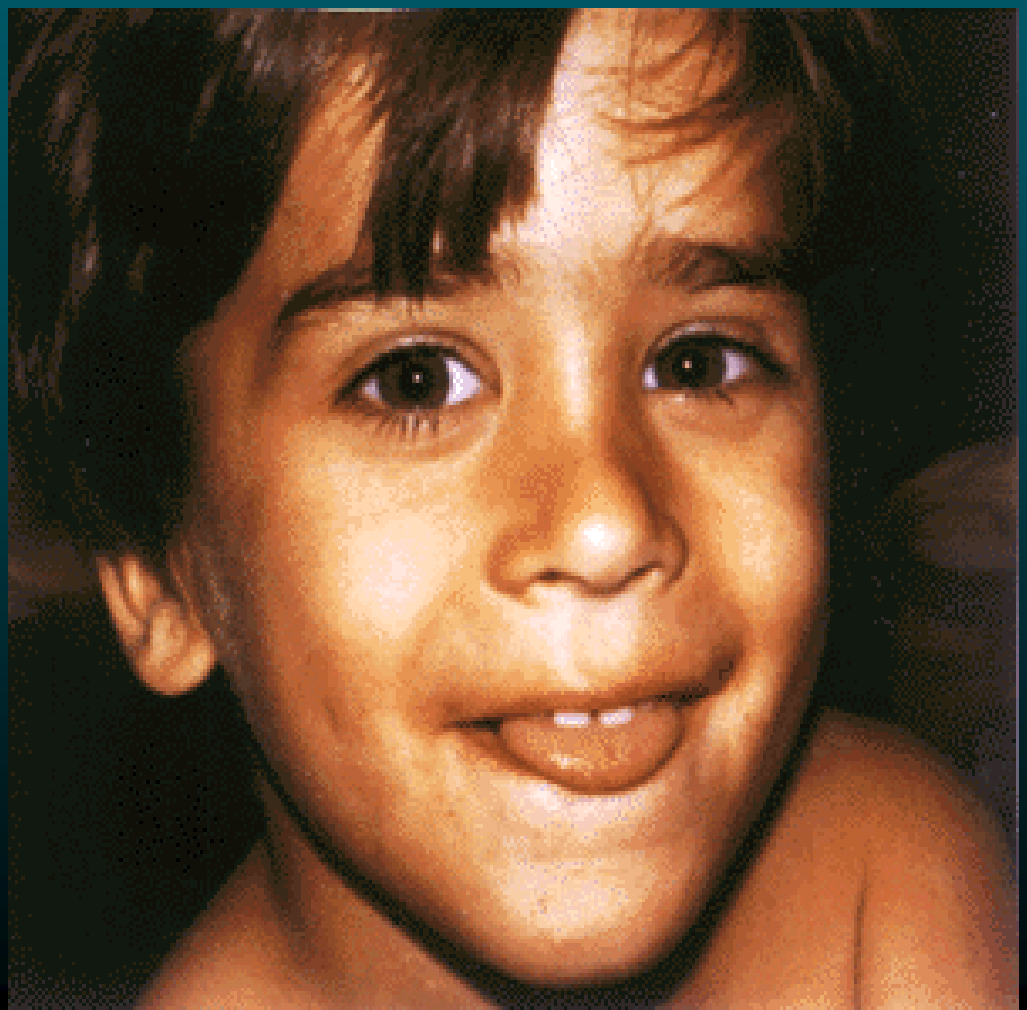
Decrease

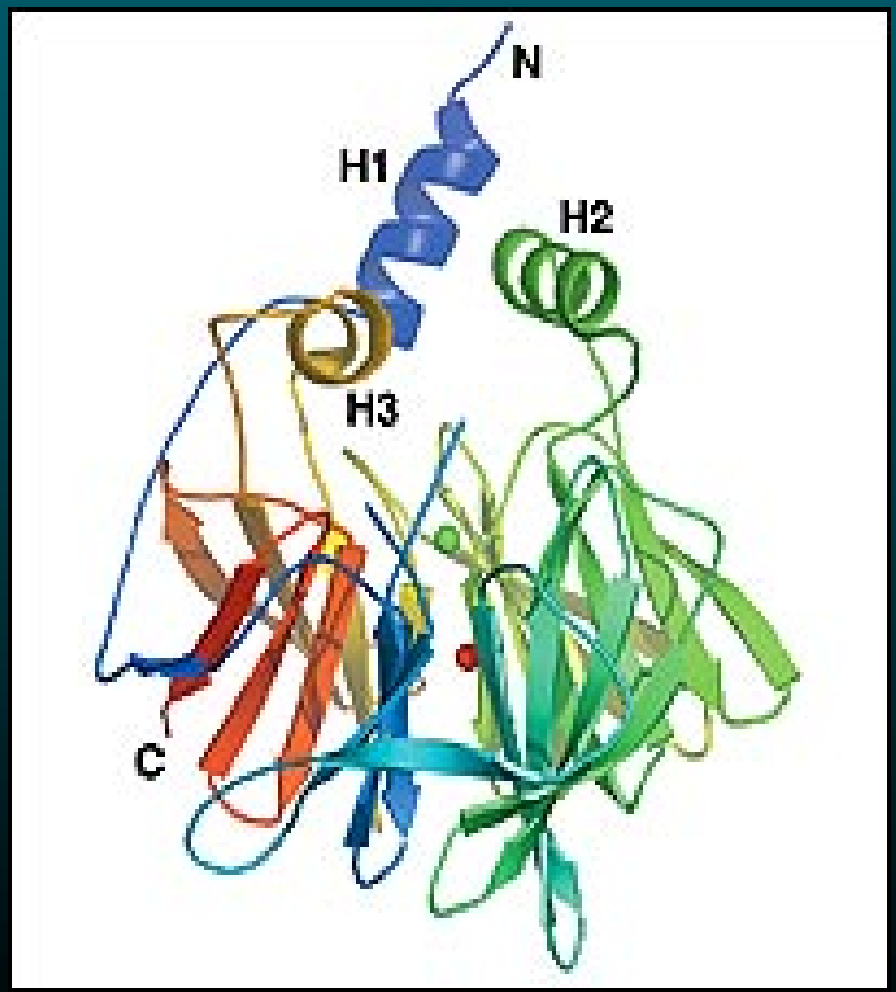
- **Vitamin use in mothers: FR=0.17 (p=0.10)**
- **Gas appliances, mothers: FR=0.40 (p=0.12)**

Huen et al. 2006. EMM.

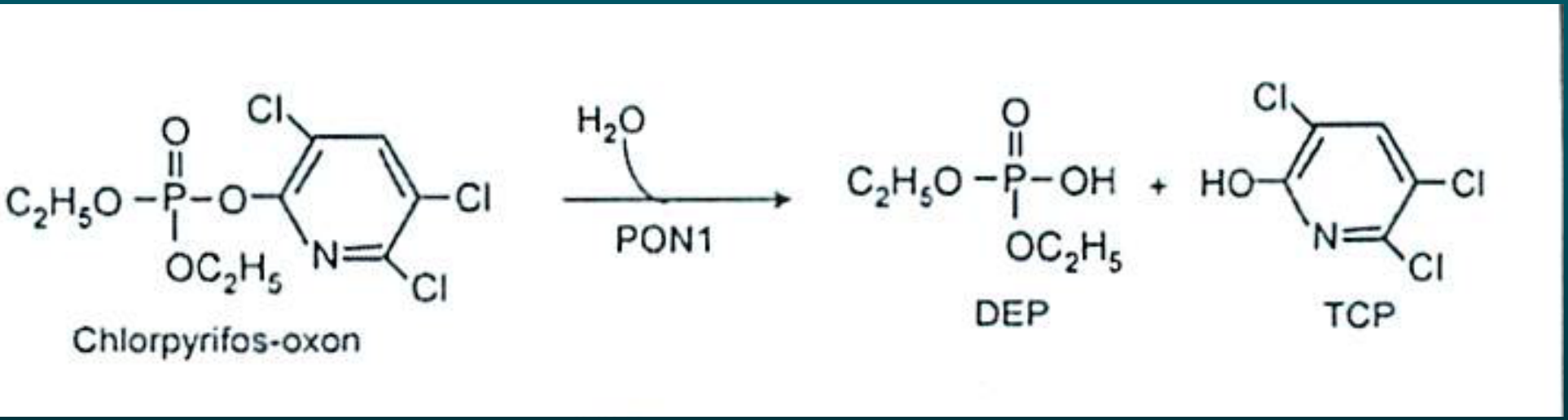
Biomarkers of Susceptibility

Paraoxonase (PON1)





Individual Susceptibility to OPs Varies by Paraoxonase **(PON1)** Activity



- **PON1 enzyme detoxifies OPs in the body and protects AChE from inhibition**
- **Detoxification depends on <u>quantity</u> and <u>efficiency</u> of enzyme**

Source: Chambers, PNAS 2008

PON1 Activity is Determined by Genetic Make-up

- *PON1* gene located on the long arm of chromosome 7
- Gene has multiple polymorphisms (SNPs)

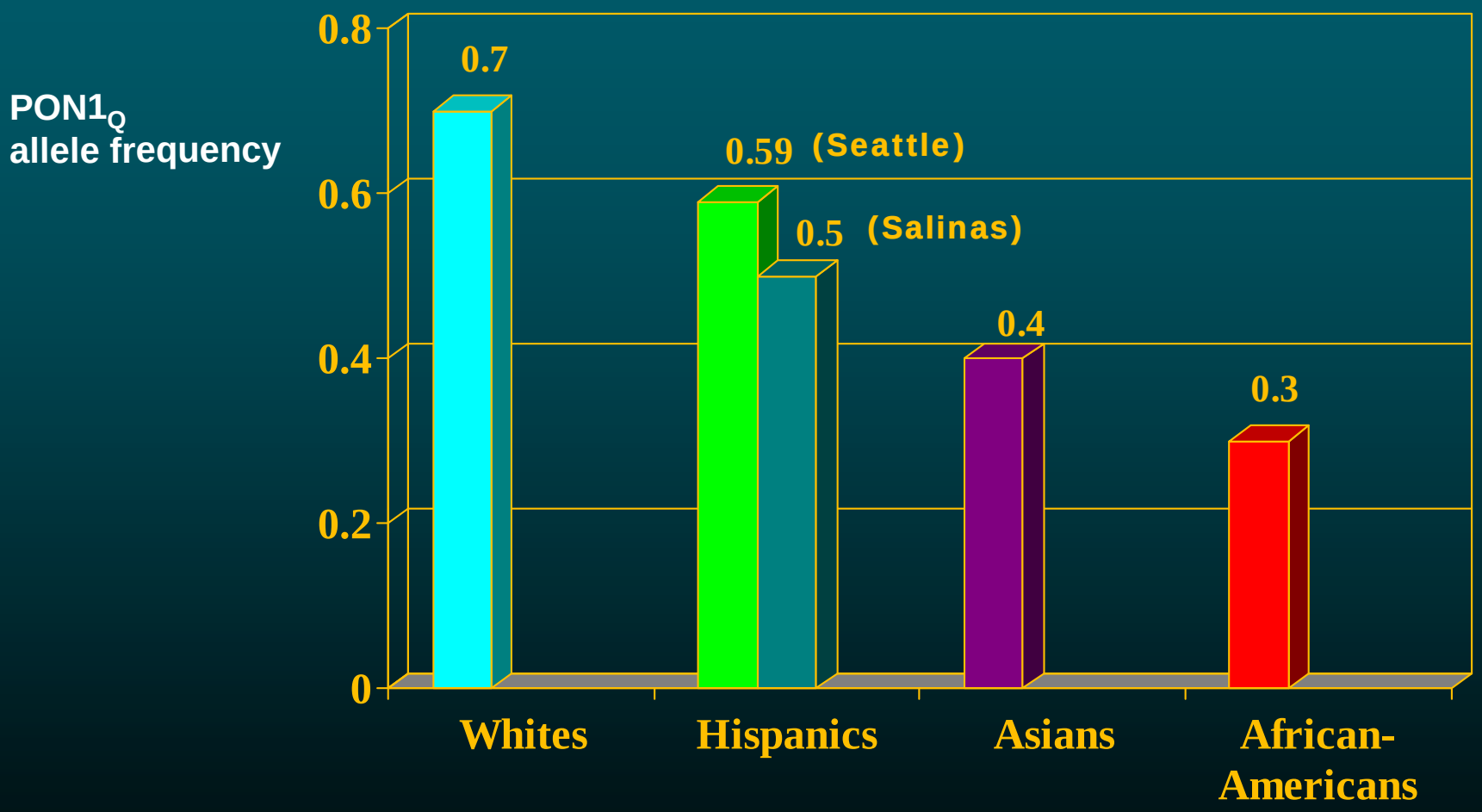
PON1$_{-108}$

  - Total quantity of enzyme
  - C allele > T allele

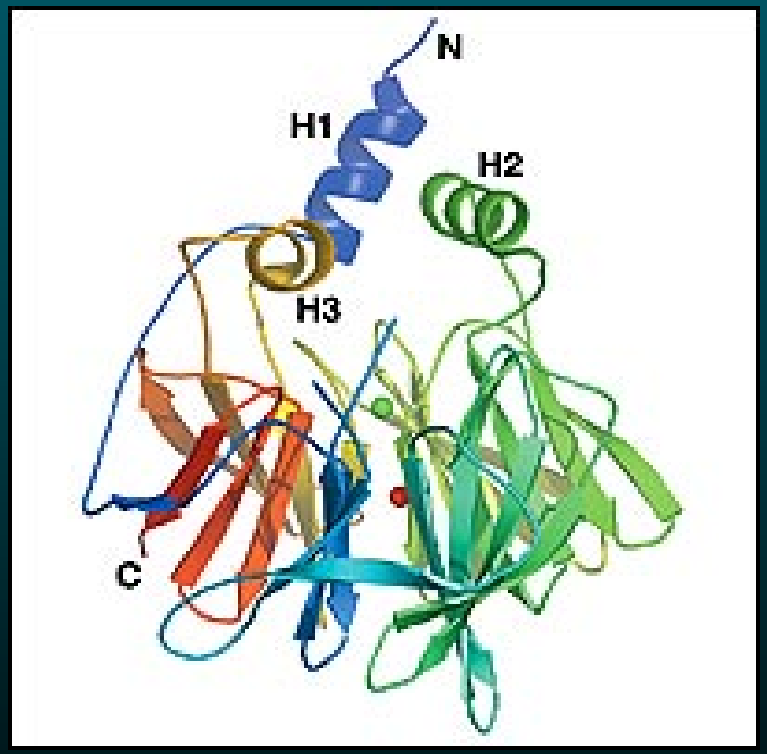
PON1$_{192}$

  - Catalytic efficiency of enzyme
  - R allele > Q allele

Ethnic Differences in PON1 gene frequency



Functional Genomics of Paraoxonase 1 (PON1)



Oxidative Stress

- cardiovascular disease
- diabetes
- rheumatoid arthritis

OP Sensitivity

- PON1 can hydrolyze oxon derivatives of OPs
- Animal models provide evidence of a protective role of PON1

PON and OP Pesticide Susceptibility

PON1 Findings:

Genetic variability (SNPs and haplotype) has greater effect in children than adults

Diazinon: 26-fold difference in susceptibility among newborns
Some newborns are up to 65 times more susceptible than adults with highest enzyme levels.

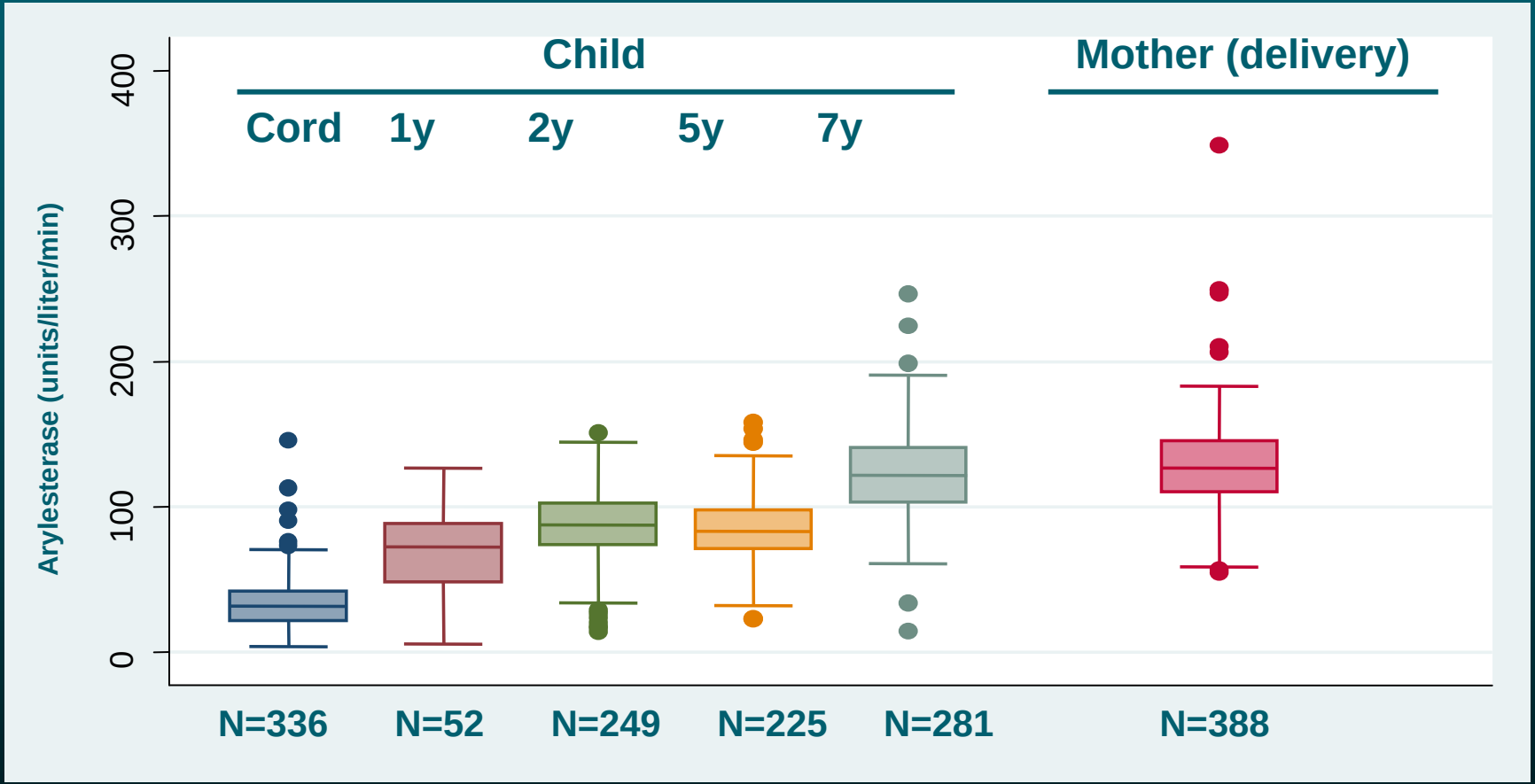
Chlorpyrifos: 50-fold difference in susceptibility among newborns
Some newborns are 130-164 times as susceptible as highest adults.

Current pesticide standards may not protect the most vulnerable.

N.Holland, C.Furlong, M.Bastaki, R.Richter, A.Bradman, A.Ho, K.Beckman, B.Eskenazi. Paraoxonase polymorphisms, haplotypes and enzyme activity in Latino mothers and newborns, EHP, 114, 985-991, 2006.

C. Furlong, N Holland*, R Richter, A Bradman, A Ho, B Eskenazi. PON1 status of farmworker mothers and children as a predictor of organophosphate sensitivity, Pharmacogenetics and Genomics, 16:183-190, 2006.* *
first co-authors

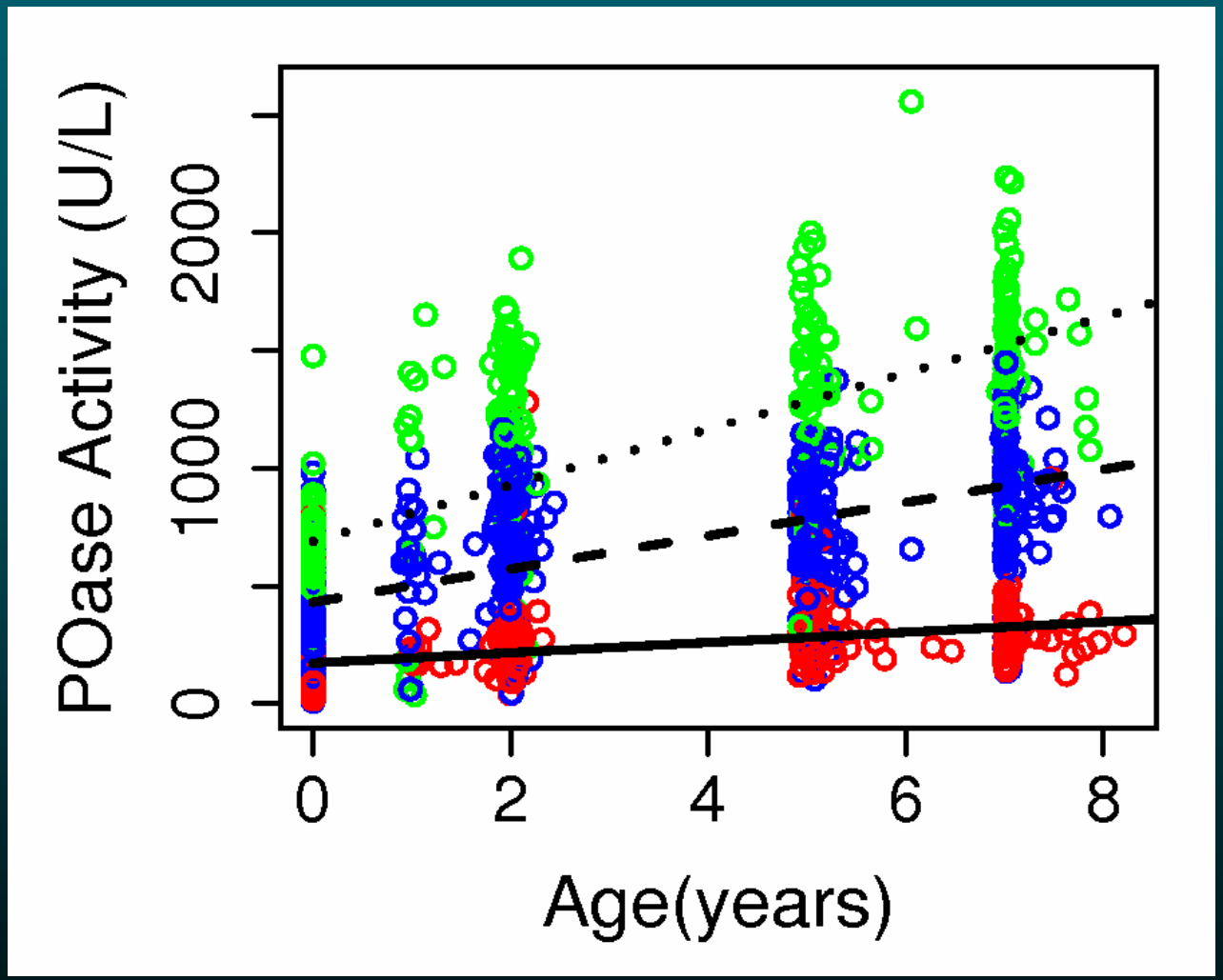
Effects of Age



• PON1 levels in children up to 7 years of age were lower than those of adults ($p<0.005$).

Holland et al.2006, Huen 2009

Genetic Influences on PON1 Ontogeny



- ***PON1 192*** **genotype significantly modified the effect of age on POase activity (p<0.0001)**

PON1 and Birth Outcomes

Health effects of PON1

- **Association of low PON1 enzyme activities in mothers with small head circumference in neonates in New York *(Berkowitz et al., 2003; 2005)***

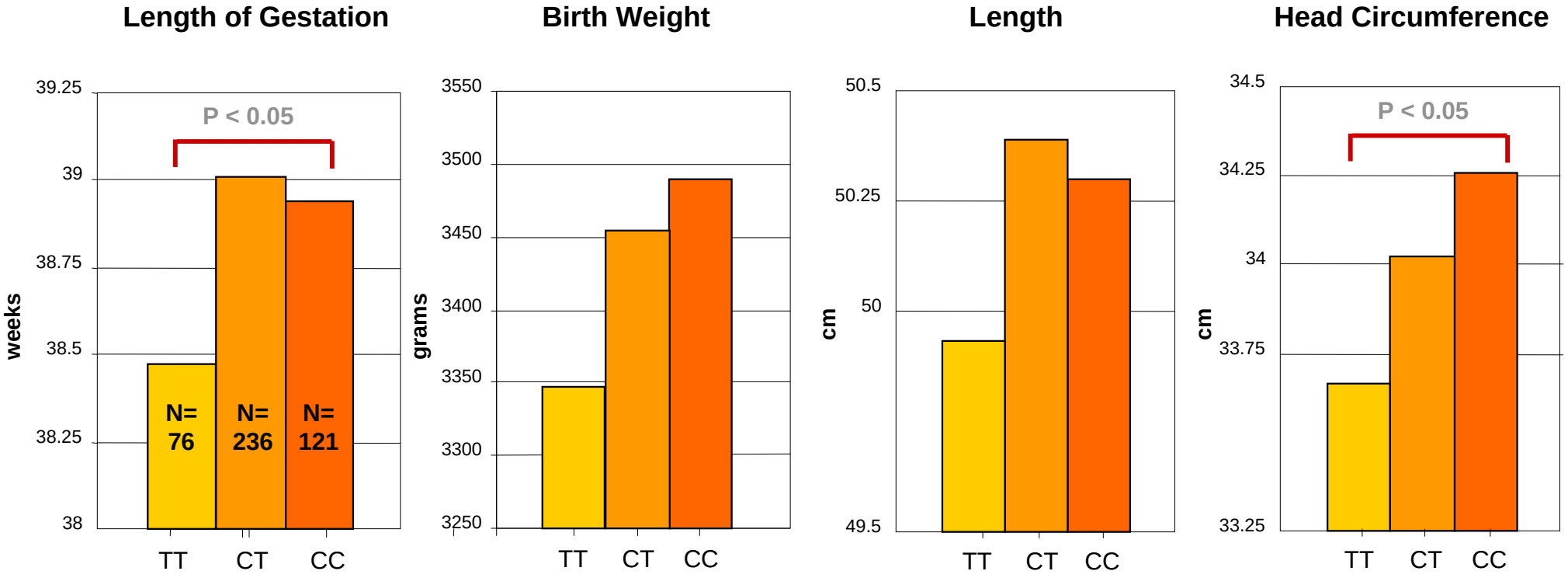
- **Low PON1 in children with autism *(Pasca et al. 2006)***

- **PON1 involved in lipid peroxidation and related health outcomes such as cardio-vascular and neuro-degenerative diseases *(rev. in Costa and Furlong, 2002)***

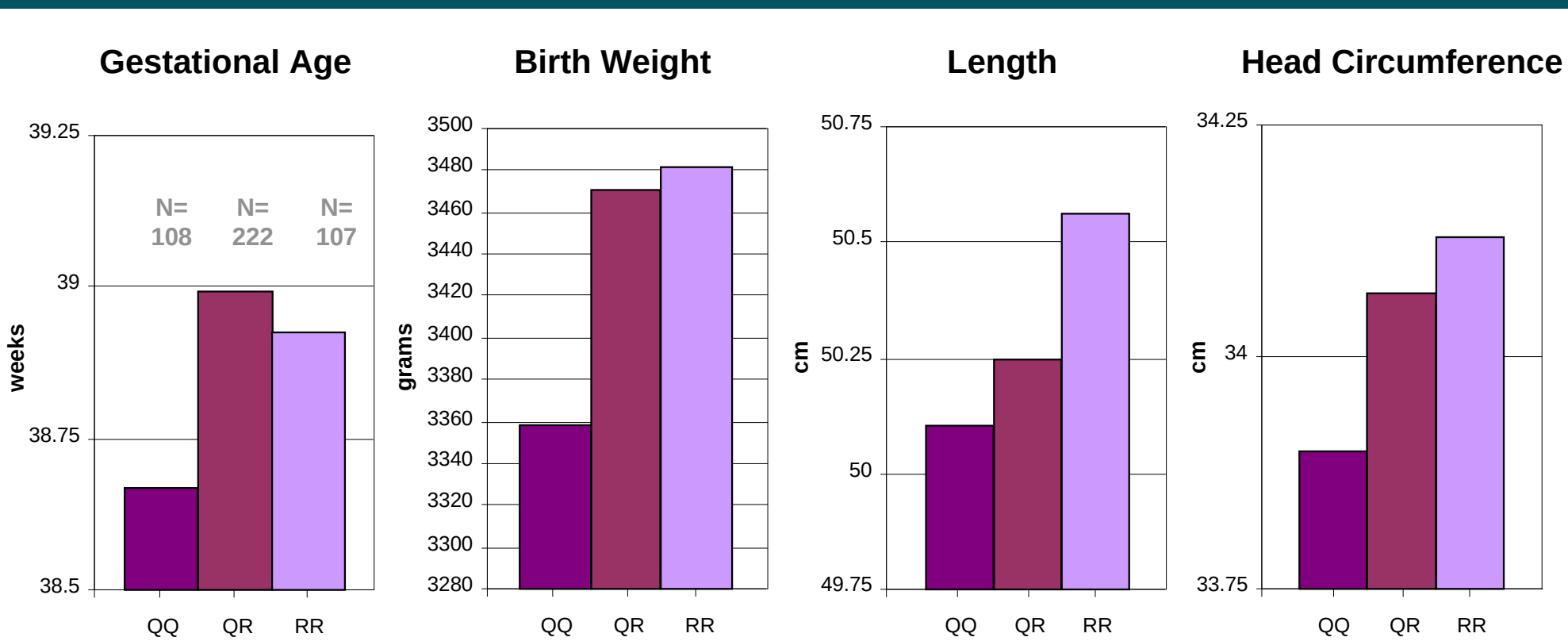
- **Associated with male infertility (*Padungtod et al.,1999*)**

- **$PON1_{192}$ associated with complications and outcomes of pregnancy (Chen et al. 2004; *Lawlor et al., 2006*)**

Child PON1$_{-108}$ Genotype and Fetal Growth (N=433)



Child PON1$_{192}$ Genotype and Fetal Growth (N=437)



Child 192

Variability and Vulnerability: Genetic Effects on Birth Outcomes

Effects of Single SNPs (child genotypes):

2 SNPs were associated with preterm birth (N=470):

- ***PON1$_{192}$QQ* vs *PON1$_{192}$RR* (OR: 3.52, p=0.03)**
- ***PON1$_{108}$TT* vs *PON1$_{108}$CC/CT* (OR:2.62 p=0.02)**

Effects of 2 SNPs in combination (child genotypes):

PON1$_{192}$QQ/PON1$_{108}$TT* vs *PON1$_{192}$RR/PON1$_{108}$CC

- **Shorter gestational age *(β=-1.03, p=0.006)***
- **Smaller head circumference *(β=-0.90, p=0.007)***

Genetic Effects beyond SNPs: Haplotypes Effects on Gestational Age

Child PON1 Haplotype	-909 -162 -108 55 192	Frequency	Beta Coefficient	95%CI	p-value
7	G A T T Q	1.1%	-2.28	(-3.35,-1.21)	<0.005
5	G A C T Q	11.1%	-0.37	(-0.74,0.005)	0.053
3	C G T T Q	13.6%	-0.33	(-0.72,0.05)	0.09
Reference	G G C T R	27.2%	---		

N=440

- 9 of the most common haplotypes (5 PON1 SNPs) were examined for associations with gestational age.
- Compared to the most frequent haplotype (Reference), haplotype 7 was associated with shorter gestational duration.

CHAMACOS Findings and Conclusions

- Pesticide exposures are widespread in CHAMACOS agricultural minority cohort
- Newborn children have lower enzyme levels (PON1, ChE) than their mothers and are more vulnerable to exposures
- Broad age- and genotype-dependent variability in susceptibility to pesticide exposure in women and children of CHAMACOS cohort
- Pesticides are associated with adverse growth and neurodevelopment outcomes

Future Directions of Biomarker Studies in Children

- Diverse ethnic and age groups
- Sophisticated collection of biological and environmental samples
- Development and application of novel biomarkers
- Longitudinal multidisciplinary programs
- Comprehensive exposure assessment: from exposure to "exposone"(*C.Wild, 2005*)

Acknowledgements

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