

# Suspected Modes of Action Affected by Pesticides Exposure: Informing an Adverse Outcomes Pathway (AOP) for Cancer

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# Adverse Outcome Pathways (AOP)

- AOPs are an important conceptual framework for organizing evidence from toxicology and **molecular epidemiology**, linking a particular exposure to an adverse outcomes.
- e.g., exposure → biomarker  
exposure → biomarker of effect → disease

# Agenda

- How can **epidemiology** and specifically **molecular epidemiology** contribute to AOP?
- Examples:
  - biomarkers of exposure,
  - telomere shortening,
  - cancer susceptibility, epigenetic,
  - biomarkers of early disease (precursors)

# Inadequacy of Earlier Case-Control or Retrospective Studies

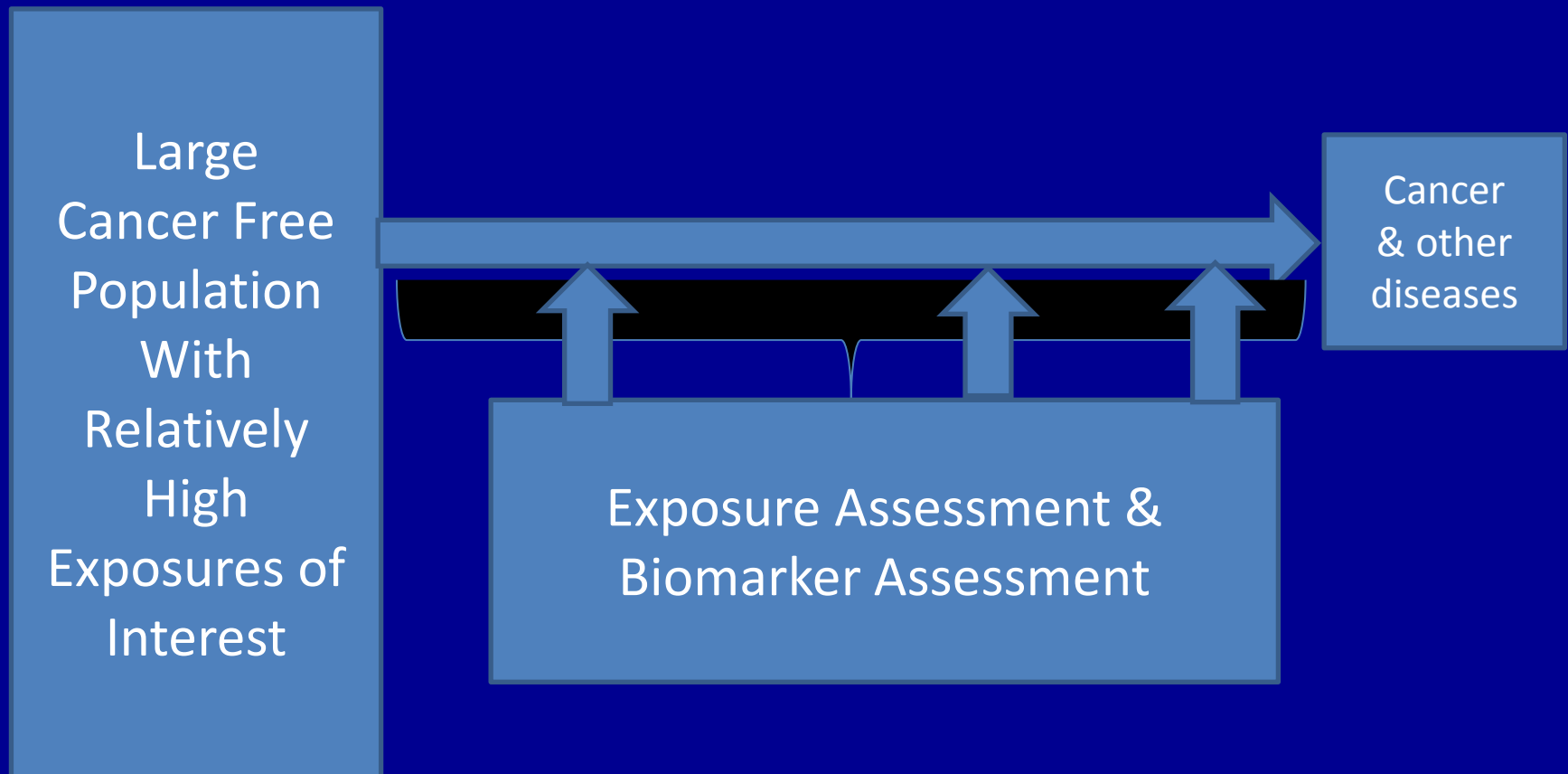
- Case-control studies:
  - Case-recall bias?
  - Was the biomarker:
    - a result of the disease?
    - disease treatment ?
    - or **exposure**?

# Prospective Study Design



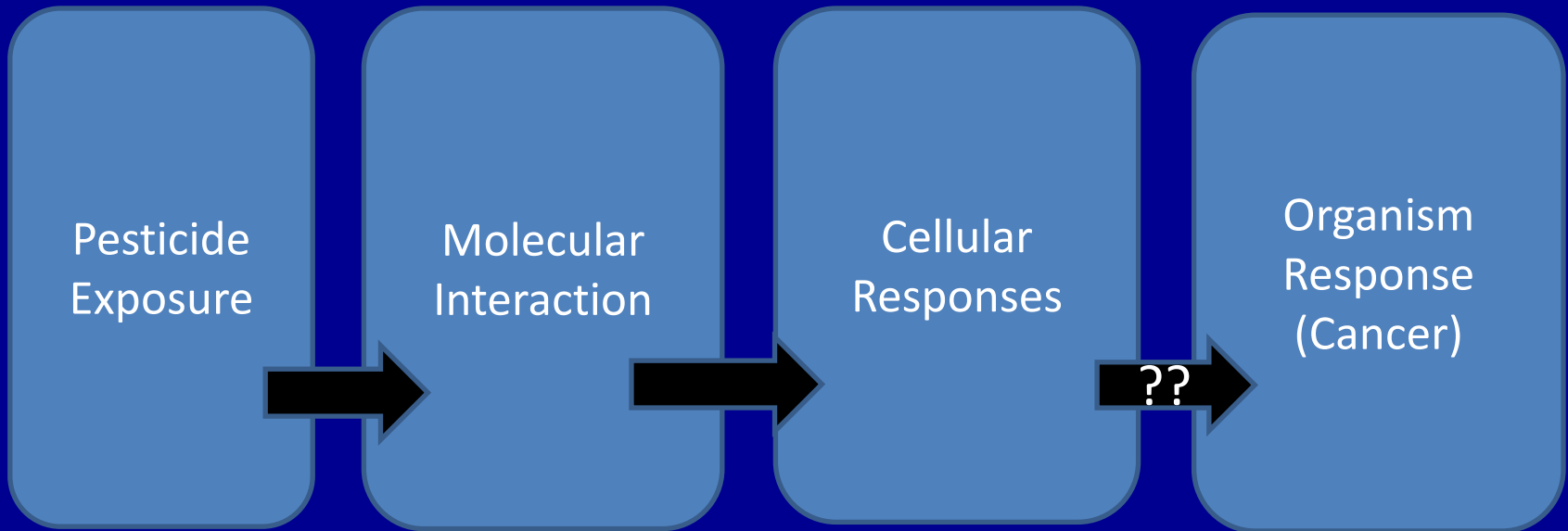
# Prospective Occupational Epidemiologic Design

- Eliminates case-recall bias & permits collection of biospecimens & ongoing exposure assessment



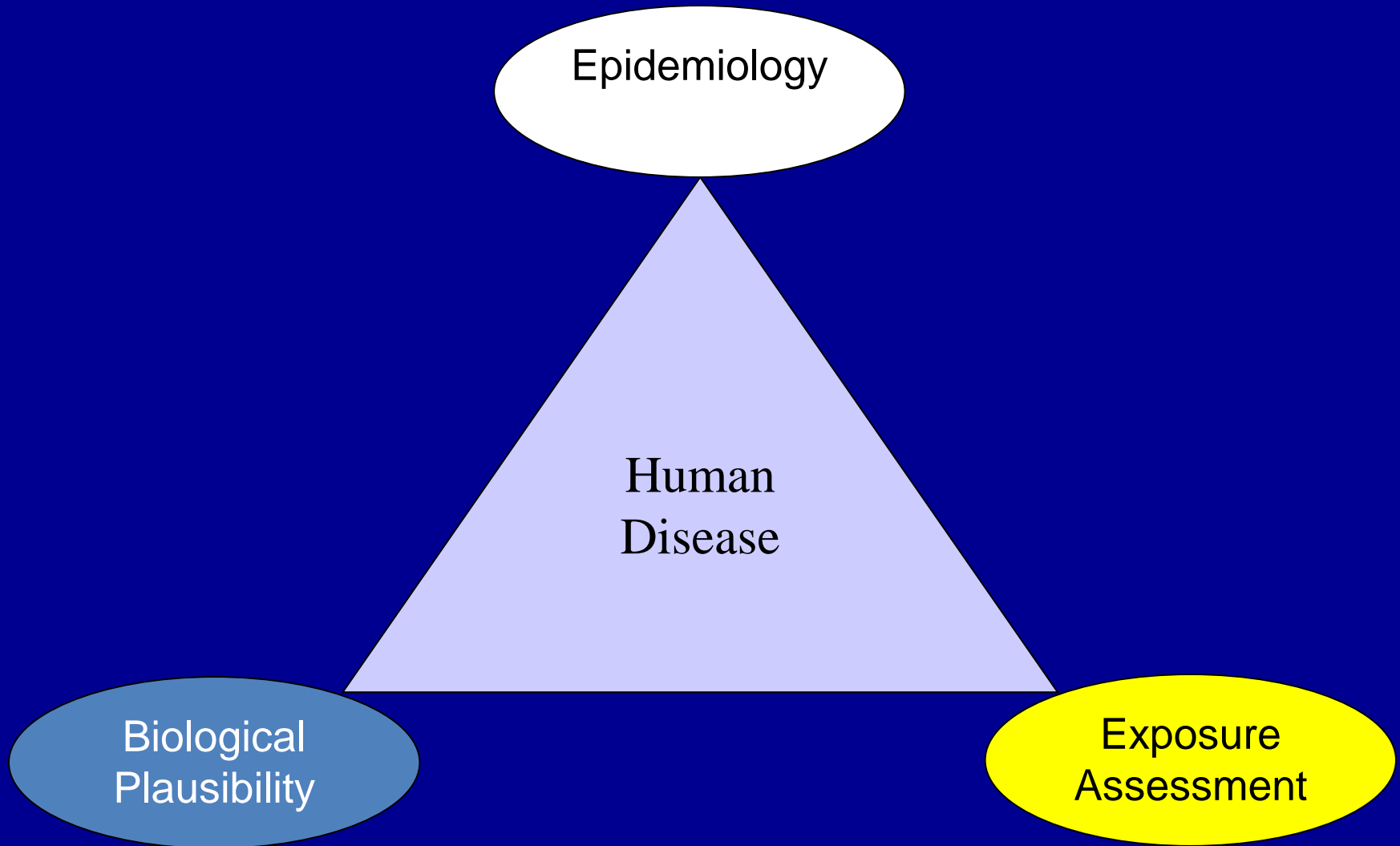
# Adverse Outcome Pathways

- Potential Contributions from epidemiology





# Logic to Establish Human Disease Associations In a Prospective Study

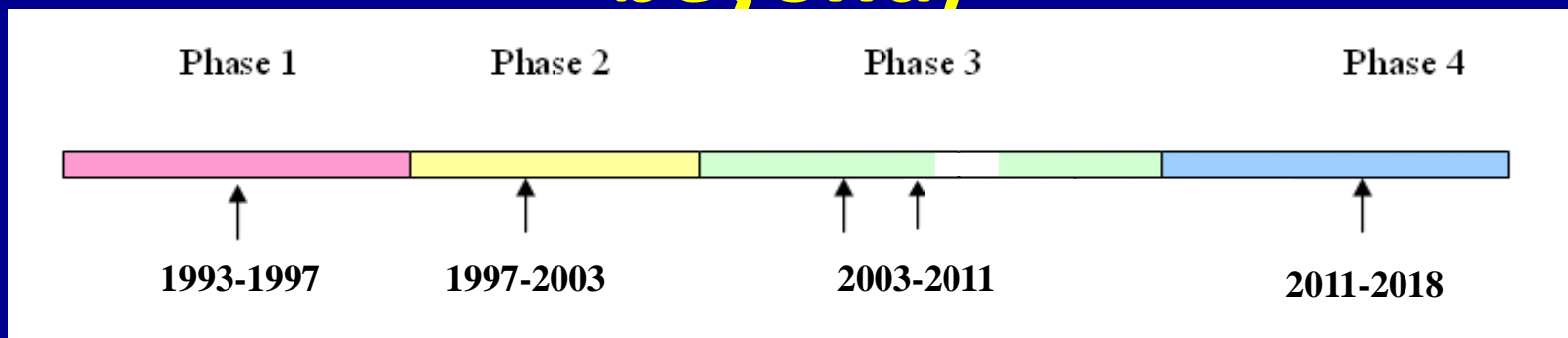


# Agricultural Health Study (AHS)

- ❖ Prospective study of
  - 52,394 private applicators (i.e., farmers)
  - 32,345 spouses of farmers
  - 4,916 commercial applicators
- ❖ Two important agricultural states (Iowa & North Carolina) in US
  - Corn, soybean and hog production in both states
  - Distinctive agriculture in North Carolina: fruits, vegetables, tobacco, cotton



# AHS Timeline 1993 to 2018 (and beyond)



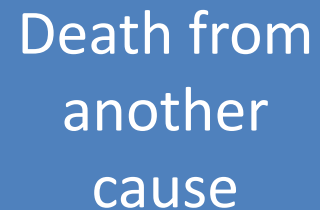
**Exposure Assessment**

**Disease follow-up, Mortality follow-up, Address follow-up**

- **Phase 1**- Enrollment questionnaire (82% of target population of private pesticide applicators enrolled)
- **Phase 2**- Follow-up questionnaire, field validation of pesticide exposures, buccal cell collection for DNA, dietary questionnaire
- **Phase 3**- Second follow-up, blood collection in sub-studies, disease etiology, begin DNA evaluation. Disease etiology.
- **Phase 4**- Disease etiology and molecular mechanisms studies

# Simple Causal Pathway

Exposure → Biomarker → Cancer



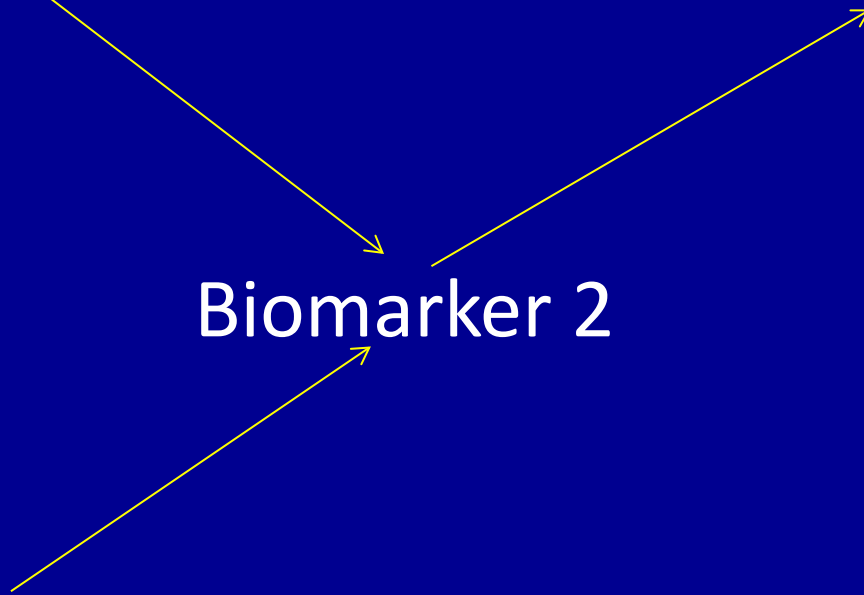
Death from  
another  
cause

# More Complex Potential Causal Pathway Typical of Epidemiology (Natural Human Experiments)

- Exposure 1 → Biomarker 1 → Cancer

Biomarker 2

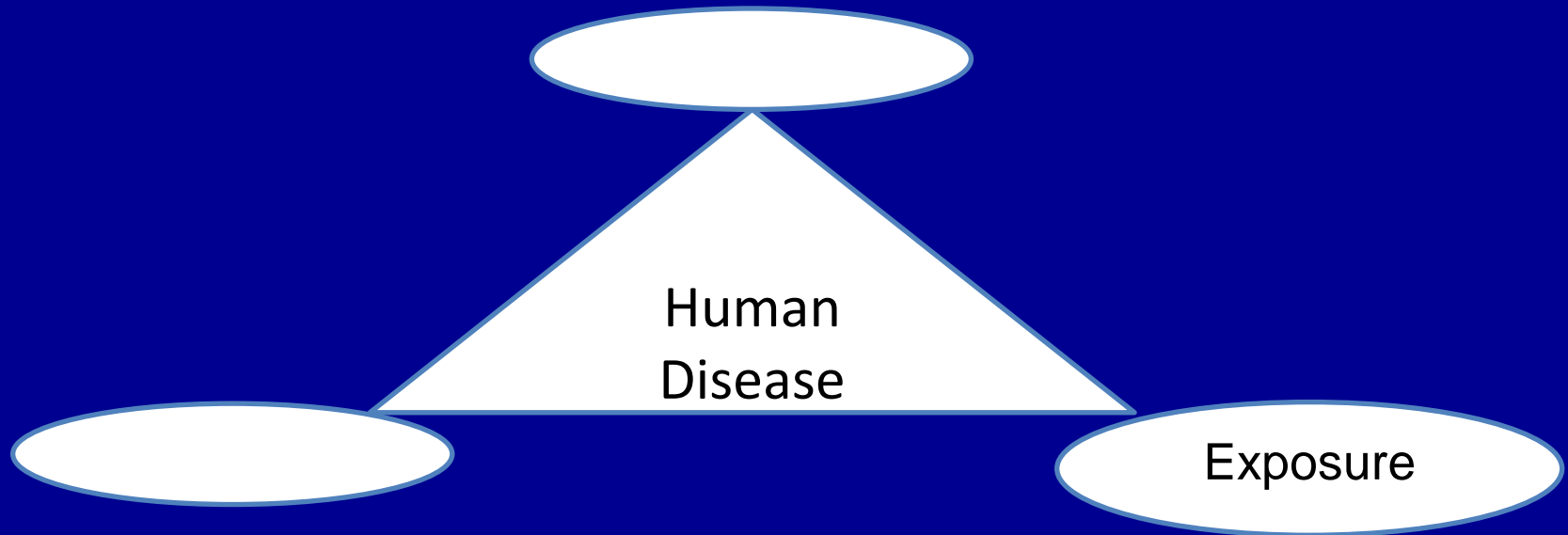
- Exposure 2 (confounding variable)



# Strengths of the AHS for Etiological/Biomarker Research

- 1) Prospective design (exposure assessed prior to cancer onset & little/no opportunity for case-recall bias)
- 2) Two important agricultural states (Iowa & North Carolina)- permitting us to evaluate consistency between states
- 3) Large cohort (89,658; Over one-million person-years of follow-up )
- 4) Little loss to cancer or mortality follow-up,
- 5) Licensed pesticide applicators (private & commercial applicators—regularly occupationally exposed, knowledgeable about their exposures).
- 6) Opportunity for ongoing exposure assessment to monitor changes in exposure and collection biospecimens over time

# Exposure Assessment

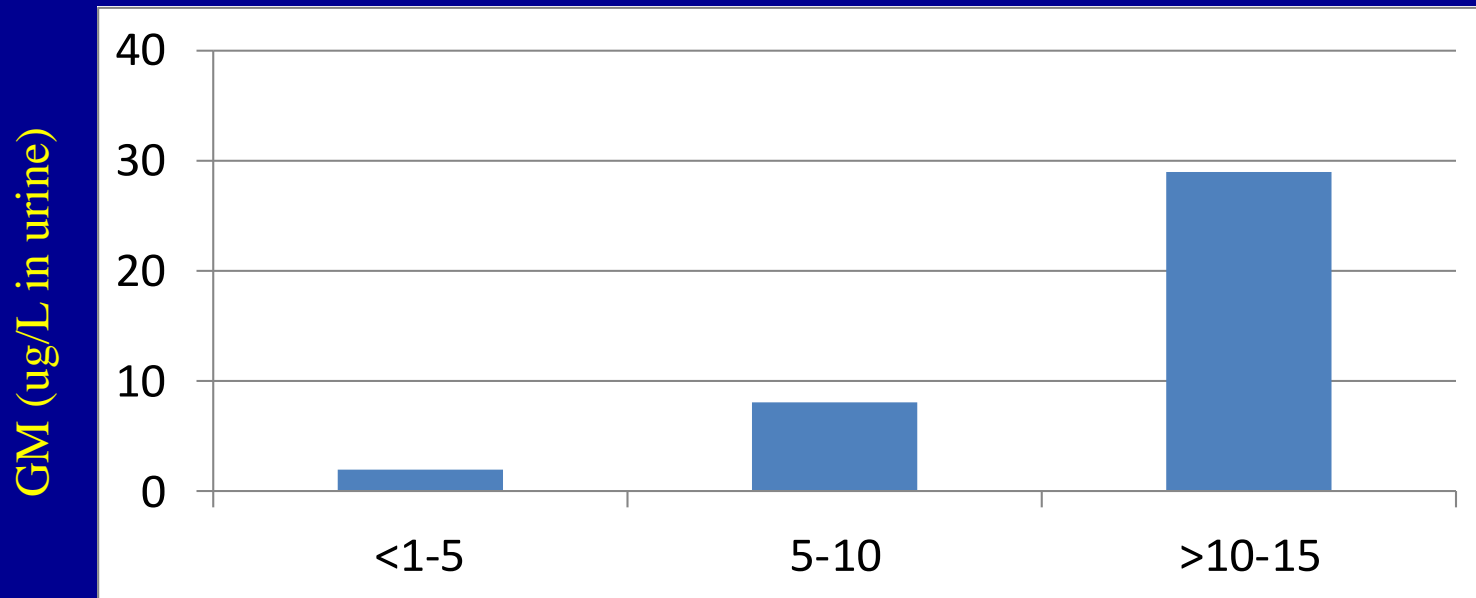


# EXPOSURE ASSESSMENT

1. Two chronic exposure metrics for long term exposures were developed
  - I. Lifetime days of pesticide use (years of use x days per year)
  
  - II. Lifetime intensity-weighted days of pesticide use (lifetime exposure days x intensity score)
  
2. Acute measure of intense event exposures (accidental spill, immersions, etc):
  - I. High Pesticide Exposure Events
  
  - II. High Pesticide Exposure Events with Symptoms
  
  - III. High pesticide Exposure Events with Symptoms and Medical Treatment



# Pesticide Concentrations Measured in Urine Samples (in ug/L) for Applicators Grouped by Algorithm Exposure Score (2,4-D)

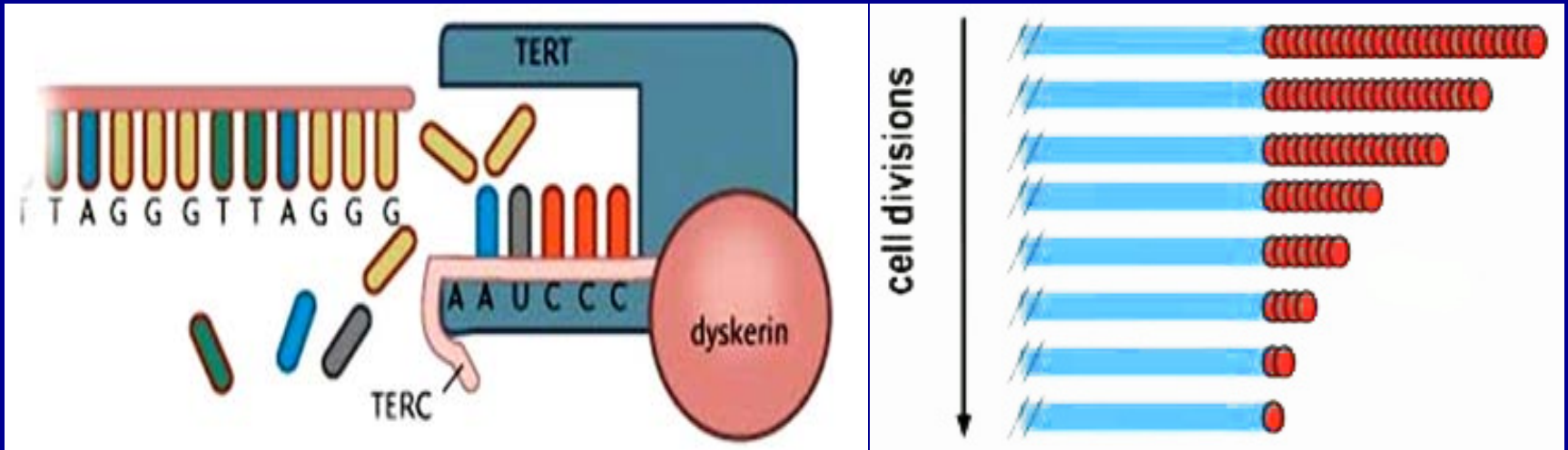
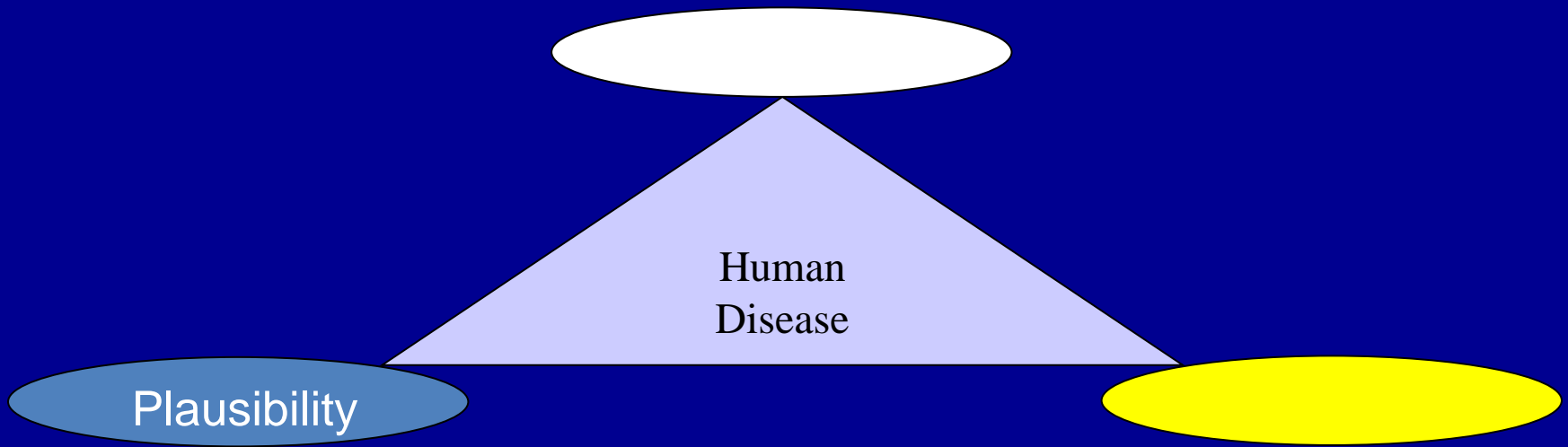


Algorithm Exposure Score (1-<5, 5-10, >10-15)

*Coble J. et al. (2005) J of Occupational and Environmental Hygiene. 2: 194-201.*

Exposure estimates from AHS questionnaires correlate well with field measurements of pesticide exposure

# Biologic Plausibility



# Biomarkers of Pesticide Exposure, Genetic Susceptibility, Oxidative Stress, DNA Damage, and Epigenetic Damage

Biomarkers	Analyte or enzyme activity assayed	Biological fluid/sample Used
<b>Pesticide Exposure</b>	<ol style="list-style-type: none"> <li>1. Pesticides and their metabolites</li> <li>2. Cholinesterase or OP-adducts</li> </ol>	<ol style="list-style-type: none"> <li>1. Urine, serum, plasma</li> <li>2. Blood</li> </ol>
	<ol style="list-style-type: none"> <li>1. Paraoxase 1 polymorphism</li> <li>2. Glutathione transferase, P450 polymor.</li> <li>3. Base-excision repair polymorphisms</li> <li>4. Nucleotide excision repair polymorphisms</li> <li>5. Other polymorphisms</li> </ol>	<ol style="list-style-type: none"> <li>1. Lipoproteins</li> <li>2. Blood lymphocytes</li> <li>3. Blood lymphocytes</li> <li>4. Blood lymphocytes</li> <li>5. Blood lymphocytes</li> </ol>
<b>Genetic Susceptibility</b>		

OP indicates organophosphate

# Biomarkers of Pesticide Exposure, Genetic Susceptibility, Oxidative Stress, DNA Damage, and Epigenetic Damage (continued)

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	Analyte or enzyme activity assayed	Biological fluid/sample Used
<b>Oxidative Stress</b>	<ol style="list-style-type: none"><li>1. Malondialdehyde, F2-isoprostanes</li><li>2. Catalase and SOD activities</li><li>3. 8-oxo or 8-OH-deoxyguanosine</li></ol>	<ol style="list-style-type: none"><li>1. Blood lymphocytes</li><li>2. RBC</li><li>3. Urine</li></ol>

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# Biomarkers of Pesticide Exposure, Genetic Susceptibility, Oxidative Stress, DNA Damage, and Epigenetic Damage (continued)

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	<b>Measure</b>	<b>Biological fluid/sample Used</b>
<b>Telomere length change</b>	<b>1. Relative Telomere Length</b>	<b>1. Buccal cell, Blood lymphocytes</b>

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# Biomarkers of Pesticide Exposure, Genetic Susceptibility, Oxidative Stress, DNA Damage, and Epigenetic Damage (continued)

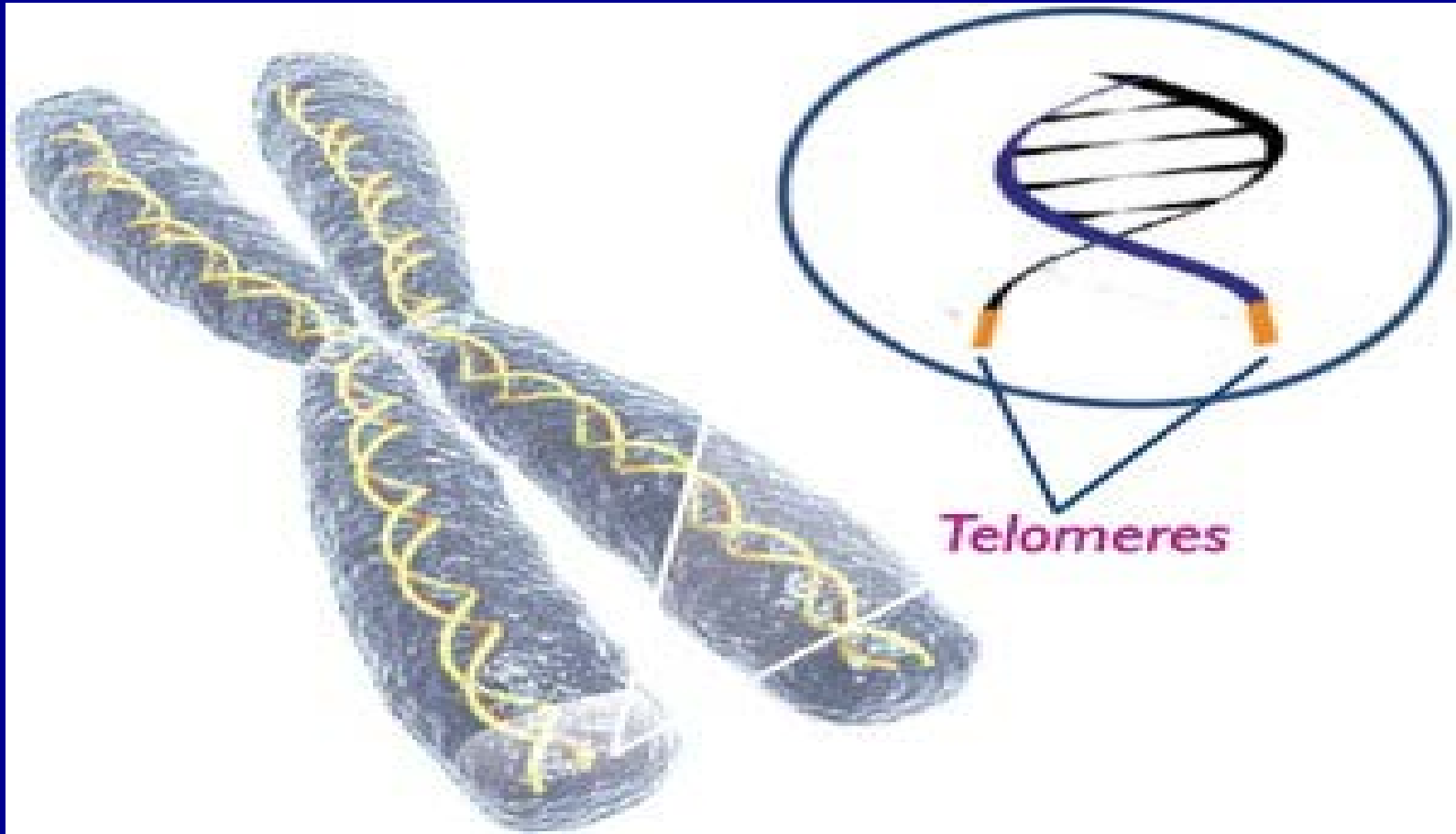
	Analyte or enzyme activity assayed	Biological fluid/sample Used
<b>DNA Damage</b>	<ol style="list-style-type: none"> <li>1. Alkaline comet assay, chromosomal aberration, sister chromatid exchange</li> <li>2. 8-oxo or 8-OH-deoxyguanosine</li> <li>3. "Challenge" assay (DNA repair phenotype)</li> </ol>	<ol style="list-style-type: none"> <li>1. Blood lymphocytes</li> <li>2. Urine</li> <li>3. Blood lymphocytes</li> </ol>
<b>Epigenetic</b>	<ol style="list-style-type: none"> <li>1. Gene specific hypermethylation</li> </ol>	<ol style="list-style-type: none"> <li>1. Blood lymphocytes.</li> </ol>

## Biomarkers of Pesticide Exposure, Genetic Susceptibility, Oxidative Stress, DNA Damage, and Epigenetic Damage (continued)

	<b>Precursor Lesions</b>	<b>Biological fluid/sample Used</b>
<b>Biologic Markers of Early Disease</b>	1. Monoclonal gammopathy of undetermined significance (MGUS) 2. Monoclonal B-cell lymphocytosis	1. Serum 2. Serum

Alavanja MCR, Ross MK, Bonner MR. CA: A Cancer J Clin; 2013;63:120-142.

# Biologic Plausibility-telomere shortening





# Buccal cell DNA

1. Buccal cells were collected from applicators from 1999-2006 using a mouthwash “swish and spit” technique (n>35,000)
2. DNA was extracted from 1,300 healthy participants who completed questionnaires on duration (years) and frequency (average days/year) of use for 48 pesticides

# Specific Pesticides and Telomere length

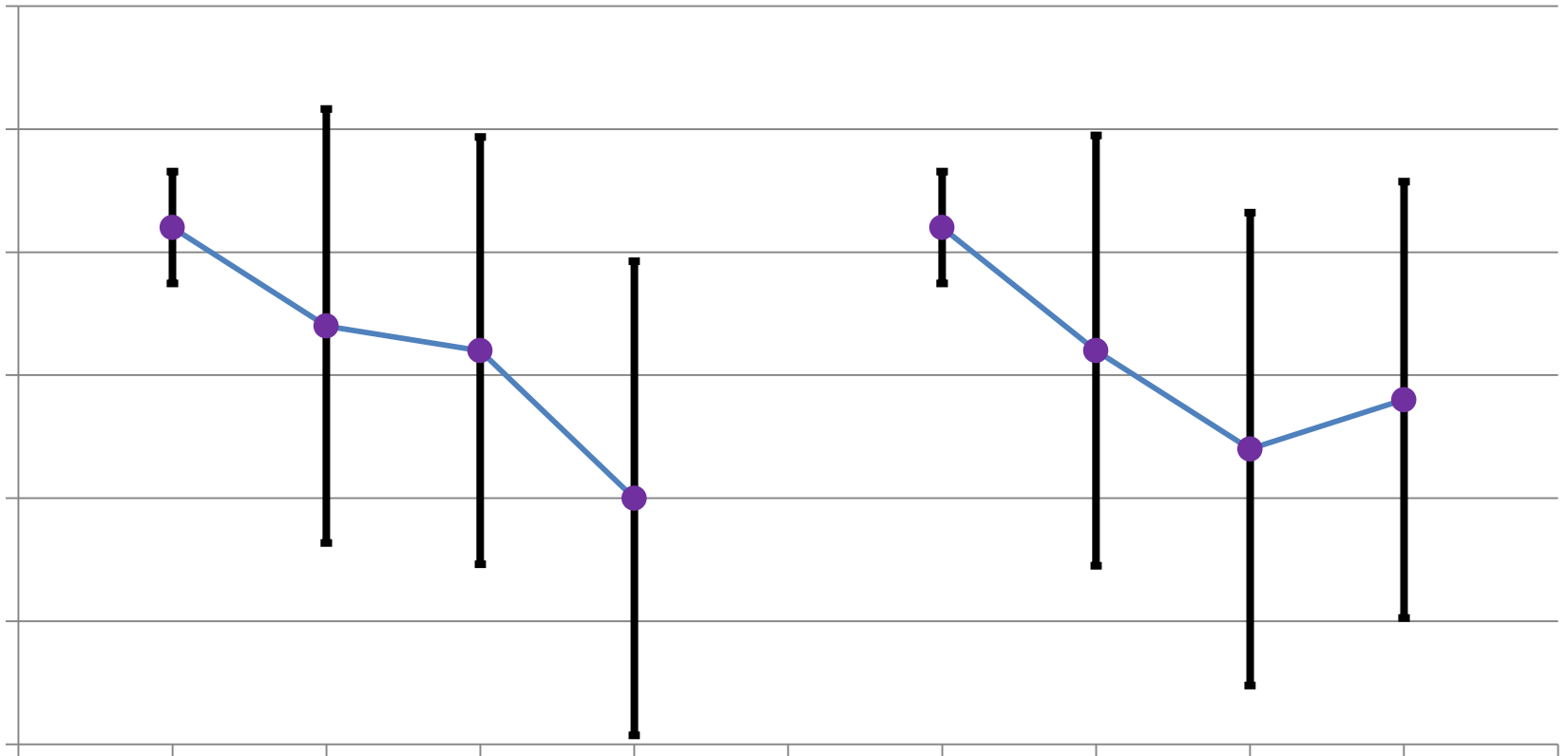
- Out of 48 pesticides examined, mean TL is inversely associated with 7 pesticide that has been previously linked to increased cancer risk:
- 4 herbicides: alachlor, metolachlor, trifluralin, and 2,4-D
- 3 insecticides: DDT, permethrin use, and toxaphene
- Other pesticides were also inversely association TL although not statistically significant (*Environ Health Perspect. Hou. L et al. 2013*)

# Pesticide (insecticide) Use and RTL

## Permethrin (poultry/livestock)

Lifetime Days

Lifetime Intensity-weighted Days

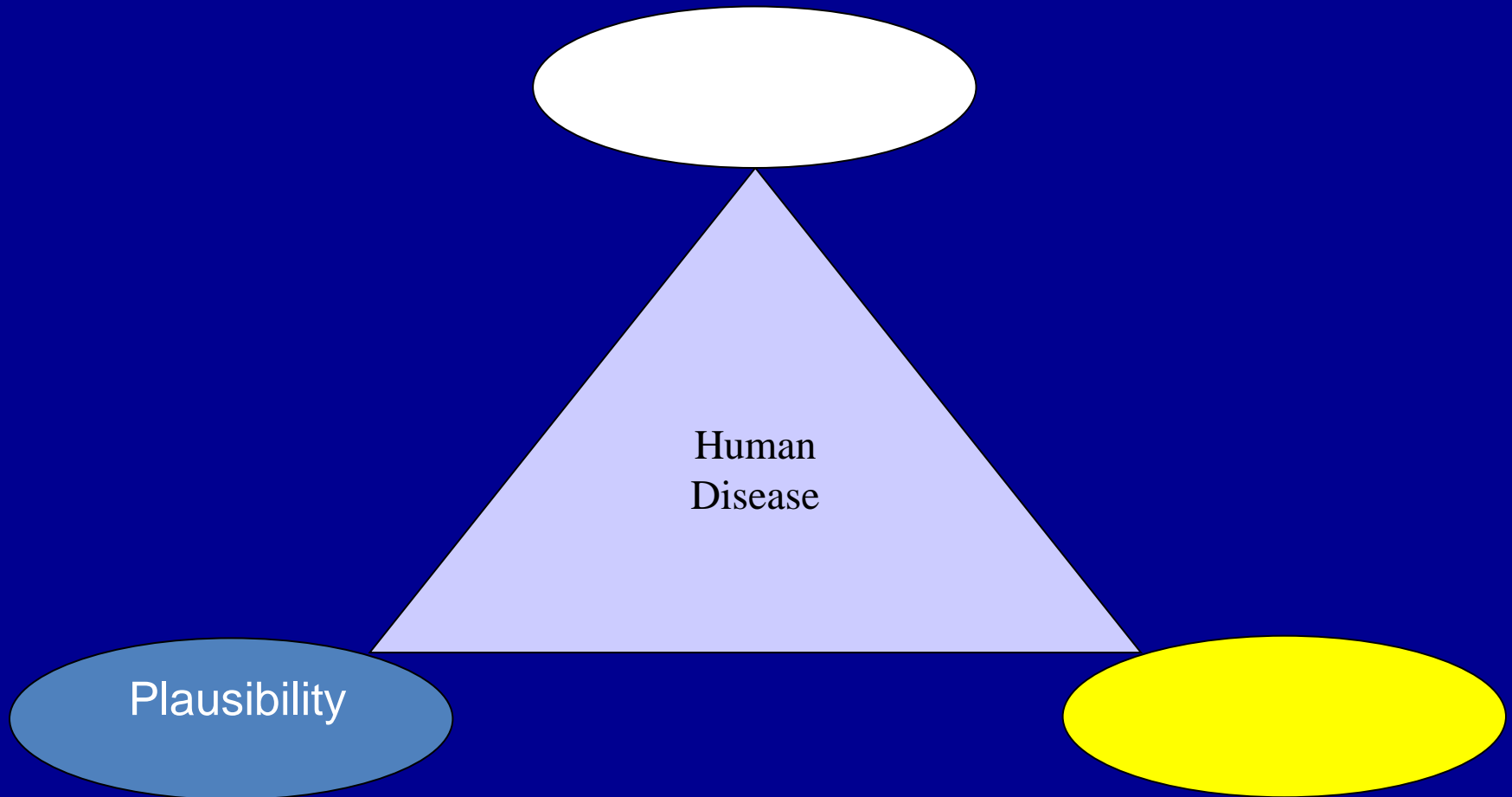


# Conclusion

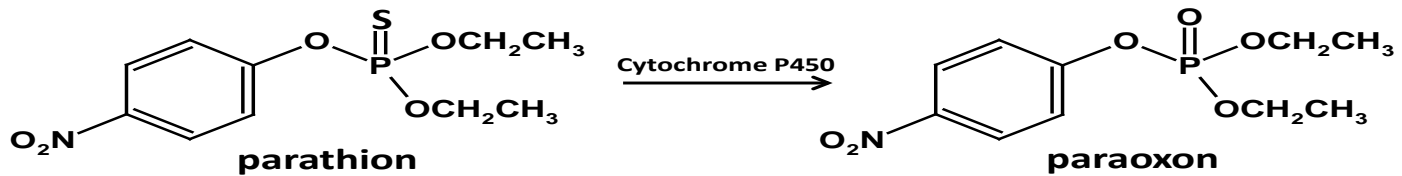
Specific pesticides may contribute to telomere shortening

Telomere shortening may serve as a mechanism for development of certain cancers

# Biologic Plausibility-genetic susceptibility (GXE)



Parathion is oxidized by cytochrome P450s to the reactive oxon metabolite, paraoxon.



# Follow-up prostate cancer study

## Chromosome 8q24 , terbufos exposure and prostate cancer risk

	No terbufos exposure	Low terbufos exposure	High terbufos exposure
Odds Ratio	1.13	1.71	2.15
95% C.I.	0.87-1.47	1.07-2.74	1.32-3.52

-Koutros, et al., *Cancer Research* 2010; 70(22):9224-9233

-previously identified variant rs4242382 [adjusted p-interaction=0.02]

-similar effect modification for **fonofos**, **coumaphos**, **phorate**, **permethrin**;

-fonofos, phorate, coumaphos and terbufos are phosphorodithioates /phosphorothioates

# GXE Prostate Cancer Observations

- **Observation:**  
Identified common specific genes that increase susceptibility to some pesticides.
  - 8q24
  - Base-excision repair
  - Nucleotide excision repair
  - Xenobiotic metabolizing
  - Lipid metabolizing
- **Follow-up:**  
Genetic testing not the answer. Control exposure is the answer.



# Biologic Plausibility-precursor conditions



# Multiple Myeloma

- A largely incurable neoplasm of plasma cells characterized by an overproduction of monoclonal immunoglobulins
- Etiology not well understood, occurs in excess among farmers (Milham S. Leukemia and multiple myeloma in farmers Am J Epidem 1971, 94(4):507-510 & Khuder SA, Mutgi AB. Meta-analyses of multiple myeloma and farming. Am J Ind Med. 1997 Nov; 32(5):510-516.)
- Highly fatal
- Monoclonal Gammopathy of Undetermined Significance (MGUS)-  
→ Multiple Myeloma

# Risk of MGUS in AHS vs. Olmstead County, MN

Population	Total, n	MGUS, n	OR (95% CI)
Olmstead County	9,469	350	1.0 (ref)
AHS cohort	555	38	1.9 (1.3-2.7)

-Landgren O et al., Blood (2009); 113(25):6386-6391

-Protein Immunology Laboratory at Mayo Clinic, Rochester, Minnesota  
(Robert Kyle, Jerry Katzmann, Vincent Rajkumar)

## Specific Pesticide Use at Enrollment and Risk of MGUS in 2008 Among 679 Male Applicators in the AHS

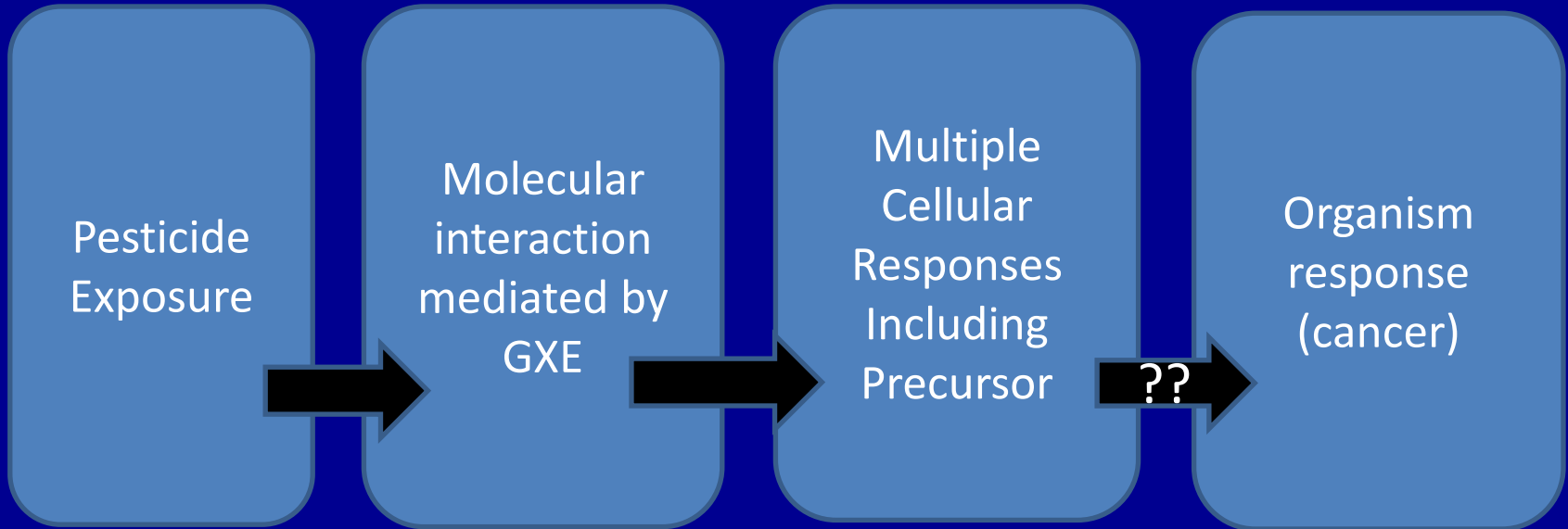
Pesticide	Exposed	Total n	Exposed n	OR (95% CI)
Dieldrin	Never	649	31	1.0 (ref)
	Ever	20	6	5.6 (1.9-16.6)
Carbon tetrachloride/ Carbon disulfide mix	Never	632	31	1.0 (ref)
	Ever	41	7	3.9 (1.5-10.0)
Chlorothalonil	Never	649	31	1.0 (ref)
	Ever	20	6	2.4 (1.1-5.3)

-Landgren O et al., Blood (2009); 113(25):6386-6391

-Protein Immunology Laboratory at Mayo Clinic, Rochester, Minnesota  
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# Adverse Outcome Pathways

- Initial Contributions from Epidemiology



Questions?

# Timeline for Hypothetical BEEA Participant

