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)

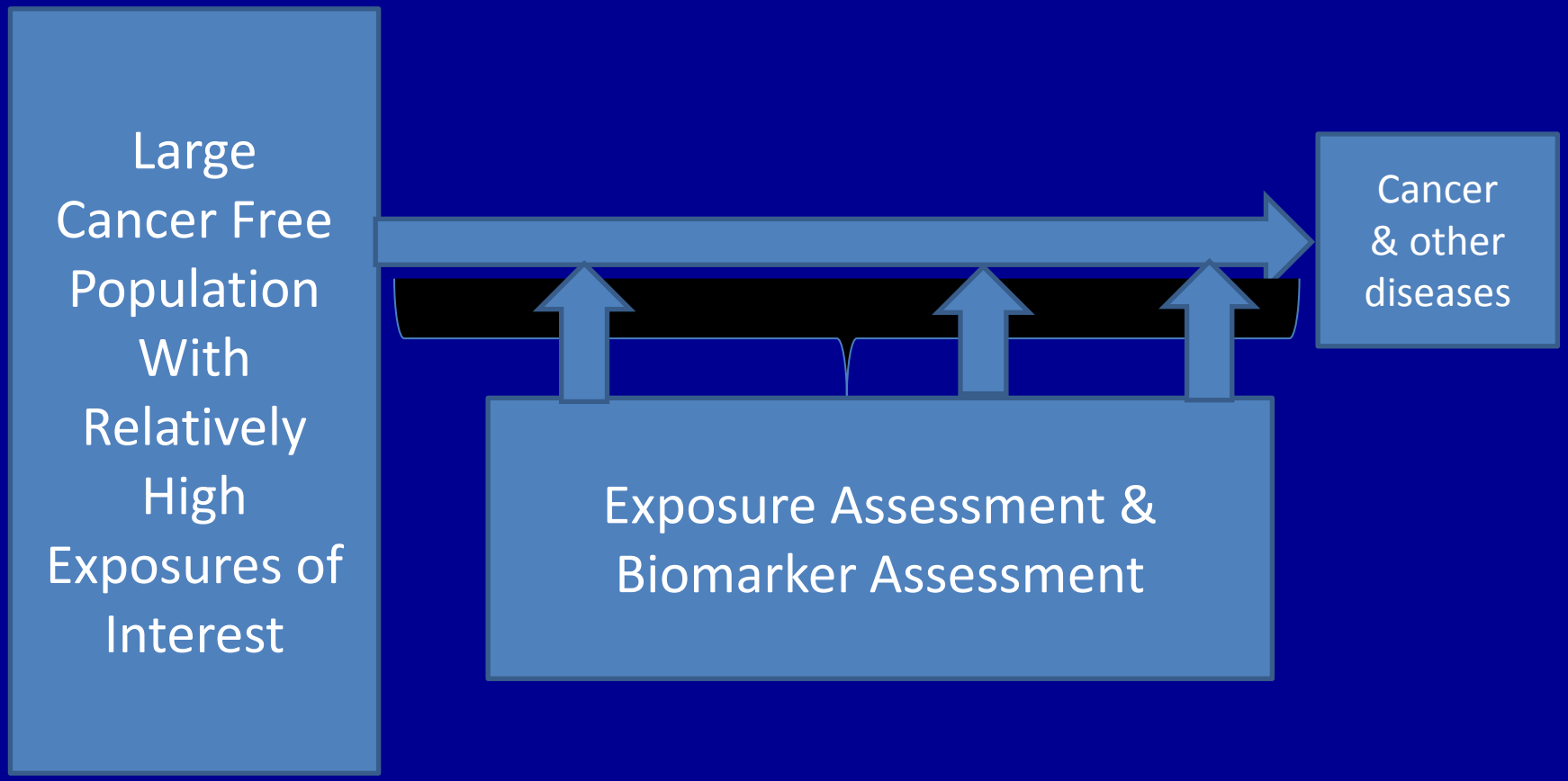
Inadequacy of Earlier Case-Control or Retrospective Studies

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

Prospective Study Design

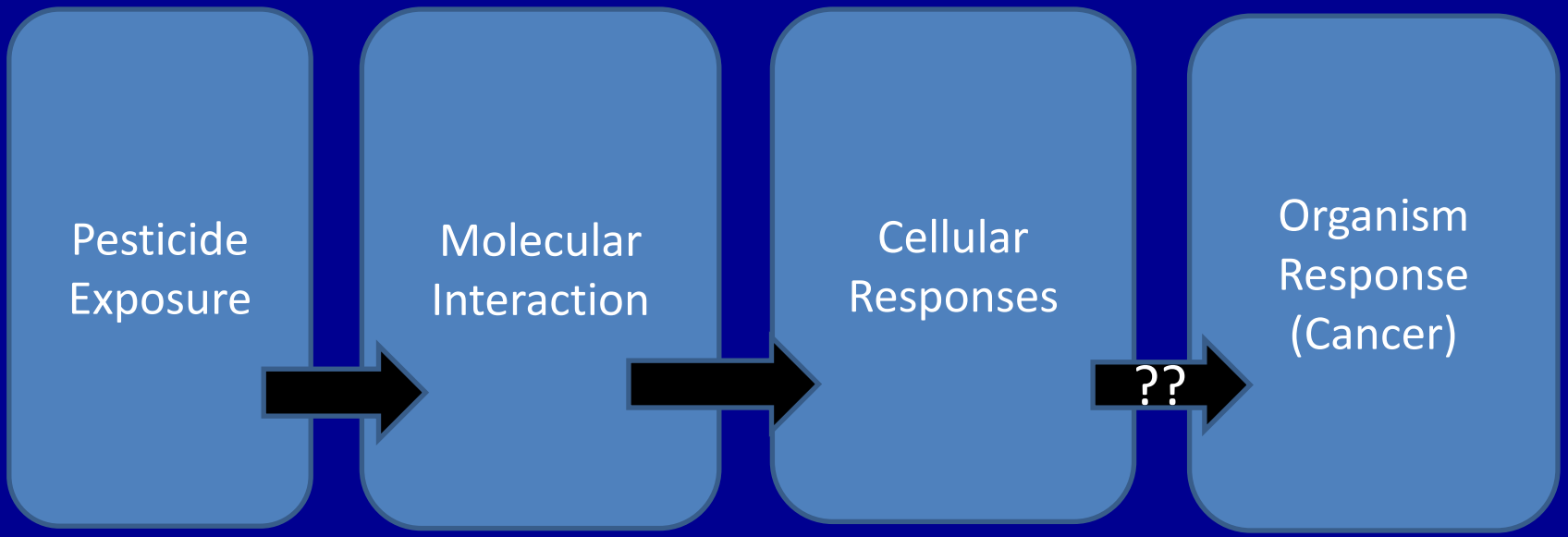
Prospective Occupational Epidemiologic Design

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

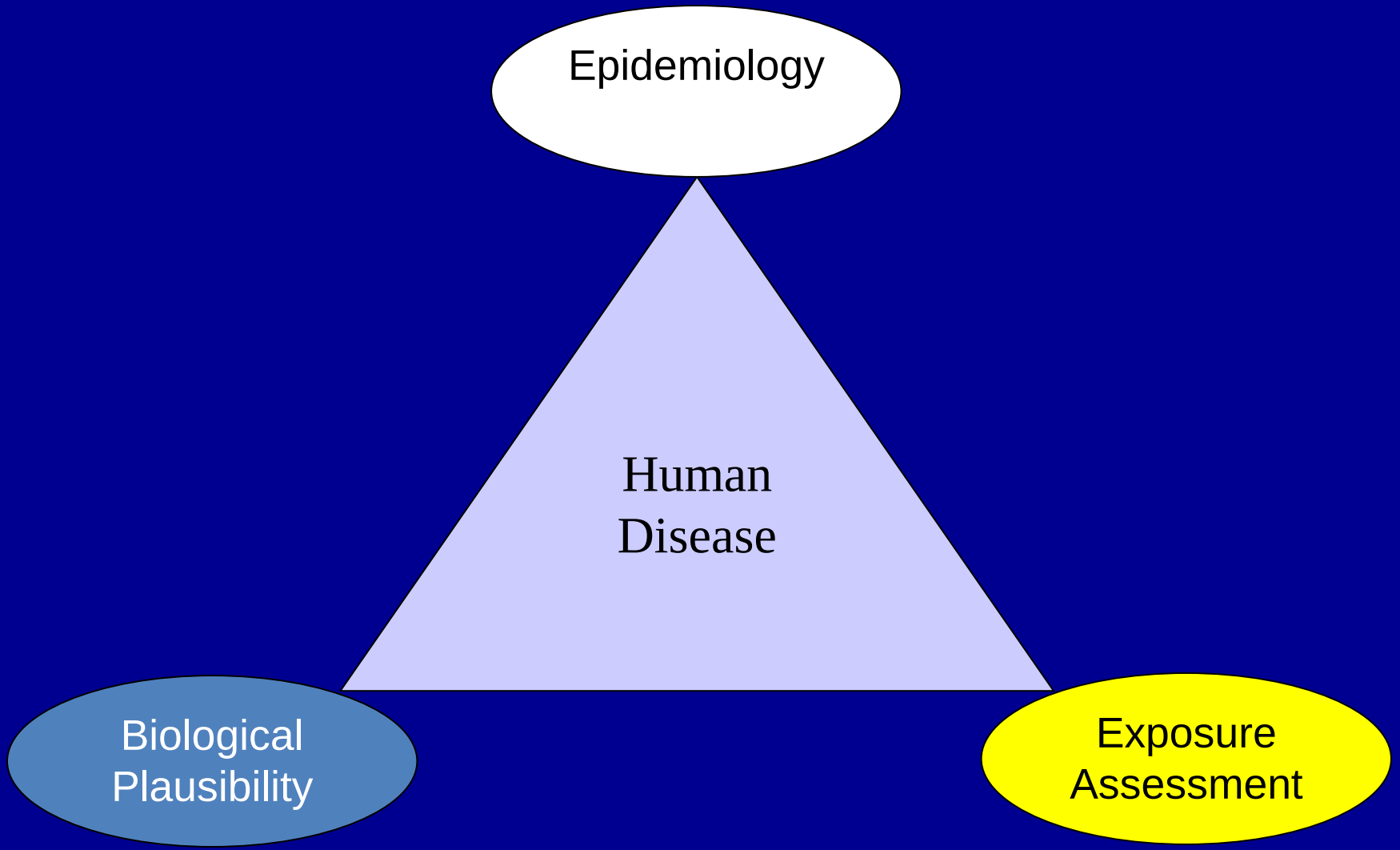


Adverse Outcome Pathways

- Potential Contributions from epidemiology



Logic to Establish Human Disease Associations In a Prospective Study

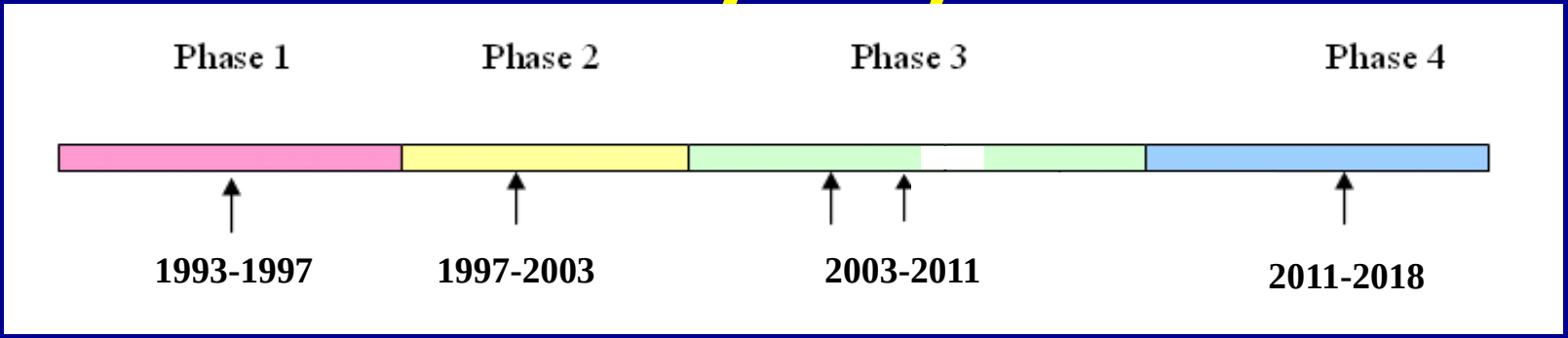


Agricultural Health Study (AHS)

- 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)

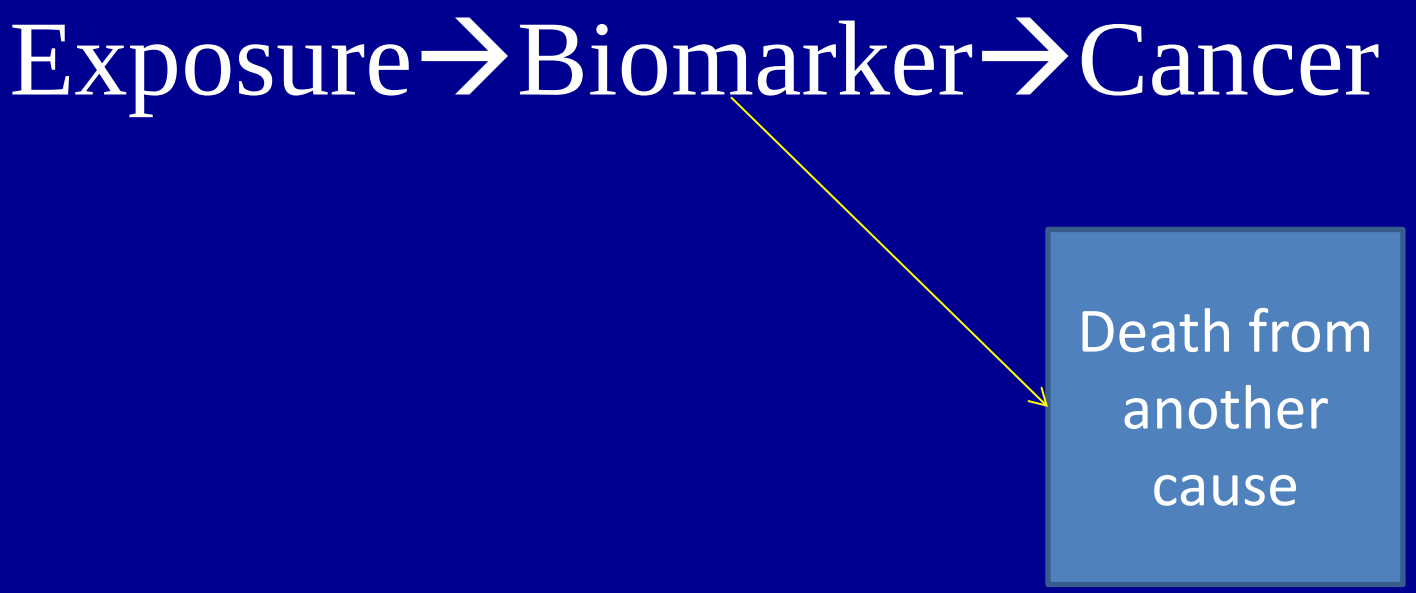


Exposure Assessment

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

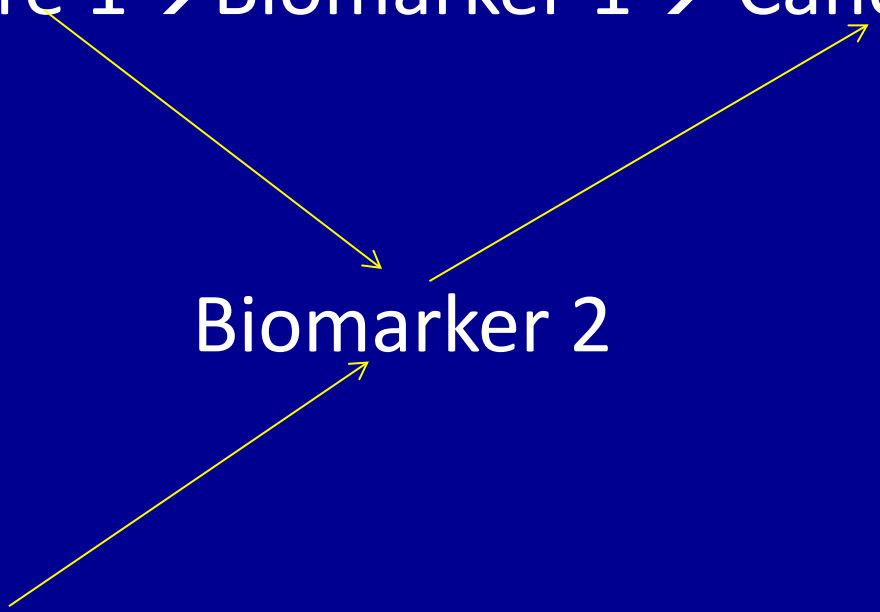
- **Phase 1**- Enrollment questionnaire (82% of target population of private pesticide applicators enrolled)
- **Phase 2**- Follow-up questionnaire, field validation of pesticide exposures, buccal cell collection for DNA, dietary questionnaire
- **Phase 3**- Second follow-up, blood collection in sub-studies,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

Simple Causal Pathway



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

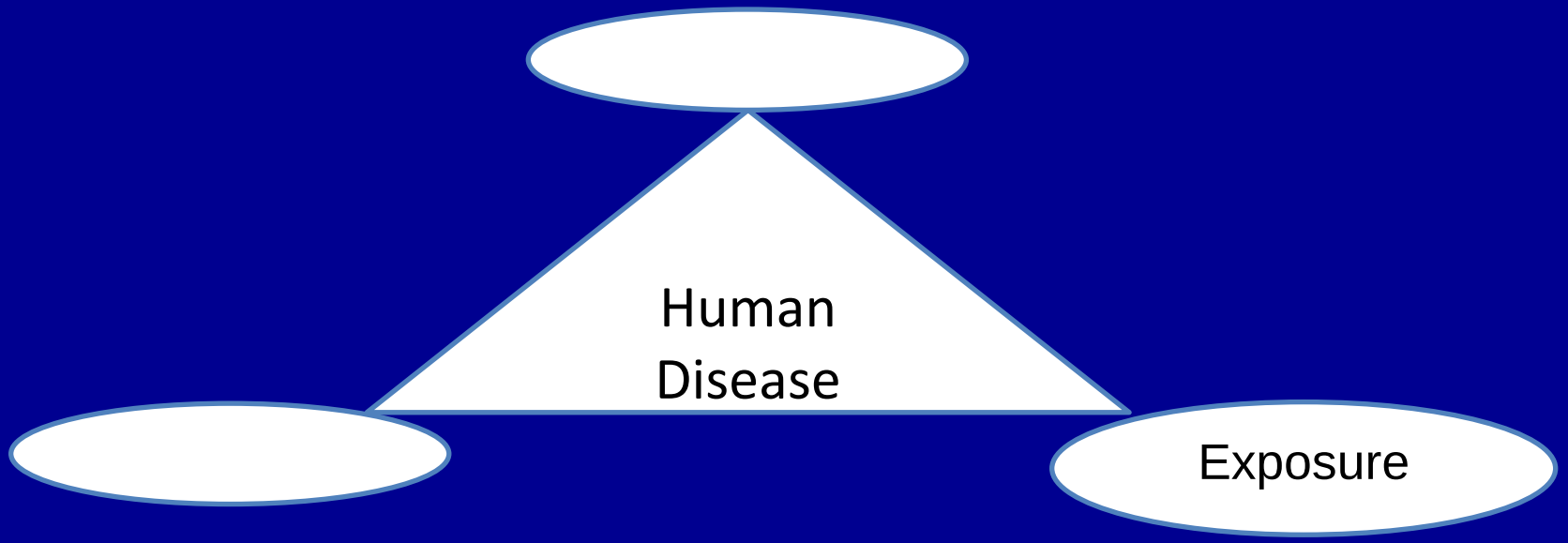
3) Large cohort (89,658; Over one-million person-years of follow-up)

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)

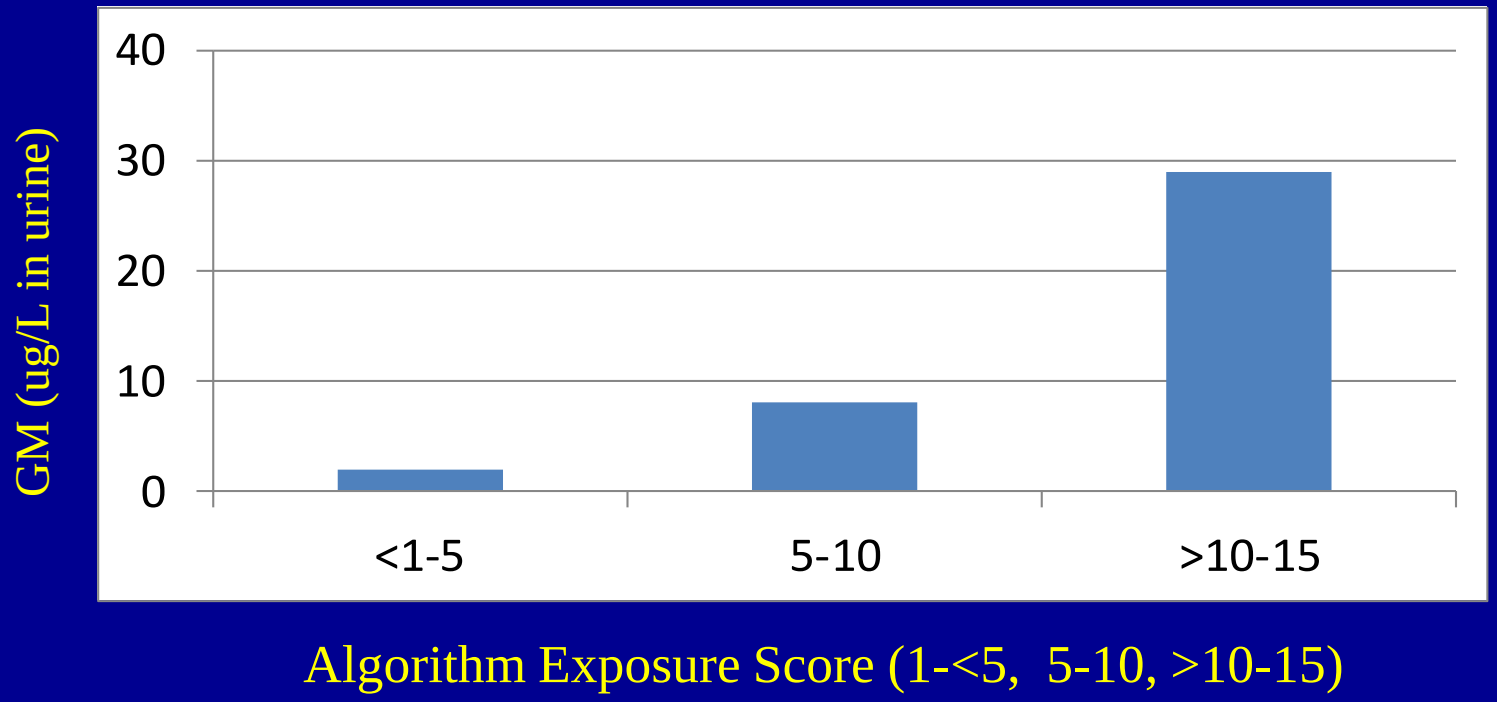
2. Acute measure of intense event exposures (accidental spill, immersions, etc):

   I. High Pesticide Exposure Events

   II. High Pesticide Exposure Events with Symptoms

   III. High pesticide Exposure Events with Symptoms and Medical Treatment

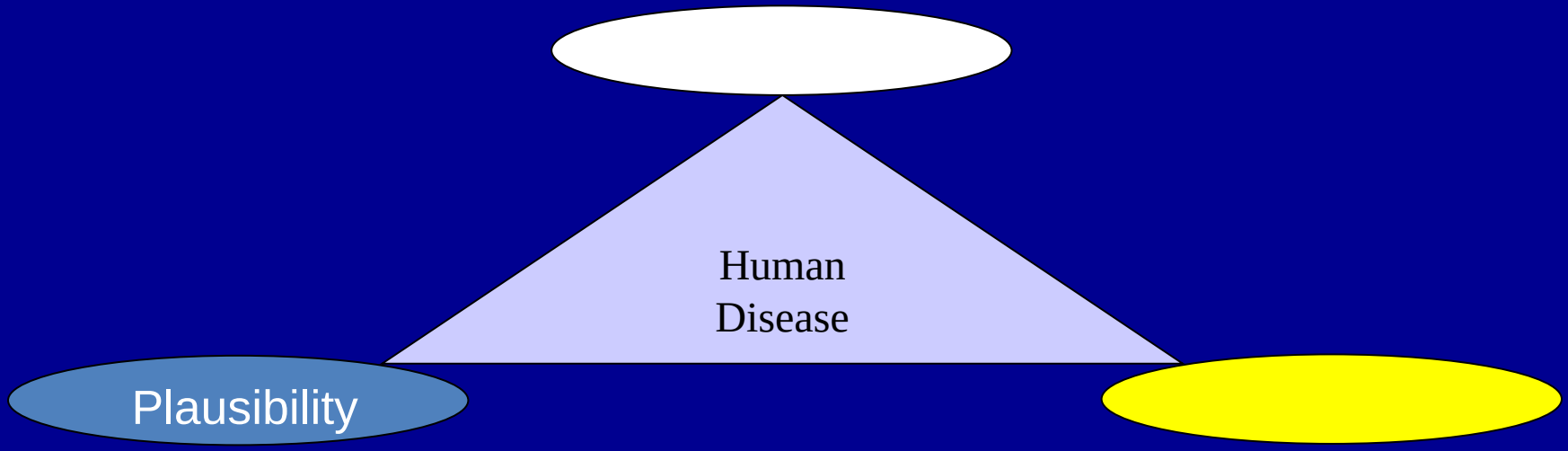
Pesticide Concentrations Measured in Urine Samples (in ug/L) for Applicators Grouped by Algorithm Exposure Score (2,4-D)

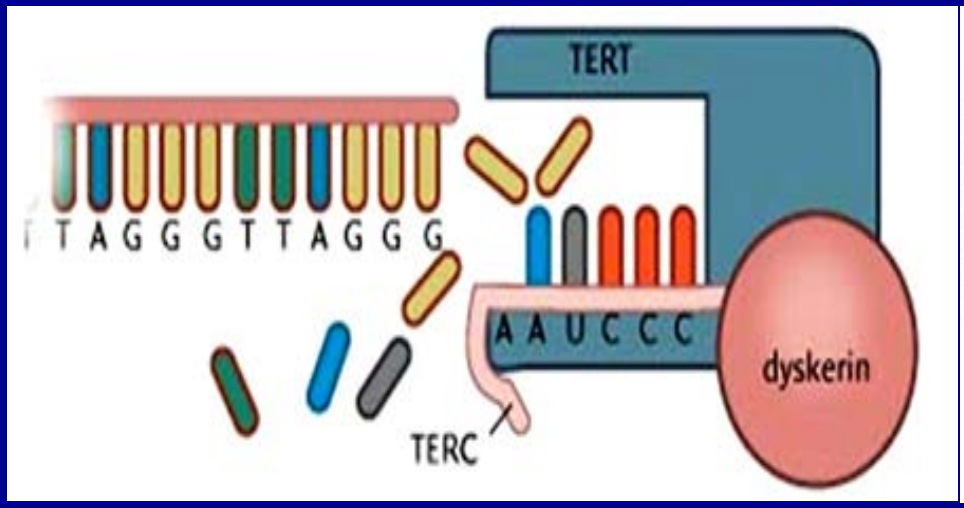


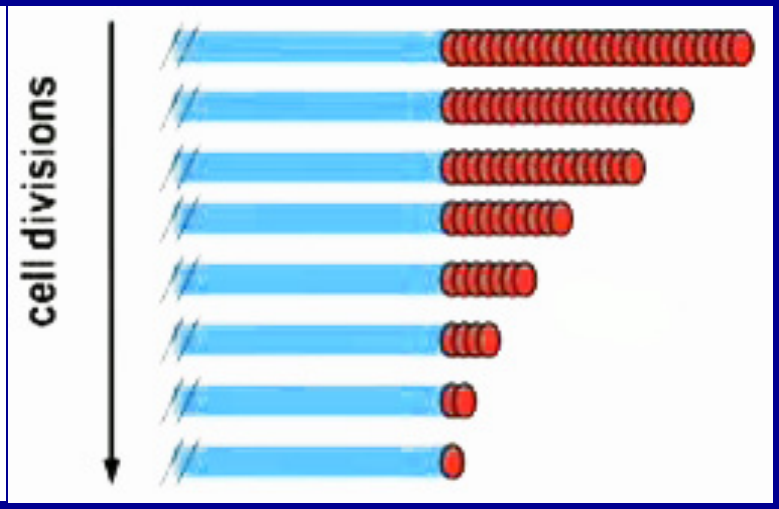
Coble J. et al. (2005) J of Occupational and Environmental Hygiene. 2: 194-201.

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
Pesticide Exposure	1. Pesticides and their metabolites 2. Cholinesterase or OP-adducts	1. Urine, serum, plasma 2. Blood
Genetic Susceptibility	1. Paraoxase 1 polymorphism 2. Glutathione transferase, P450 polymor. 3. Base-excision repair polymorphisms 4. Nucleotide excision repair polymorphisms 5. Other polymorphisms	1. Lipoproteins 2. Blood lymphocytes 3. Blood lymphocytes 4. Blood lymphocytes 5. Blood lymphocytes

OP indicates organophosphate

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

	Analyte or enzyme activity assayed	Biological fluid/sample Used
Oxidative Stress	1. Malondialdhehyde, F2-isoprostanes 2. Catalase and SOD activities 3. 8-oxo or 8-OH-deoxyguanosine	1. Blood lymphocytes 2. RBC 3. Urine

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

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

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

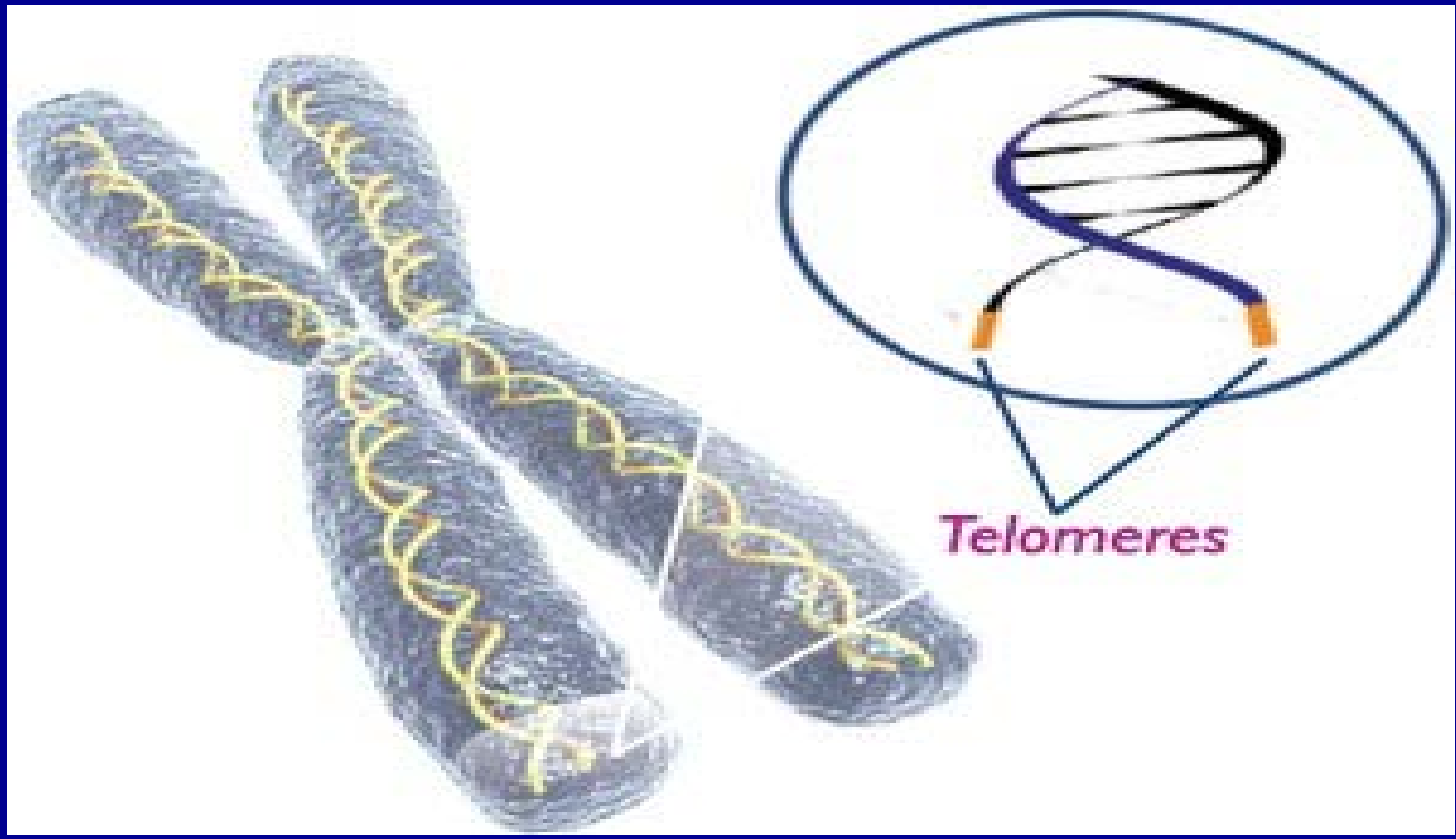
	Analyte or enzyme activity assayed	Biological fluid/sample Used
DNA Damage	1. Alkaline comet assay, chromosomal aberration, sister chromatid exchange 2. 8-oxo or 8-OH-deoxyguanosine 3. “Challenge” assay (DNA repair phenotype	1. Blood lymphocytes 2. Urine 3. Blood lymphocytes
Epigenetic	1. Gene specific hypermethylation	1. Blood lymphocytes.

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

	Precursor Lesions	Biological fluid/sample Used
Biologic Markers of Early Disease	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

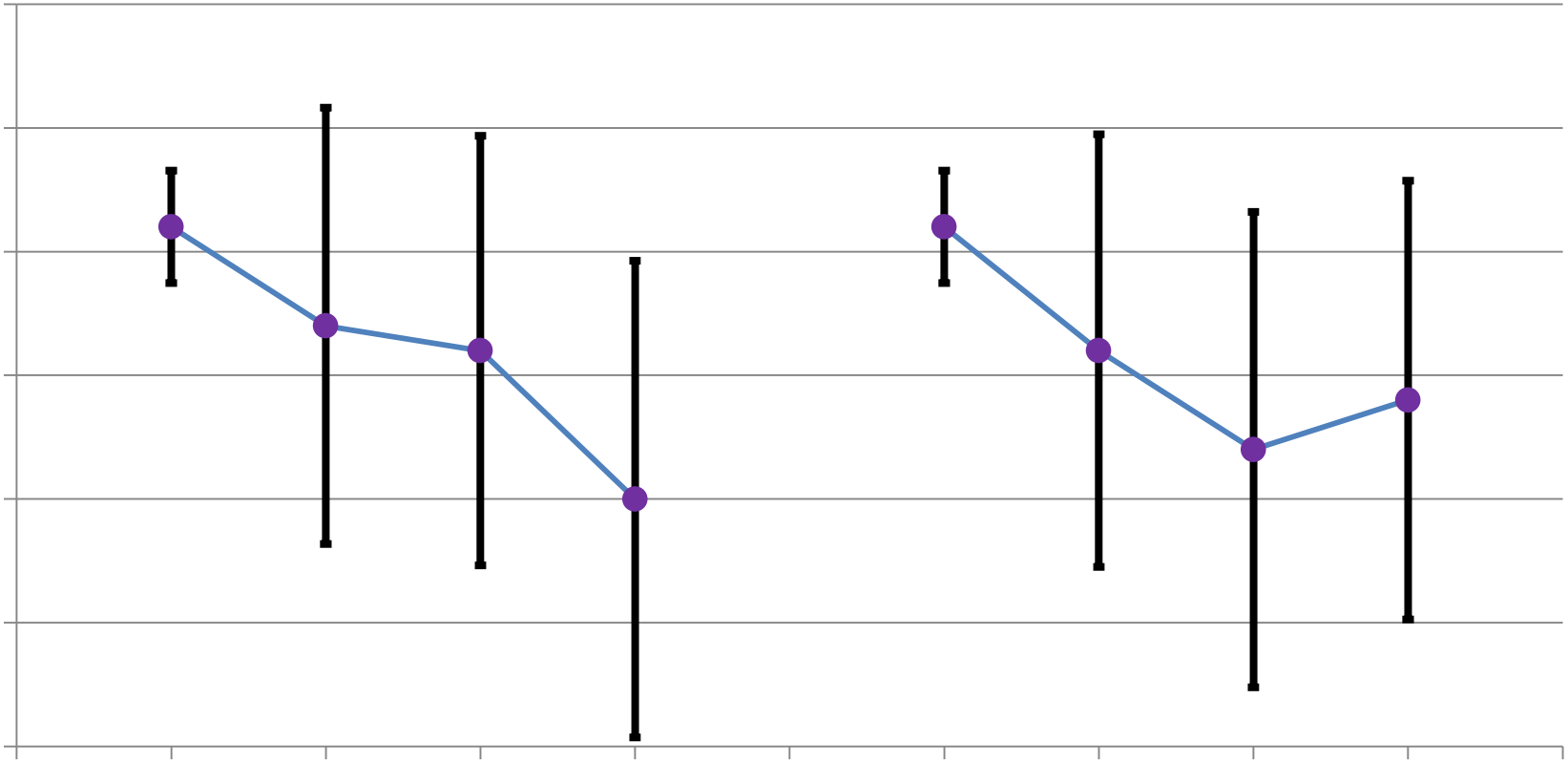
- Out of 48 pesticides examined, mean TL is inversely associated with 7 pesticide that has been previously linked to increased cancer risk:
- 4 herbicides: alachlor, metolachlor, trifluralin, and 2,4-D
- 3 insecticides: DDT, permethrin use, and toxaphene

- 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**

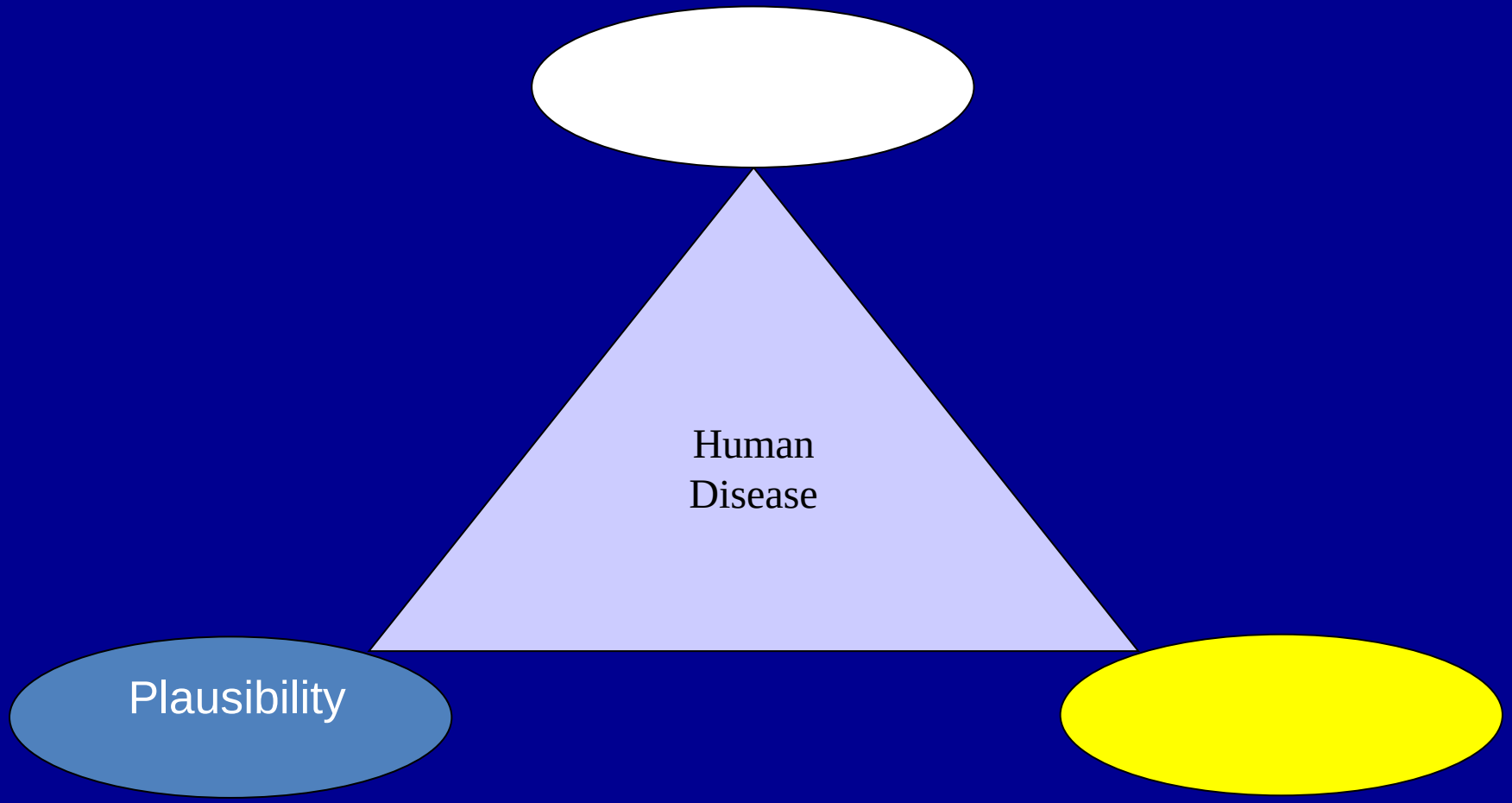


Conclusion

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

- Monoclonial 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)

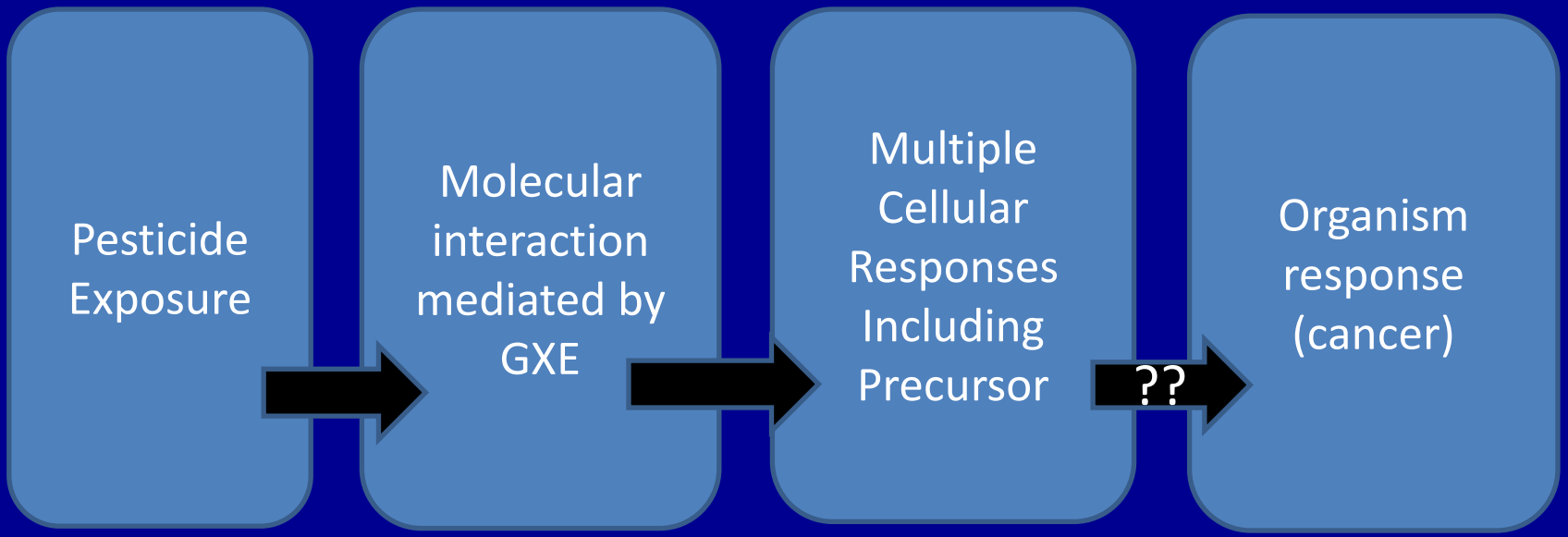
Specific Pesticide Use at Enrollment and Risk of MGUS in 2008 Among 679 Male Applicators in the AHS

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 (Robert Kyle, Jerry Katzmann, Vincent Rajkumar)

Adverse Outcome Pathways

- Initial Contributions from Epidemiology



Questions?

Timeline for Hypothetical BEEA Participant