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 $\rightarrow$ biomarker
  exposure $\rightarrow$ biomarker of effect $\rightarrow$ 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

Large Cancer Free Population With Relatively High Exposures of Interest

Exposure Assessment & Biomarker Assessment

Cancer & other diseases
Adverse Outcome Pathways

- Potential Contributions from epidemiology

Pesticide Exposure → Molecular Interaction → Cellular Responses → Organism Response (Cancer)
Logic to Establish Human Disease Associations In a Prospective Study

Epidemiology

Human Disease

Biological Plausibility

Exposure Assessment
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 $\rightarrow$ Biomarker 1 $\rightarrow$ Cancer

• 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

Human Disease

Exposure
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)

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

Human Disease

Plausibility

[Diagram showing molecular structures and cell divisions]
<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Analyte or enzyme activity assayed</th>
<th>Biological fluid/sample Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pesticide Exposure</strong></td>
<td>1. Pesticides and their metabolites</td>
<td>1. Urine, serum, plasma</td>
</tr>
<tr>
<td></td>
<td>2. Cholinesterase or OP-adducts</td>
<td>2. Blood</td>
</tr>
<tr>
<td>1. Paraoxase 1 polymorphism</td>
<td>1. Lipoproteins</td>
<td></td>
</tr>
<tr>
<td>2. Glutathione transferase, P450 polymor.</td>
<td>2. Blood lymphocytes</td>
<td></td>
</tr>
<tr>
<td>5. Other polymorphisms</td>
<td>5. Blood lymphocytes</td>
<td></td>
</tr>
<tr>
<td><strong>Genetic Susceptibility</strong></td>
<td></td>
<td></td>
</tr>
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<td>1. Paraoxase 1 polymorphism</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3. Base-excision repair polymorphisms</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Nucleotide excision repair polymorphisms</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Other polymorphisms</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Analyte or enzyme activity assayed</th>
<th>Biological fluid/sample Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidative Stress</strong></td>
<td></td>
</tr>
<tr>
<td>1. Malondialdehyde, F2-isoprostanes</td>
<td>1. Blood lymphocytes</td>
</tr>
<tr>
<td>2. Catalase and SOD activities</td>
<td>2. RBC</td>
</tr>
<tr>
<td>3. 8-oxo or 8-OH-deoxyguanosine</td>
<td>3. Urine</td>
</tr>
</tbody>
</table>
Biomarkers of Pesticide Exposure, Genetic Susceptibility, Oxidative Stress, DNA Damage, and Epigenetic Damage (continued)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Biological fluid/sample Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere length change</td>
<td>1. Relative Telomere Length</td>
</tr>
<tr>
<td></td>
<td>1. Buccal cell, Blood lymphocytes</td>
</tr>
</tbody>
</table>
### Biomarkers of Pesticide Exposure, Genetic Susceptibility, Oxidative Stress, DNA Damage, and Epigenetic Damage (continued)

<table>
<thead>
<tr>
<th>DNA Damage</th>
<th>Analyte or enzyme activity assayed</th>
<th>Biological fluid/sample Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alkaline comet assay, chromosomal aberration, sister chromatid exchange</td>
<td>1. Blood lymphocytes</td>
<td></td>
</tr>
<tr>
<td>2. 8-oxo or 8-OH-deoxyguanosine</td>
<td>2. Urine</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Epigenetic</th>
<th>Analyte or enzyme activity assayed</th>
<th>Biological fluid/sample Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Gene specific hypermethylation</td>
<td>1. Blood lymphocytes</td>
<td></td>
</tr>
</tbody>
</table>
### Precursor Lesions

<table>
<thead>
<tr>
<th>Biologic Markers of Early Disease</th>
<th>Biological fluid/sample Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Monoclonal gammopathy of undetermined significance (MGUS)</td>
<td>1. Serum</td>
</tr>
<tr>
<td>2. Monoclonal B-cell lymphocytosis</td>
<td>2. Serum</td>
</tr>
</tbody>
</table>

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)
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)

Human Disease

Plausibility
Parathion is oxidized by cytochrome P450s to the reactive oxon metabolite, paraoxon.
Follow-up prostate cancer study

<table>
<thead>
<tr>
<th>Chromosome 8q24 , terbufos exposure and prostate cancer risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>Odds Ratio</td>
</tr>
<tr>
<td>95% C.I.</td>
</tr>
</tbody>
</table>

-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


- Highly fatal

- Monoclonial Gammopathy of Undetermined Significance (MGUS) → Multiple Myeloma
## Risk of MGUS in AHS vs. Olmstead County, MN

<table>
<thead>
<tr>
<th>Population</th>
<th>Total, n</th>
<th>MGUS, n</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olmstead County</td>
<td>9,469</td>
<td>350</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>AHS cohort</td>
<td>555</td>
<td>38</td>
<td>1.9 (1.3-2.7)</td>
</tr>
</tbody>
</table>

-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

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Exposed</th>
<th>Total n</th>
<th>Exposed n</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dieldrin</td>
<td>Never</td>
<td>649</td>
<td>31</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td></td>
<td>Ever</td>
<td>20</td>
<td>6</td>
<td>5.6 (1.9-16.6)</td>
</tr>
<tr>
<td>Carbon tetrachloride/Carbon disulfide mix</td>
<td>Never</td>
<td>632</td>
<td>31</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td></td>
<td>Ever</td>
<td>41</td>
<td>7</td>
<td>3.9 (1.5-10.0)</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td>Never</td>
<td>649</td>
<td>31</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td></td>
<td>Ever</td>
<td>20</td>
<td>6</td>
<td>2.4 (1.1-5.3)</td>
</tr>
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Adverse Outcome Pathways

• Initial Contributions from Epidemiology

Pesticide Exposure → Molecular interaction mediated by GXE → Multiple Cellular Responses Including Precursor → Organism response (cancer)
Questions?
Timeline for Hypothetical BEEA Participant

30 years of exposure

1980
• 20 yrs old
• Began pesticide application

1994
• 34 yrs old
• Cancer free
• Phase I Questionnaire

1999
• 39 yrs old
• Cancer free
• Phase II Questionnaire

2004
• 44 yrs old
• Cancer free
• Phase III Questionnaire

2010
• 50 yrs old
• Cancer free: at BEEA screening
• Enrolled BEEA