Chronic Neurologic Effects of Pesticides: Results from the Agricultural Health Study

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Pesticides: Definition

- “A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.” (US EPA)
- Classify by use

- Classify by chemistry (insecticides)
  - Organophosphates
  - Carbamates
  - Pyrethroids
  - Organochlorines
Pesticide Products

- Formulations: active ingredients plus ‘inerts’
  - >16,000 formulations
  - >1,000 active ingredients
  - “Inerts” may also be neurotoxic
- 1.2 billion lb active ingredient used annually in US
  - 5.3 billion lb worldwide
- Occupational vs environmental exposure
  - Similar chemicals
  - Formulations or quantities may differ

Pesticide Neurotoxicity

- Insecticides designed to be neurotoxicants

- Acute high-level exposure is neurotoxic in humans
  - Symptoms are obvious and severe (poisoning)
- 1980s – 1990s: poisoning can have long-term sequelae
  - Direct effect of pesticides?
Questions

- Effects of chronic exposure without poisoning?
- Most research on organophosphates -- effects of other chemicals?
- Relevant aspects of exposure?
  - Duration? Intensity?
  - Application methods? Protective equipment?
  - Interaction with other exposures?
- Which outcomes are affected?

Overview

- Agricultural Health Study
  - Design
  - Exposure assessment

- Neurologic dysfunction and disease
  - Neurologic symptoms
  - Macular degeneration
  - Parkinson’s disease
The Agricultural Health Study
National Cancer Institute
Environmental Protection Agency
National Institute of Environmental Health Sciences

Phase 1
1993-1997

Private Applicators
Enrollment Q
(n~52,400, 84%)

Private Applicators
Supplemental Q
(n~23,000, 44%)

Spouses
Spouse Q
(n~32,300, 72%)

Phase 2
1999-2003

Private Applicators
Telephone Interview
(n~33,500, 64%)

Spouses
Telephone Interview
(n~23,800, 74%)
Characteristics of Cohort at Enrollment

<table>
<thead>
<tr>
<th></th>
<th>Applicators</th>
<th>Spouses</th>
</tr>
</thead>
<tbody>
<tr>
<td>State (% Iowa)</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td>Median age (years)</td>
<td>46</td>
<td>40</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>97</td>
<td>1</td>
</tr>
<tr>
<td>Race (% white, not Hispanic)</td>
<td>96</td>
<td>98</td>
</tr>
<tr>
<td>Education (% &gt;high school)</td>
<td>41</td>
<td>54</td>
</tr>
<tr>
<td>Smoking (% ever, lifetime)</td>
<td>47</td>
<td>28</td>
</tr>
<tr>
<td>Alcohol use (% ever, previous year)</td>
<td>65</td>
<td>46</td>
</tr>
<tr>
<td>Pesticide use (% ever, lifetime)</td>
<td>99</td>
<td>44</td>
</tr>
</tbody>
</table>

Exposure Assessment (Phase 1)

- General pesticide exposure
  - Ever use; duration, intensity
  - Application methods
  - Personal protective equipment
  - Acute high intensity exposure
  - Pesticide-related medical care; poisoning
- 50 specific pesticides
- Level of exposure variable within cohort
- Substudies using biomarkers validate questionnaire data
Neurologic Dysfunction and Disease

- Neurologic symptoms
- Macular degeneration
- Parkinson’s disease

Neurologic Symptoms

- Early evidence of neurologic dysfunction, before clinical signs are apparent
- Multiple functional domains
Symptom Study Design

- Cross-sectional study based on Phase 1 data
- Applicators who completed take-home questionnaire (n=18,782)
- Outcome: multiple symptoms in year before enrollment
  - Cases: ≥10 symptoms (20%)
  - Controls: <10 symptoms (80%)
- Referent for exposure: internal comparison
  - More exposed vs less exposed applicators


High-Exposure Events

<table>
<thead>
<tr>
<th>Event Description</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>Adjusted OR * (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever had event involving high personal exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No event</td>
<td>76</td>
<td>88</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Mainly dermal exposure</td>
<td>13</td>
<td>8</td>
<td>1.8 (1.6-2.0)</td>
</tr>
<tr>
<td>Inhalation, ingestion</td>
<td>11</td>
<td>4</td>
<td>3.0 (2.7-3.5)</td>
</tr>
</tbody>
</table>

* Adjusted for age, state, education, cigarette smoking and alcohol use
### Pesticide-Related Medical Care

<table>
<thead>
<tr>
<th></th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>Adjusted OR <em>(95% CI)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever sought medical care for pesticide-related illness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>89</td>
<td>95</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Doctor visit</td>
<td>9</td>
<td>4</td>
<td>2.3 (2.0-2.6)</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>2</td>
<td>1</td>
<td>2.0 (1.4-2.7)</td>
</tr>
<tr>
<td>Ever diagnosed with pesticide poisoning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>96</td>
<td>98</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td>2</td>
<td>2.5 (2.0-3.1)</td>
</tr>
</tbody>
</table>

* Adjusted for age, state, education, cigarette smoking, and alcohol use

### Insecticide Use

![Graph showing the relationship between lifetime days of use and odds ratio](image-url)
### Specific Chemicals

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Number evaluated</th>
<th>Number with OR &gt; 1.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphates</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Organochlorines</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Carbamates</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Fungicides</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Fumigants</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Herbicides</td>
<td>18</td>
<td>3</td>
</tr>
</tbody>
</table>

### Application Methods

<table>
<thead>
<tr>
<th>Level of Exposure</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>Adjusted OR * (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crop insecticides – No</td>
<td>19</td>
<td>20</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Low methods only</td>
<td>58</td>
<td>60</td>
<td>1.0 (0.9-1.1)</td>
</tr>
<tr>
<td>Low and high</td>
<td>23</td>
<td>20</td>
<td>1.4 (1.2-1.6)</td>
</tr>
<tr>
<td>Herbicides – No</td>
<td>4</td>
<td>6</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Low methods only</td>
<td>21</td>
<td>31</td>
<td>0.9 (0.7-1.1)</td>
</tr>
<tr>
<td>Low and high</td>
<td>74</td>
<td>63</td>
<td>1.5 (1.2-1.8)</td>
</tr>
<tr>
<td>Fungicides – No</td>
<td>70</td>
<td>73</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Low methods only</td>
<td>16</td>
<td>14</td>
<td>1.3 (1.2-1.4)</td>
</tr>
<tr>
<td>Low and high</td>
<td>14</td>
<td>13</td>
<td>1.4 (1.3-1.6)</td>
</tr>
</tbody>
</table>

* Adjusted for age, state, education, cigarette smoking, and alcohol use
Summary

- Cumulative lifetime use of insecticides associated with increased risk of having ≥10 symptoms in prior year
- Strong and consistent effects for organophosphates and organochlorines
- Accounting for recent pesticide use did not change association with cumulative use
- Effects present in applicators with no history of pesticide poisoning or high exposure events
- Use of high exposure application methods associated with increased risk

Age-Related Macular Degeneration (AMD)

- Leading cause of blindness in older adults in the developed world
- Risk associated with genetic polymorphisms
- Environmental risk factors: smoking
Retinal Degeneration and Pesticides

- **Rationale:** Animal studies suggest organophosphate exposure damages retina
- **Cross-sectional study (Phase 1)**
- **Case definition:** self-reported physician diagnosis of retinal or macular degeneration
- **Applicators:** 154 cases, 17,804 controls (Kamel et al 2000)
  - Fungicides OR = 1.8 (1.3-2.6)
  - Organophosphates OR = 1.6 (0.9-2.9)
  - Organochlorines OR = 1.5 (1.1-2.2)
  - Carbamates OR = 1.6 (1.1-2.4)
- **Spouses:** 281 cases, 29,657 controls (Kirrane et al 2005)
  - Fungicides OR = 1.9 (1.2-3.1)

Genes, Environment, and AMD

- **Case-control study of incident cases (Phase 2 and 3)**
  - Exclude cases from Phase 1
  - Applicators and spouses
- **Confirm self-reported macular degeneration using physician questionnaire and retinal fundus photographs**
- **Controls:** remaining cohort
- **Exposure data from Phase 1 (prospective)**
- **Field work completed:** ~160 confirmed cases

Montgomery et al, in progress
### AMD and Pesticides

<table>
<thead>
<tr>
<th></th>
<th>Adjusted OR * (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
</tr>
<tr>
<td>Organochlorines</td>
<td>2.2 (1.3-3.8)</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>2.6 (1.1-6.1)</td>
</tr>
<tr>
<td>Carbamates</td>
<td>1.2 (0.7-1.9)</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td>1.4 (0.8-2.4)</td>
</tr>
<tr>
<td>Fungicides</td>
<td>1.4 (0.9-2.2)</td>
</tr>
<tr>
<td>Fumigants</td>
<td>1.0 (0.6-1.6)</td>
</tr>
<tr>
<td>Herbicides</td>
<td>NC</td>
</tr>
</tbody>
</table>

* Adjusted for age, state and smoking. NC, not calculated.

### Parkinson’s Disease (PD)

- Progressive neurodegenerative disease affecting movement
- Cardinal signs: tremor, slow movement, rigidity, postural instability
- Symptoms: stooped posture, shuffling gait, soft voice, small handwriting
- Genetic variants in early onset PD
- Environmental risk factors: pesticides
PD and Pesticides

- Why pesticides?
  - Rural residence, farming associated with PD
  - MPTP causes parkinsonism -- structurally similar to paraquat

- Over 50 epidemiologic studies of pesticides and PD
  - Meta-analysis: relative risk ~1.9
- Animal and mechanistic studies
- Data sparse, lacking detail

PD and Pesticides in AHS

- Case-control study of incident PD
- Case definition: self-report of physician diagnosis
  - Exclude those with PD in Phase 1
  - Incident PD in Phase 2 (n = 78 )
  - Applicators and spouses
- Controls: remaining Phase 2 cohort (n = 55,931)
- Exposure data from Phase 1 (prospective)
## PD and High Intensity Exposure

<table>
<thead>
<tr>
<th>High exposure event</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>84</td>
<td>85</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Washed within 1 hour</td>
<td>6</td>
<td>9</td>
<td>1.1 (0.3-4.7)</td>
</tr>
<tr>
<td>Washed after 1 hour</td>
<td>10</td>
<td>6</td>
<td>1.7 (0.5-5.9)</td>
</tr>
</tbody>
</table>

* Adjusted for age and state

## PD and Cumulative Lifetime Use

<table>
<thead>
<tr>
<th>Days of use</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-64</td>
<td>28</td>
<td>47</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>65-200</td>
<td>14</td>
<td>16</td>
<td>1.2 (0.5-2.6)</td>
</tr>
<tr>
<td>201-396</td>
<td>23</td>
<td>18</td>
<td>1.7 (0.8-3.5)</td>
</tr>
<tr>
<td>397-7000</td>
<td>35</td>
<td>19</td>
<td>2.3 (1.2-4.5)</td>
</tr>
</tbody>
</table>

* Adjusted for age, state, and person
Farming and Movement Evaluation Study

- Collaboration with Carlie Tanner (Parkinson’s Institute)
- Address limitations of AHS analysis
  - Self-reported diagnosis
- Nested case-control study
- Diagnosis confirmed by neurologist
- Telephone interview for additional exposure data
- Fieldwork complete
  - N=115 cases, 384 controls
- Data analysis in progress

Summary

- AHS provides unique and powerful setting to study neurologic effects of pesticide exposure
- Address fundamental questions
  - Which outcomes are affected
  - Relevant aspects of exposure
  - Role of modifying factors
- Chronic moderate exposure has neurologic effects in humans
ALS and Lead Exposure

- Long-standing hypothesis, but data are sparse
- New England ALS Study
  - Case-control study, 1993-1996
  - 110 cases and 39 controls (men and women)
  - Blood and bone lead levels
- Veterans with ALS and Lead Exposure (VALE)
  - Case-control study, 2007-2008
  - Based on the National Registry of Veterans with ALS
  - 184 cases and 194 controls (veterans)
  - Blood lead levels
  - Bone turnover biomarkers

New England ALS Study
Blood and Bone Lead Levels

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>ALS Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.9 (1.4-2.6)</td>
<td>3.6 (0.6-21)</td>
</tr>
<tr>
<td>Patella</td>
<td>15 (10-20)</td>
<td>23 (15-25)</td>
</tr>
<tr>
<td>Tibia</td>
<td>10 (5-15)</td>
<td>12 (8-16)</td>
</tr>
</tbody>
</table>

Kamel et al 2002
Veterans with ALS and Lead Exposure (VALE)
Blood Lead Levels

- Overall OR 1.9 (1.3-2.7)
- Stratified by CTX
  \[\leq\text{median}: 2.8 (1.4-5.5)\]
  \[>\text{median}: 1.6 (1.1-2.4)\]
- Stratified by ALAD genotype
  - ALAD1-1: 2.0 (1.3-2.9)
  - ALAD2-x: 1.2 (0.4-3.1)

Fang et al, under review

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Acknowledgments

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  - Duke University: Silke Schmidt
## Symptoms Studied in AHS

<table>
<thead>
<tr>
<th>Domain</th>
<th>Symptoms (% in year before enrollment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affect</td>
<td>Anxiety (52), irritability (37), depression (27)</td>
</tr>
<tr>
<td>Cognition</td>
<td>Memory (24), concentration (20)</td>
</tr>
<tr>
<td>Sensory</td>
<td>Numbness (27), night vision (12), blurred vision (10), smell/taste (6)</td>
</tr>
<tr>
<td>Motor</td>
<td>Twitches (17), weakness (15), balance (12), tremor (11), speech (4)</td>
</tr>
<tr>
<td>Autonomic</td>
<td>Nausea (27), appetite (18), sweating (17), heart rate (15)</td>
</tr>
<tr>
<td>Other</td>
<td>Headache (68), fatigue (58), insomnia (43), dizziness (28), loss of consciousness (2)</td>
</tr>
</tbody>
</table>