Pesticides, Agent Orange and Parkinson's Disease

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Director SW PADRECC and UCLA Movement Disorders
Epidemiology Studies

- Study disease clusters
  - Small population
- Case-control and Population-based studies
  - Larger population
- Prospective studies
  - Huge population
The MPTP Story

- 1976: Barry Kidston in Maryland acute Parkinsonism trying to make MPPP (an analogue of Demerol).
- 1982: 4 patients in Santa Clara with acute Parkinsonism. MPTP discovered to be the cause.
How MPTP causes Parkinsonism

MAO-B

MPTP → MPP⁺ → DAT → MPP⁺
MPTP AND STRUCTURALLY-RELATED COMPOUNDS

MPTP
MPP+ (CYPERQUAT)
PARAQUAT
4-PHENYL PYRIDINE
Farming, Pesticides, and PD

- Most studies have found positive associations.
  - Farming: OR 1.4 (95% CI = 1.1-1.9)
  - Well water: OR 1.3 (95% CI = 0.9-1.6)
  - Professional pesticide use: OR 1.9 (95% CI = 1.5-2.5)

Elbaz and Tranchant. 2007
Pesticide Use and PD

Brown et al. 2006
Pesticides and PD

- Associations found in 4 continents (North America, Europe, Asia, Australia).
- Appears to be independent of farming

**BUT**

- Methodological concerns
  - Recall bias
  - Control population
- Problems identifying individual agents
Honolulu-Asia Aging Study

- Prospective cohort of 8006 Japanese-American men born 1900-1919
- Years on plantation assessed in 1965
- Self report on pesticide exposure (occupation and home exposure) assessed in 1971
- 116 incident cases

Addressing Recall Bias: Prospective Cohort Study
Age-adjusted Incidence of PD* by Years of Plantation Work (follow-up = 30 yrs)

<table>
<thead>
<tr>
<th>Years of Plantation Work</th>
<th>Incidence Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>1-10</td>
<td>5.4</td>
</tr>
<tr>
<td>11-20</td>
<td>9.2</td>
</tr>
<tr>
<td>&gt;20</td>
<td>10.3</td>
</tr>
</tbody>
</table>

*Rate per 10,000 person-years
Test for Trend - p=0.011
Age-adjusted Incidence of PD* by Years of Pesticide Exposure (follow-up = 24 yrs)

*Rate per 10,000 person-years
Test for Trend - p=0.100
Cancer Prevention Study II
Nutrition Cohort

- 143,325 individuals
- Enrolled in 1992
- Exposure was assessed by a questionnaire (yes or no, length of exposure?)
- Case identification by questionnaire, medical records then reviewed.

Ascherio et al. 2006
Cancer Prevention Study II
Nutrition Cohort

Ascherio et al. 2006
Prospective Cohort Studies

- **Strengths**
  - Large study population
  - Controlled for many possible confounds (e.g. smoking, NSAIDs)
  - Prospective (no recall bias)

- **Weaknesses**
  - Little info on duration, intensity and type of exposures
  - No info on specific pesticides
  - Potential misdiagnosis of cases

Ascherio et al. 2006
Agricultural Health Study

- Prospective cohort study examining relationship between pesticide use and human disease
- 52,000 pesticide applicators and 32,000 spouses from Iowa and N. Carolina enrolled 1993-97
- Detailed pesticide use information
- PD participants almost twice as likely to be exposed to \(2,4,5\)-T and to trifualin than non-PD (control) participants

Kamel et al. 2007
Agricultural Health Study

Strengths
- Incident cases were prospective
- Identified specific pesticides
- Controlled for other confounds

Weaknesses
- Small number of cases (self reported), limited power
- Exposures self reported (agents, durations etc)
- Diagnosis self reported

Kamel et al. 2007
Parkinson’s (PEG) Study (Beate Ritz MD, Ph.D.)

Goal:

- Identify all newly diagnosed cases of PD over a 4-year period (2001-2005)
  400 cases
  400 population controls and 200-300 (unaffected) sibling controls

- Establish a lifetime history of exposure to pesticides
  recollections of residential history
  historical exposure records for pesticide use (PUR)

Collect biological samples

- DNA for genotyping
- Organochlorine serum analyses
- Lymphocytes for GEP and oxidative stress markers
Determining Pesticides Exposure

![Downwind Herbicide Deposition Graph]

- Extremely coarse-very coarse spray, 3 mph wind
- Medium spray, 3 mph wind
- Extremely coarse-very coarse spray, 10 mph wind
- Medium spray, 10 mph wind

Effect level for 50% of young plants
Table 4. Associations with PD by number of risk alleles* in the 5′and 3′ region of DAT and residential paraquat and maneb exposure in the California Central Valley study

<table>
<thead>
<tr>
<th>Risk Alleles</th>
<th>Zero/Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n= 286)</td>
<td>Controls (n= 319)</td>
</tr>
<tr>
<td>0</td>
<td>65</td>
<td>80</td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>117</td>
</tr>
<tr>
<td>2+</td>
<td>122</td>
<td>122</td>
</tr>
</tbody>
</table>

\(^a\) Odds Ratio (OR) adjusted for age (continuous), race, years of schooling, smoking (ever/never)

Ritz et al, 2009
How can we prove that a toxin contributes to the pathogenesis of PD?

- A plausible mechanism of action.
- Association between a toxin and PD in epidemiological studies.
- Recapitulation of behavioral and pathological features in cellular and animal models.
Mitochondrial Dysfunction and PD

- MPTP is a complex I inhibitor
- Decreased complex I and II in brains and peripheral platelets in PD
- Mitochondrial-associated genes and PD (PINK1, DJ1, POLG, cybrids)
Rotenone Exposure and PD

- Residential use more common than commercial use.
- A few case-control studies support increase incidence but not conclusive.
  - Dhillon et al 2008: OR 10.9 (2.5-48)
  - Agriculture Health Study: OR 1.7 (few cases)
  - Anecdotal reports
Mechanisms of Rotenone Toxicity

- Complex I inhibition leads to oxidative stress and energy failure at low concentrations
- Proteasome inhibition
- Microtubule inhibition
Chronic systemic pesticide exposure reproduces features of Parkinson’s disease

Ranjita Betarbet, Todd B. Sherer, Gillian MacKenzie, Monica Garcia-Osuna, Alexander V. Panov and J. Timothy Greenamyre
Progression of Parkinson's Disease Pathology Is Reproduced by Intragastric Administration of Rotenone in Mice

Francisco Pan-Montojo et al. PLOS One 2010
Proteasome Dysfunction and PD

- Proteasome-associated genes and PD (Parkin, UCH L1)
- Decreased activity in brains and blood in PD
- Alpha-synuclein is at least partially degraded by the proteasome.
Pesticides that Lead to UPS Inhibition

**Rotenone**
Complex I inhibitor

**Ziram and other dithiocarbamates**
Fungicide
Multivalent interactions

**Benomyl**
Fungicide (Benzimidazole)
Binds to tubulin

**Dieldrin and Endosulfan**
Organochlorines (epoxicide)
Inhibits GABA-gated chloride channels

Wang et al, 2006
Ziram and Related Compounds

- Dimethyl- and diethyldithiocarbamates
- Widely used fungicides on fruits and nuts.
- Approximately 15 million lbs were used in the US in 2002.
# Ziram Exposure and PD (PEG Study)

Table 3. Ambient Occupational and Residential Maneb, Ziram, and Paraquat Exposure by Time Window of Exposure and Age of Central Valley of California Study Population

<table>
<thead>
<tr>
<th>Ziram and paraquat exposure</th>
<th>Occupational**</th>
<th></th>
<th>Residential ***</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1974-1999 Time Window</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 years old or younger</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No exposure to ziram or paraquat</td>
<td>28</td>
<td>53</td>
<td>1.00 ref</td>
<td>21</td>
</tr>
<tr>
<td>Ziram or paraquat exp only</td>
<td>30</td>
<td>29</td>
<td>1.90 (0.92,3.94)</td>
<td>35</td>
</tr>
<tr>
<td>Ziram and paraquat exp</td>
<td>19</td>
<td>5</td>
<td>5.97 (1.94,18.33)</td>
<td>21</td>
</tr>
<tr>
<td>Over 60 years old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No exposure to ziram or paraquat</td>
<td>137</td>
<td>141</td>
<td>1.00 ref</td>
<td>103</td>
</tr>
<tr>
<td>Ziram or paraquat exp only</td>
<td>84</td>
<td>76</td>
<td>1.17 (0.78,1.76)</td>
<td>113</td>
</tr>
<tr>
<td>Ziram and paraquat exp</td>
<td>64</td>
<td>37</td>
<td>1.93 (1.18,3.15)</td>
<td>69</td>
</tr>
</tbody>
</table>

Systemic Administration of Ziram

![Graph showing T-Turn (Inverse Transform) vs. Time (Weeks) for Saline and NaDMDC.](image)

<table>
<thead>
<tr>
<th>region</th>
<th>2W</th>
<th>2 + 9W</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>treated</td>
<td>control</td>
</tr>
<tr>
<td>TH immunoreactivity fiber density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dorsolateral</td>
<td>1515 ± 207</td>
<td>1456 ± 221</td>
</tr>
<tr>
<td>dorsomedial</td>
<td>1048 ± 149</td>
<td>843 ± 117</td>
</tr>
<tr>
<td>ventrolateral</td>
<td>1439 ± 142</td>
<td>1290 ± 140</td>
</tr>
<tr>
<td>ventromedial</td>
<td>815 ± 103</td>
<td>696 ± 62</td>
</tr>
</tbody>
</table>

**p ≤ 0.05**

Chou et al, 2009
Other Pesticides and PD

- Paraquat
  - Associated with increased risk of PD (in combination with maneb and ziram)
  - Mechanism: Redox-cycling?
  - Animal model; DA cell loss and behavior

- Benomyl
  - Associated with increased risk of PD (PEG and Ag Health)
  - Mechanisms: UPS-I, ALDH-I, and MT-I
  - Primary culture DA loss but no animal models
What about Agent Orange?
“...the Department of Veterans Affairs plans to add Parkinson’s disease, ischemic heart disease and hairy-cell leukemia to the growing list of illnesses presumed to have been caused by Agent Orange, the toxic defoliant used widely in Vietnam.”
Agent Orange

- Code name for a defoliant herbicide used during the Vietnam War.
- Name derived from orange drums it is shipped in
- Mixture of 2 herbicides:
  - 2,4-dichlorophenoxyacetic acid (2,4-D)
  - 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).
- Contaminant: 2,3,7,8-TCDD (dioxin)
Herbicide use during Vietnam War

- 86% from large scale spraying operations (operation Ranch Hand) – good documentation
- Rest from trucks and back packs – poor documentation

Source: Science 2007; 315:176-79
TABLE 4. Ever use of specific pesticides by self-reported Parkinson’s disease (PD) cases and controls identified in the Agricultural Health Study at enrollment in 1993–1997 (prevalent PD) or at follow-up in 1999–2003 (incident PD)*

<table>
<thead>
<tr>
<th>Pesticide classification†</th>
<th>Chemical†</th>
<th>Prevalent PD</th>
<th>Incident PD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Herbicides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroacetanilide</td>
<td>Alachlor</td>
<td>30</td>
<td>25,599</td>
</tr>
<tr>
<td></td>
<td>Metolachlor</td>
<td>22</td>
<td>22,202</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>Dicamba</td>
<td>26</td>
<td>23,847</td>
</tr>
<tr>
<td>Dinotroaniline</td>
<td>Pendimethalin</td>
<td>23</td>
<td>21,386</td>
</tr>
<tr>
<td></td>
<td>Trifuralin</td>
<td>31</td>
<td>25,787</td>
</tr>
<tr>
<td>Imidazolinone</td>
<td>Imazethapyr</td>
<td>19</td>
<td>20,461</td>
</tr>
<tr>
<td>Mixture</td>
<td>Petroleum oil</td>
<td>22</td>
<td>22,295</td>
</tr>
<tr>
<td>Organophosphorus</td>
<td>Glyphosate</td>
<td>45</td>
<td>46,687</td>
</tr>
<tr>
<td>Phenoxyacetate</td>
<td>2,4-D‡</td>
<td>47</td>
<td>40,405</td>
</tr>
<tr>
<td></td>
<td>2,4,5-T‡</td>
<td>16</td>
<td>9,824</td>
</tr>
<tr>
<td></td>
<td>2,4,5-TP‡</td>
<td>4</td>
<td>4,229</td>
</tr>
<tr>
<td>Quaternary ammonium</td>
<td>Paraquat</td>
<td>14</td>
<td>11,266</td>
</tr>
<tr>
<td>Thiocarbamate</td>
<td>EPTC‡</td>
<td>6</td>
<td>9,160</td>
</tr>
<tr>
<td></td>
<td>Butylate</td>
<td>17</td>
<td>14,726</td>
</tr>
<tr>
<td>Sulfonyl urea</td>
<td>Chlorimuron-ethyl</td>
<td>16</td>
<td>17,552</td>
</tr>
<tr>
<td>Triazine</td>
<td>Atrazine</td>
<td>40</td>
<td>35,377</td>
</tr>
<tr>
<td></td>
<td>Cyazinamide</td>
<td>30</td>
<td>19,702</td>
</tr>
<tr>
<td>Triazineone</td>
<td>Metribuzin</td>
<td>28</td>
<td>20,879</td>
</tr>
</tbody>
</table>
Agent Orange Neurotoxicity

- **2,4-D**: mechanism of injury is unknown and evidence for effects on dopaminergic system is inconsistent
  - Inhibits microtubule assembly in cerebellar granule cell culture
  - Mild, transient locomotor effect in acutely exposed rats
  - Striatal dopamine depletion in rats administered intracerebral injection, but no toxicity noted in mice administered sub q injection.
- **TCDD**: mechanism of injury is unknown but linked to oxidative stress
Conclusions: Do pesticides contribute to the pathogenesis of PD?

- Plausible mechanisms of action. **YES**
- Association between a toxin and PD in epidemiological studies. **YES**
- Recapitulation of behavioral and pathological features in cellular and animal models. **YES**
Question & Answers

Thank You