The Issue

Paraquat exposure has been implicated in the literature as a potential contributory factor in Parkinson's disease (PD) in humans.

- There is a chemical structure association made in the literature between paraquat and MPTP shown to induce symptomology of Parkinson's disease in humans.
- There are literature epidemiology reports of an association between working with pesticides (and paraquat) and Parkinson's disease.
- There are literature reports of an experimental model for PD in the C57Bl/6 mouse where paraquat administered intraperitoneally causes a reduction in the number of neurones in the brain, together with in some studies a reduction in levels of dopamine in the brain and behavioural changes.

What we cannot say

- Paraquat does not enter the brain
- Paraquat does not cause any changes in the brain
- Paraquat only causes effects in the mouse
- The mouse data on paraquat are not relevant to humans
- People are not exposed to paraquat
- There are no data reporting that paraquat may be associated with PD in humans
- The data show that paraquat does not cause PD in humans

Current position

Epidemiology

- The evidence from case reports, case series and case-control studies is mixed and without consistent exposure-response or chemical-specific pattern.
- A major problem is the absence of detailed and validated exposure information.
- Currently, the data from epidemiology studies do not provide sufficient evidence to support a causal association between exposure to agricultural chemicals, including paraquat, and PD.
Animal studies

- The mouse has been developed as a sensitive investigative tool to examine chemicals for changes considered relevant to PD.
- Paraquat administered to the mouse by the intraperitoneal (ip) route leads to a limited reduction in neuronal cell number in the S nigra.
- There are no consistent data from the mouse model indicating effects from paraquat dosing required as part of the progression and expression of PD (dopamine changes or behavioural effects).
- There is limited evidence for paraquat causing a reduction in neuronal cell number in species other than the mouse.
  - CTL has examined the rat using a protocol taken from the literature that claimed an effect. There was no reduction in neuronal cell number in our study.
- The relevance of the observations in this investigative model to humans who may be exposed to low levels of paraquat mainly by the dermal/oral route is considered to be limited.
- There is no evidence that paraquat causes biological changes related to PD by a relevant route for human exposure, or at doses approaching those to which humans may be exposed.

Structural association with MPTP

- The structural association of paraquat to MPTP is superficial and is inappropriate for drawing any conclusions on potential similar activity in regard to PD.

Ongoing work

- External labs will continue to generate and report data:
  - Epidemiology
    - Ag Health study USA
    - Farm workers (x2?) France
    - DEFRA (Geoparkinson study) UK
  - Animal studies
    - Di Monte
    - Corey Slechta
    - Others
  - Syngenta
    - Evaluation of PQ in the rat (negative)
    - Kinetics of PQ uptake into the brain of the mouse
Table 4: Prevalence of specific antibodies by self-reported Parkinson’s disease (PD) case and control identified in the Agricultural Health Study. Study enrollment in 1982–1987 (prevalent PD in a follow-up in 1993–1997 (incident PD)).

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>No. Exposed Cases/Total Cases</th>
<th>No. Exposed Controls/Total Controls</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hertzman 1990</td>
<td>Canada</td>
<td>30/369</td>
<td>0/0</td>
<td>1.29 (0.97-1.71)</td>
<td>0.09</td>
</tr>
<tr>
<td>Hertzman 1994</td>
<td>Canada</td>
<td>10/192</td>
<td>6/100</td>
<td>1.61 (0.96-2.67)</td>
<td>0.07</td>
</tr>
<tr>
<td>Seidler 1996</td>
<td>Germany</td>
<td>4/380</td>
<td>0/0</td>
<td>1.17 (0.94-1.47)</td>
<td>0.20</td>
</tr>
<tr>
<td>Liou 1997</td>
<td>Taiwan</td>
<td>31/120</td>
<td>22/240</td>
<td>0.91 (0.63-1.30)</td>
<td>0.69</td>
</tr>
<tr>
<td>Kuopio 1999</td>
<td>Finland</td>
<td>3/123</td>
<td>5/246</td>
<td>0.79 (0.38-1.66)</td>
<td>0.54</td>
</tr>
<tr>
<td>Engel 2001*</td>
<td>USA</td>
<td>20/49</td>
<td>0/0</td>
<td>1.05 (0.59-1.90)</td>
<td>0.85</td>
</tr>
<tr>
<td>Kamel 2001**</td>
<td>USA</td>
<td>7/55</td>
<td>7/2288</td>
<td>1.57 (0.69-3.63)</td>
<td>0.53</td>
</tr>
<tr>
<td>Firestone 2005</td>
<td>USA</td>
<td>7/250</td>
<td>7/388</td>
<td>1.67 (0.82-3.40)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

*Cross-sectional study  **Conference presentation
The Optical Fractionator Method of Stereology

\[ N = \text{Sum} \times \frac{1}{ssf} \times \frac{1}{asf} \times \frac{1}{tsf} \]

Proposed Exponent Protocol

1) Toxicokinetic Study (single dose of 7.4 & 74 \( \mu \)mol/kg - propose to dose the Gramoxone formulation)

- PND 5 mice
  - Saline
  - 0.3 mg/kg PQ
  - PQ + 1 mg/kg maneb
    - i.p. & oral

- Adult mice
  - Saline
  - 10 mg/kg PQ
  - PQ + 30 mg/kg maneb
    - i.p., oral, dermal & inhalation
      - (direct application to the nasal epithelium)

Concentration of PQ in plasma and different brain regions using \([^{14}\text{C}]\)-PQ and autoradiography (WBA for young mice)

2) Use PBPK modelling to determine the equivalent human exposure doses via the relevant routes (dermal / oral - seem to think inhalation route is relevant).

3) Toxicology Study (i.p. dosing to mice using the Cory-Slechta dosing regimen)

- PND 5-19 mice
  - Saline
  - PQ
  - PQ + maneb (leave till 8 months old)

- PND 5-19 & as adults (8 months old)
  - Saline
  - PQ
  - PQ + maneb

- Adult mice (8 months old)
  - Saline
  - PQ
  - PQ + maneb
  - PQ + maneb (leave till 16 months old)

Neuronal Cell Loss

(Stereology - Jim O'Callaghan (CDC); silver staining - Bob Switzer (Neuroscience Associates) & Daryl Thake (Monsanto))

3 different doses to both male & female mice:
(i) Human equivalent dose (from PBPK modelling)
(ii) Mid dose
(iii) "High" Cory-Slechta dose (0.3 or 10 mg/kg PQ, 1 or 30 mg/kg maneb)

<table>
<thead>
<tr>
<th>Dose</th>
<th>10%</th>
<th>35%</th>
<th>8%</th>
<th>YES</th>
<th>NO</th>
<th>YES+</th>
<th>NO</th>
<th>SYNGENTA CTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td>YES</td>
<td></td>
<td>NO</td>
<td>YES+</td>
<td>SYNGENTA CTL</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td>YES</td>
<td></td>
<td>NO</td>
<td>YES+</td>
<td>SYNGENTA CTL</td>
</tr>
<tr>
<td>5 mg/kg for 4 wks</td>
<td></td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
<td>SYN Ge nTA CT L</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>25-24%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>61%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>28%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>26%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>26-35%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg for 4 wks</td>
<td>8%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 10 mg/kg</td>
<td>14%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 10 mg/kg</td>
<td>21%</td>
<td>10%</td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 10 mg/kg</td>
<td>31%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 10 mg/kg</td>
<td>38%</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 10 mg/kg (for 6 wks)</td>
<td>9%</td>
<td>7%</td>
<td></td>
<td>NO</td>
<td>NO</td>
<td>SYNGENTA CTL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 x 10 mg/kg (for 12 wks)</td>
<td></td>
<td></td>
<td></td>
<td>YES</td>
<td></td>
<td>NO</td>
<td>YES</td>
<td>SYNGENTA CTL</td>
</tr>
</tbody>
</table>
Nigrostriatal dopaminergic system

- Anterior
- Cerebrum
- Striatum
- Reduction in dopamine concentration
- Substantia nigra pars compacta (dopaminergic neurones)
- Posterior
- Brain stem
- Behavioural deficits; reduced motor activity
- Reduction in dopaminergic neurones

Apparent Structural Similarity Of Paraquat With MPTP & MPP^+

Paraquat
- Hydrophilic, charged di-cation
- Monoamine, uncharged lipophilic molecule

MPTP
- Charged, neurotoxic metabolite of MPTP

MPP^+
- Charged, neurotoxic metabolite of MPTP