Cutting the Mustard: Evidence of VX Nerve Agent Use from Contaminated White Mustard Plants

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Aim

To determine if natural flora can be used to enable the sensitive detection of organophosphorus nerve agents
Introduction

• The ability to detect the presence of CWAs in environmental samples is a highly valuable capability
  – Allegations of use
  – Land remediation
  – Incident management

• Current detection is based upon the analysis of soil samples
  – Sampling considerations
  – Extraction problems
  – Leaching issues
Chemical Warfare Agent Hydrolysis

\[
\begin{align*}
\text{sarin} & \quad \text{fast} \quad \text{H}_2\text{O} \quad \rightarrow \quad \text{iPMPA} \\
\text{soman} & \quad \text{fast} \quad \text{H}_2\text{O} \quad \rightarrow \quad \text{PMPA} \\
\text{VX} & \quad \text{fast} \quad \text{H}_2\text{O} \quad \rightarrow \quad \text{EMPA}
\end{align*}
\]
Introduction

• Initial Study
  – Contaminated seeds grown in compost with VX
  – Plants were harvested after 5, 9, 16 and 28 days
  – Four plants were analysed per time point
  – Plant material and soil analysed for VX and degradation products EMPA and MPA

• Advanced Study
  – Contaminated seeds grown in three different types of soil (sandy, loam and clay)
  – Seeds were contaminated with VX (Route 1) and with EMPA & MPA (Route 2)
  – Plants were harvested after 8, 16, 33 and 45 days
  – Ten plants were analysed per time point
  – Plant material and soil analysed for VX and degradation products EMPA and MPA
Initial Study
Initial Study Experimental

Seeds placed in seed tray and contaminated with 1 ml of 250 µg ml⁻¹ solution of VX and iPMPA

Immediately watered with 10 ml of local borehole water and subsequently at 24 h intervals

Placed under a lighting and timing system that provided light for 10 h a day

LC-MS (Agilent, 6530 Q-ToF)

GC-MS (Agilent, 6890-5973)

100 µl

1 ml Derivatisation

Plant and soil samples were defrosted. Prepared and extracted with ethanol and filtered

Plants harvested at 5, 9, 16 and 28 days. Plants were separated from the soil and both plants and soil were immediately frozen and stored at -40 °C

INNOVATIVE THINKING FOR THE REAL WORLD
Results (1) - Plants

VX concentration in plants spiked with VX

<table>
<thead>
<tr>
<th>Harvest Time Point</th>
<th>DAY 5</th>
<th>DAY 9</th>
<th>DAY 16</th>
<th>DAY 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (ng/ml)</td>
<td>30.00</td>
<td>80.00</td>
<td>20.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Conc$^n$ ng/ml

During the harvest time points, VX concentration was observed to be significantly higher on DAY 9 compared to other days. The chart also indicates a decrease in VX concentration as the harvest time progresses, with the lowest concentration observed on DAY 28.
Results (2) - Plants

Phosphonic acid concentration in plants spiked with VX

- EMPA
- MPA

[Bar chart showing concentration of EMPA and MPA over different harvest time points (DAY 5, DAY 9, DAY 16, DAY 28).]
Results (3) - Soil

Detection of EMPA and MPA in soils with high organic content is challenging.
- Levington Compost (suited for full plant nourishment) was used.
- Due to absorption and retention of VX.
- Checked by spiking EMPA and MPA directly onto soil and extracting with a) ethanol and b) basification of the sample.
- EMPA and MPA were not detectable.
Initial Study Conclusions

- VX concentration in plants increased up to Day 9 and then fell by Day 28
- VX was detectable at sub ng/ml levels
- EMPA and MPA (degradation products) show a similar profile to VX
- VX concentration in the soil remained constant until Day 9 and then plummeted by Day 16, falling further by Day 28
  - Consistent with other studies where VX hydrolysis, once started, occurs autocatalytically
- VX concentration in plants increased whilst VX was available for uptake and decreased as the soil concentration decreased
- EMPA and MPA were unable to be detected in soil (extraction difficulties) but detectable in plant material
- This methodology can provide evidence of VX use that would otherwise be unavailable via established approaches
Advanced Study
Advanced Study Questions

• Can we expand the detection window for VX?

• What is the impact on the type of soil on uptake of VX and degradation products?

• Does the plant selectively break down VX or is non-selective hydrolysis involved?

• Can plants be used to bioremediate land contaminated with nerve agents?

• Advanced Study
  • Contaminated seeds grown in three different types of soil (sandy, loam and clay)
  • Seeds were contaminated with VX (Route 1) and with EMPA & MPA (Route 2)
  • Plants were harvested after 8, 16, 33 and 45 days
  • Ten plants were analysed per time point
  • Plant material and soil analysed for VX and its degradation products, EMPA and MPA
Seeds placed in three types of soil (sand, clay and loam) and spiked with 1 ml of a 250 µg ml⁻¹ aqueous solution of VX

Seeds immediately watered with 10 ml local borehole water and at 24 h intervals and illuminated with 10 h light per day

Plants harvested at 8, 16, 33 and 45 days and immediately frozen at -40 °C

Plants defrosted. Pulverised, extracted with ethanol, filtered through a 0.45 µm filter and concentrated under nitrogen to 1 ml

100 µl

10 µl derivatisation

LC-MS (Agilent, 6530 Q-ToF)

GC-MS (Agilent, 6890-5973)
Soil Types

• Nerve agents absorb rapidly onto soil particles

• Generally the smaller the particle size the longer a nerve agent persists due to absorption over a larger surface area

• Relationship between particle size and persistence is complex as it depends upon organic content, clay content and pH

• Soil organic matter has amino, carboxyl and hydroxyl functional groups that provide hydrogen bonding sites for nerve agents. More organic matter = more sites
Results (1) - Plants

- Initial absorption of VX on Day 8 appeared most efficient in sand, less efficient in loam and least efficient in clay.
- Order predicted by considerations of soil structures and chemical retention properties.
- By Day 16 uptake has converged and follows the similar profile.
Results (2) - Plants

- VX spiked plants enable detection of EMPA out to 45 days
- There is a large profile difference across the different soil types
- Large variation made statistically relevant conclusions difficult
Results (3) - Plants

- VX spiked plants enable detection of MPA out to 45 days
- MPA content rises sharply in plants grown in clay from Day 16 – 45 (50 ng – 180 ng)
- To explain the EMPA and MPA results, we performed studies growing plants in soil spiked with EMPA and MPA
Results (4) – EMPA and MPA Plants

- Plants grown in EMPA soil did not contain any measurable EMPA.
- MPA was detectable in plants grown in MPA contaminated soil.
- This data, interestingly, suggests EMPA detected in plants grown in VX contaminated soil originated from metabolism of VX.
Results (5) – EMPA and MPA Plants

• Mechanism currently unknown, unable to find reports of OP hydrolase enzymes

• VX and MPA absorbed by roots but EMPA is not?

• Positive correlation sometimes exists between the water solubility of a substance and its systemic activity
### Results (6) – EMPA and MPA Plants

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Vapour pressure (mmHg)</th>
<th>Water solubility (g/l)</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt; (25 °C)</th>
<th>Log K&lt;sub&gt;ow&lt;/sub&gt;</th>
<th>Uptake by white mustard</th>
</tr>
</thead>
<tbody>
<tr>
<td>VX</td>
<td>7.0 × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>30</td>
<td>9.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+2.09</td>
<td>Y</td>
</tr>
<tr>
<td>EMPA</td>
<td>3.6 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>180</td>
<td>2.76</td>
<td>-1.15</td>
<td>N</td>
</tr>
<tr>
<td>MPA</td>
<td>2.0 × 10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>&gt;1000</td>
<td>2.38, 7.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2.28</td>
<td>Y</td>
</tr>
<tr>
<td>iPMPA</td>
<td>3.4 × 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>48</td>
<td>1.98</td>
<td>NA</td>
<td>Y</td>
</tr>
</tbody>
</table>

- VX exists in a protonated cationic form that is most likely to be absorbed and translocated by plants in loam and sandy soils and in a neutral form that is most likely to be absorbed and translocated by plants in clay soil.

- EMPA and MPA will exist in their soil in the anionic form.

- EMPA is less stable than MPA. However *Sinapis alba* seems to selectively uptake certain alkyl methylphosphonic acids.
Advanced Study Conclusions

• VX concentration in plants was detectable out to 45 Days post contamination

• VX was detectable at sub ng/ml levels

• EMPA and MPA were detectable in plants 45 Days after exposure to VX

• No EMPA was detectable in plants contaminated with phosphonic acid degradation products

• EMPA detectable in plants grown in VX contaminated soil may originate from selective metabolism within the plant

• Further studies are required (including expanding the range of plants used) to understand and exploit this effect
Overall Conclusions

• *Sinapis alba* acts as a time capsule for VX, EMPA and MPA and allows the detection of this nerve agent for a long period of time after use

• Analytical methodology is simple (ethanol extraction) and could be applicable to on-site testing and analysis

• Plant material is easy to find and collect. Could also be analysed via sensors responding to plant physiological changes

• Soil analysis can be hit and miss. Using plants is more reliable and should apply to all parts of the world (maximum utility for attribution and deterrence)

• Extends current methods for detecting use of chemical agents

• Selective breakdown pathway for VX opens up possibility using quick growing plants for bioremediation of contaminated land
External Dissemination

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