Phototrophic Microorganisms: A Potent Biodegrading Community Whose Contribution to Pesticide Fate is Currently Overlooked

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Background (1)

- Syngenta has been interested in the role of phototrophic organisms (algae and macrophytes) in active metabolism of CPPs since 1998
  
  - First publication on *Lambda*-cyhalothrin in 2001

- This was followed by development of a modified OECD308 study under non-UV light, to allow the investigation of algal metabolism in isolation from photolysis
  
  - First used on the fungicide, Isopyrazam
  
  - This showed significant enhancement of degradation
Background (2)

- A post doctoral research project was then conducted to investigate across a range of chemistries
  - Clear enhancement of degradation observed in five of the six test compounds
  - Extent of enhancement ranged from 1.5 – 20 x, depending on the compound
  - Sub-communities of the water column biomass selectively cultured and metabolic potential assessed with one compound (Fludioxonil)
  - All phototrophic communities and individual species shown to be metabolically competent
  - Non-phototrophic communities (bacteria and fungi) did not degrade fludioxonil under the conditions used.
From the precedent observed in aquatic systems, the obvious question was:
- *Is metabolism by soil algae also a significant process which is not captured by OECD307 regulatory soil degradation studies?*

Higher tier studies were therefore developed to investigate this.

These higher tier aquatic and soil studies were conducted in the regulatory study package for the new Syngenta fungicide, Solatenol™ (Benzovindiflupyr)
- This represents the first comprehensive investigation of the role of algae in agrochemical metabolism
Metabolism in Pure Algal Cultures

- In order to conclusively demonstrate the capability of algae to metabolise Benzovindiflupyr, a $^{14}$C pure culture experiment was conducted
  - Two species were used
    - *Scenedesmus quadricauda* (Chlorophyta)
    - *Anabaena cylindrica* (Cyanophyta)
- Significant metabolism was observed in both species
Aquatic Studies – Tiered Approach

Tier 1 – Regulatory OECD308

- $^{14}$C study in continuous darkness

Tier 2 – Modified OECD308

- $^{14}$C study in fluorescent light/dark cycle. Otherwise identical design to regulatory study

Tier 3 – Outdoor Microcosm

- $^{14}$C study under natural UK summer conditions. Integrated system in which all degradation processes can occur
Aquatic Studies – Tier 1 (Regulatory OECD308)

- Relatively rapid dissipation from water column to sediment
- Very little degradation in the sediment

<table>
<thead>
<tr>
<th>Compartment</th>
<th>SFO DT&lt;sub&gt;50&lt;/sub&gt; (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Column</td>
<td>18</td>
</tr>
<tr>
<td>Total System</td>
<td>427</td>
</tr>
</tbody>
</table>

- Compound is not readily degraded by heterotrophic communities
Aquatic Studies – Tier 2 (Modified OECD308)

- Significantly faster dissipation from the water column and lower peak % in sediment
  - Metabolism by planktonic algae
- Significant degradation also observed in sediment
  - Metabolism by algae on sediment surface

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<th>Compartment</th>
<th>SFO DT&lt;sub&gt;50&lt;/sub&gt; (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Column</td>
<td>4</td>
</tr>
<tr>
<td>Total System</td>
<td>52</td>
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</tbody>
</table>

![Graph showing % Applied Radioactivity as Parent over days for Total System, Water Column, and Sediment compartments.](image)
Aquatic Studies – Tier 3 (Outdoor Microcosm)

- Dissipation from the water column similar to modified OECD308
- Lower peak % in sediment and degradation again observed
- Route of metabolism consistent with modified OECD308 and pure algal cultures
  - But different from aqueous photolysis
- Metabolism by phototrophs is the dominant process

<table>
<thead>
<tr>
<th>Compartment</th>
<th>SFO DT(_{50}) (Days)</th>
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<tbody>
<tr>
<td>Water Column</td>
<td>4.3</td>
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<tr>
<td>Total System</td>
<td>15.1</td>
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</table>
Aquatic Studies – Overview

- Water Column
- Sediment
- Total System

Graphs showing the percentage of applied radioactivity as parent (Total System) over days for different conditions:
- Regulatory OECD308
- Modified OECD308 (non-UV Light)
- Outdoor Microcosm
Soil Studies – Tiered Approach

Tier 1 – Regulatory OECD307

\(^{14}\text{C}\) study using sieved soil in continuous darkness

Tier 2 – Undisturbed Cores

\(^{14}\text{C}\) study in either fluorescent or UV light/dark cycle. Moisture supplied from below to simulate groundwater

Tier 3 – \(^{14}\text{C}\) Field Study

\(^{14}\text{C}\) bare soil TFD study under natural US summer conditions.
Soil Studies – Tier 1 (Regulatory OECD307)

- Very slow degradation in all soils tested

<table>
<thead>
<tr>
<th>Soil</th>
<th>SFO DT&lt;sub&gt;50&lt;/sub&gt; (Days)</th>
</tr>
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<tbody>
<tr>
<td>Soil 1</td>
<td>&gt;1000</td>
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<tr>
<td>Soil 2</td>
<td>514</td>
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<tr>
<td>Soil 3</td>
<td>550</td>
</tr>
<tr>
<td>Soil 4</td>
<td>924</td>
</tr>
<tr>
<td>Soil 5</td>
<td>940</td>
</tr>
</tbody>
</table>

- Compound is not readily degraded by heterotrophic soil communities
Soil Studies – Tier 2 (Undisturbed Cores)

- Parallel testing of undisturbed cores in the dark, non-UV light and Suntest (to allow assessment of photolysis)
  - No photolysis observed in regulatory thin soil layer study
- Slow degradation in the dark (confirming OECD307 results)
- Degradation rapid under non-UV light/dark cycle (algal metabolism)
- Not degraded any quicker under UV light (confirming photolysis is negligible).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>SFO DT$_{50}$ (Days)</th>
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<tbody>
<tr>
<td>Dark</td>
<td>349</td>
</tr>
<tr>
<td>Non-UV Light</td>
<td>36</td>
</tr>
<tr>
<td>UV Light</td>
<td>35</td>
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</tbody>
</table>

% Applied Radioactivity as Parent

Days

Days

non-UV Light

Suntest

Darkness
● The rate of degradation observed under field conditions over the first 60 elapsed days (130 time-step normalised days) is matched by that observed in the soil cores under non-UV light/dark regime

● Field degradation can be attributed to metabolism by the surface algal communities
Soil Studies – Overview

- Regulatory OECD307
- Undisturbed Cores (non-UV Light)
- Radiolabelled Field Dissipation
Conclusions

- Recent research and the studies reported herein demonstrate that algae can be significant contributors to the biotransformation of pesticides in both aquatic and terrestrial ecosystems.
- Current regulatory laboratory studies do not capture this autotrophic mechanism, as they are conducted in continuous darkness.
- For some compounds, autotrophic degradation may be the most significant loss mechanism.
- Simple studies can be conducted in the laboratory to investigate whether this mechanism is significant for a given compound.
  - These studies can also provide useful data for the “conceptual model” of field behaviour.
- Without this data, assessments of persistence will be made on the basis of an incomplete understanding of a compound’s environmental fate.
Thank you for your attention