Evaluation of a novel test design to determine uptake of chemicals by plant roots

Experiences with uptake testing

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Outline

- Introduction
- Study design
- Results from ring test with 1,2,4-triazole in wheat
- Uptake studies with various crops/compounds combinations
- Comparison with former studies
- Summary and outlook
Intro: Purpose of testing a new design?

- Increased reproducibility of uptake measurements
- Determination of translocation from (soil) solution into the plant
- Formula to derive input parameter for e-fate models (leaching)
- Proposal to regulatory authorities
- Way forward to more robust regulatory decision making?
Introduction
Plant Uptake of chemicals

After entering the plant via the root hairs, a chemical can follow:
Apoplastic pathway via the cell walls
Symplastic pathway via the plasmodesma
Transcellular pathway from vacuole to vacuole
Uptake in environmental fate models

- Decreases mass of chemical in soil available for leaching
- Mass removed from soil depends on:
  - concentration in the liquid phase
  - transpiration
  - potential of a compound to be taken up via plant roots
- Potential for uptake via root is described by a single parameter, PUF** or TSCF*, that describes the ratio of concentrations of a chemical in different compartments.

**PUF**: Plant Uptake Factor
*TSCF*: Transpiration Stream Concentration Factor
**RCF**: Root Concentration Factor

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**TSCF**: Transpiration Stream Concentration Factor
**RCF**: Root Concentration Factor
*K_{OM}*: Distribution Coefficient Soil Organic Matter and Porewater
Introduction
Calculation of Uptake Factors

Uptake in aerial part (shoots)

\[
TSCF = \ln\left(1 - \frac{m_{\text{shoots}}}{m_{\text{shoots}} + m_{\text{sol-8}}}\right)
\]

\[
= \ln\left(\frac{V_{\text{sol-0}}}{V_{\text{sol-2}}}\right)
\]

Uptake in whole plant (roots & shoots)

\[
PUF = \ln\left(\frac{m_{\text{sol-8}}}{m_{\text{sol-2}}}\right)
\]

\[
= \ln\left(\frac{V_{\text{sol-8}}}{V_{\text{sol-2}}}\right)
\]

\[m_{\text{sol-2}}:\] mass of test chemical in solution at the end of the equilibration phase (Day 2) [µg]
\[m_{\text{sol-8}}:\] mass of test chemical in solution at the end of the experiment (Day 8) [µg]
\[V_{\text{sol-0}}:\] volume of nutrient solution at the start of the equilibration phase (Day 0), after removal of aliquot L
\[V_{\text{sol-2}}:\] volume of nutrient solution at the end of the equilibration phase (Day 2), after removal of aliquot L
\[V_{\text{sol-8}}:\] volume of nutrient solution at the end of the experiment (Day 8), after removal of aliquot L
\[m_{\text{shoots}}:\] mass of test chemical in shoots (Day 8) [µg]
Plant uptake: study design

Use of radioactivity enables the detection of total translocated amount

Test solution: volume, mass test item, pH, O₂

Test item (¹⁴C) application

Plant material: ¹⁴C - mass balance (shoots, roots, root wash), biomass

Day 0

Day 2 (t₀)

Day 4 (t₁)

Day 8 (t_ end)

Experimental phase

Cultivation

Pre-conditioning (10 days)

Equilibration

Experiment

Substrate

Perlite

Nutrient solution

Nutrient solution + test item

Application and sampling

BBCH 12

BBCH ~ 21

Day 2

Day 4

Day 8

Application and sampling
Results from ring test with 1,2,4-triazole in wheat

*Lab #1 and #3 failed to sample at Day 2 and therefore PUF values could not be calculated.*
# Application of quality criterion “biomass” to PUF and TSCF values (1,2,4-triazole in wheat)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Confidence Interval (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PUF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUF (n=39) without quality check</td>
<td>0.73</td>
<td>(0.64 - 0.82)</td>
</tr>
<tr>
<td>PUF (n=33) with quality check “biomass”</td>
<td>0.65</td>
<td>(0.57 - 0.73)</td>
</tr>
<tr>
<td>(biomass factor &gt;= 1.739 OR biomass factor &lt; 1.739 and initial biomass &gt; 1.55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TSCF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSCF (n=49) without quality check</td>
<td>1.03</td>
<td>(0.76 - 1.3)</td>
</tr>
<tr>
<td>TSCF (n=39) with quality check “biomass”</td>
<td>0.64</td>
<td>(0.58 - 0.70)</td>
</tr>
<tr>
<td>(only replicates with biomass increase of &gt; 0.67 g over 8 d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:** PUF≈TSCF, narrow confidence interval
Suitable for other substances and crops?
Review of 14 data sets

- 11 compounds → broad range of different chemical classes
  - log $K_{ow}$: -1.5 up to 2
  - molecular mass: 69 up to 563 g/mol
  - Three ionic compounds: A (pka 0.23), H (pka 3.58) and G (pka 4.06)
- 3 plant species
- Compound-crop combinations

<table>
<thead>
<tr>
<th>Substance</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* 1,2,4-Triazole, round robin test
XV Symp. PC, Piacenza 2015
A to H: mol. weight <370 g/mol
I to K: mol. weight >393 g/mol
Uptake studies with various crops/compounds combinations

- Uptake is correlated with transpiration (mol. weight ≤ 363 g/mol)
- Uptake decreases when mol. weight > 394 g/mol
Hypothesis:
If plants are comparable (size, growth, transpiration), then species per se does not play a major role.
### Summary of study results

<table>
<thead>
<tr>
<th>Substance</th>
<th>Plant</th>
<th>MW&lt;sup&gt;(1)&lt;/sup&gt; [g/mol]</th>
<th>Log Kow</th>
<th>PUF (± SD)</th>
<th>Confidence Interval (95%)</th>
<th>TSCF (± SD)</th>
<th>Confidence Interval (95%)</th>
<th>Radioactive recovery [%]</th>
<th>WUE [g/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>potato</td>
<td>114</td>
<td>0.56</td>
<td>0.57 ± 0.20</td>
<td>(0.46, 0.68)</td>
<td>0.76 ± 0.15</td>
<td>(0.66, 0.74)</td>
<td>95.8</td>
<td>97.3</td>
</tr>
<tr>
<td>B</td>
<td>potato</td>
<td>169</td>
<td>1.31</td>
<td>0.68 ± 0.02</td>
<td>(0.65, 0.69)</td>
<td>0.67 ± 0.01</td>
<td>(0.65, 0.69)</td>
<td>99.8</td>
<td>30.5</td>
</tr>
<tr>
<td>C</td>
<td>wheat</td>
<td>141</td>
<td>-0.58</td>
<td>0.64 ± 0.19</td>
<td>(0.57, 0.71)</td>
<td>0.67 ± 0.18</td>
<td>(0.61, 0.73)</td>
<td>98.0</td>
<td>35.9</td>
</tr>
<tr>
<td>D</td>
<td>wheat</td>
<td>141</td>
<td>-0.13</td>
<td>0.69 ± 0.16</td>
<td>(0.51, 0.87)</td>
<td>0.69 ± 0.06</td>
<td>(0.64, 0.73)</td>
<td>99.7</td>
<td>54.9</td>
</tr>
<tr>
<td>E</td>
<td>wheat</td>
<td>217</td>
<td>-1.54</td>
<td>0.65 ± 0.12</td>
<td>(0.55, 0.75)</td>
<td>0.37 ± 0.03</td>
<td>(0.34, 0.4)</td>
<td>96.4</td>
<td>14.9</td>
</tr>
<tr>
<td>F</td>
<td>wheat</td>
<td>369</td>
<td>0.11</td>
<td>0.31 ± 0.07</td>
<td>(0.25, 0.37)</td>
<td>0.2 ± 0.02</td>
<td>(0.18, 0.22)</td>
<td>98.3</td>
<td>30.9</td>
</tr>
<tr>
<td>G</td>
<td>tomato</td>
<td>141</td>
<td>-0.18</td>
<td>0.60 ± 0.04</td>
<td>(0.55, 0.75)</td>
<td>0.33 ± 0.13</td>
<td>(0.31, 0.35)</td>
<td>99.9</td>
<td>51.6</td>
</tr>
<tr>
<td>H</td>
<td>tomato</td>
<td>217</td>
<td>0.60</td>
<td>0.60 ± 0.07</td>
<td>(0.55, 0.67)</td>
<td>0.33 ± 0.02</td>
<td>(0.31, 0.35)</td>
<td>96.1</td>
<td>35.3</td>
</tr>
<tr>
<td>I</td>
<td>tomato</td>
<td>394</td>
<td>0.10</td>
<td>0.61 ± 0.08</td>
<td>(0.63, 0.71)</td>
<td>0.31 ± 0.13</td>
<td>(0.28, 0.34)</td>
<td>99.9</td>
<td>47.0</td>
</tr>
<tr>
<td>J</td>
<td>tomato</td>
<td>549</td>
<td>-1.10</td>
<td>0.02 ± 0.03</td>
<td>(0.00, 0.04)</td>
<td>0.01 ± 0.00</td>
<td>(0.00, 0.01)</td>
<td>109.1</td>
<td>37.0</td>
</tr>
<tr>
<td>K</td>
<td>tomato</td>
<td>563</td>
<td>-0.75</td>
<td>0.09 ± 0.04</td>
<td>(0.05, 0.13)</td>
<td>0.01 ± 0.00</td>
<td>(0.01, 0.01)</td>
<td>95.7</td>
<td>40.5</td>
</tr>
</tbody>
</table>

- **Successful application to non-ionic and ionic compounds.**
- Recovery rates and radio-chemical purity were high in the present studies suggesting that chemical loss processes (e.g. volatilisation and metabolism) did not affect TSCF calculations.
- **WUE confirmed good plant growth/health.**
- Small range of confidence intervals show the robustness and reliability of the study design.
- Precise TSCF determination CI range from 0.01-0.12.
Comparison of TSCF values from different studies

- High uptake of polar compounds with masses of less than 200 g/mol
- Negligible uptake of compounds with masses of greater than 394 g/mol
Conclusion on TSCF predictability

Compounds with log $K_{ow}$ -2 to 2
- Briggs curve showed parallelism with always lower TSCF values
- Dettenmaier: overestimation of TSCF (for small highly water soluble polar chemicals)?
How could the new test design be used?

- Qualitative indication of plant uptake ➔ PUF/TSCF > 0
- Tier 0: ZERO!
- Tier 1: TSCF according to Briggs et al. 1982:
  Reasons: EFSA 2013, FOCUS 2000; Lamshöft 2017 (in prep.,)
- Tier 2: Experimental TSCF:
  [Reason: EFSA 2013]
  Proposal from ECPA/IVA:
  a: average value from test with surrogate plants (wheat and tomato) or
  b: average value from tests with selected crops (e.g. herbicides)
Summary and outlook
Test design to determine plant uptake

- What it is for
  - Environmental fate modelling
  - Measure variables to calculate PUF and TSCF

- Experiences so far
  - Checked for applicability, intra-/inter-laboratory variability (round robin test 2015)
  - Review of tests with different compounds using wheat, tomato and potato

- Next steps
  - Implementation as an OECD guideline
  - Publication in a peer-reviewed scientific journal (ongoing)
Thank You!

Acknowledgement:
Fraunhofer Institute IME, Ricerca Biosciences, Utah State University, RLP Agroscience, Smithers Viscient, Fera Science Ltd., Eurofins GmbH, Bayer AG, Syngenta AG conducted the round robin test.
Back-up slide
Coefficient of variation or confidence interval for small numbers?

<table>
<thead>
<tr>
<th>TSCF</th>
<th>Substance 1</th>
<th>Substance 2</th>
<th>Substance 3</th>
<th>Substance 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate 1</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>0</td>
<td>0.6</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Replicate 3</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Replicate 4</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>0.08</td>
<td>0.53</td>
<td>0.48</td>
<td>0.98</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>66.67</td>
<td>9.52</td>
<td>10.53</td>
<td>5.13</td>
</tr>
<tr>
<td>Standard error of mean</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>95% confidence interval, lower limit</td>
<td>0.03</td>
<td>0.48</td>
<td>0.43</td>
<td>0.93</td>
</tr>
<tr>
<td>95% confidence interval, upper limit</td>
<td>0.12</td>
<td>0.57</td>
<td>0.52</td>
<td>1.02</td>
</tr>
<tr>
<td>95% confidence interval, range</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>