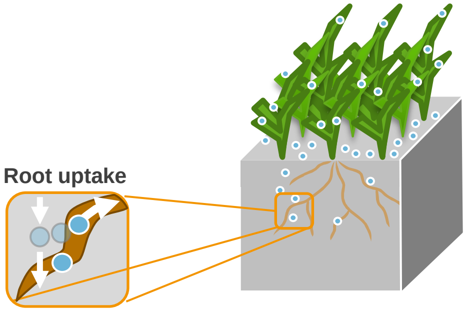
Evaluation of a novel test design to determine uptake of chemicals by plant roots

Experiences with uptake testing



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ECPA/IVA Working Group “Plant Uptake Factor”, Presentation at Conference on Pesticide Behaviour, York, UK, Sep. 2017

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

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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

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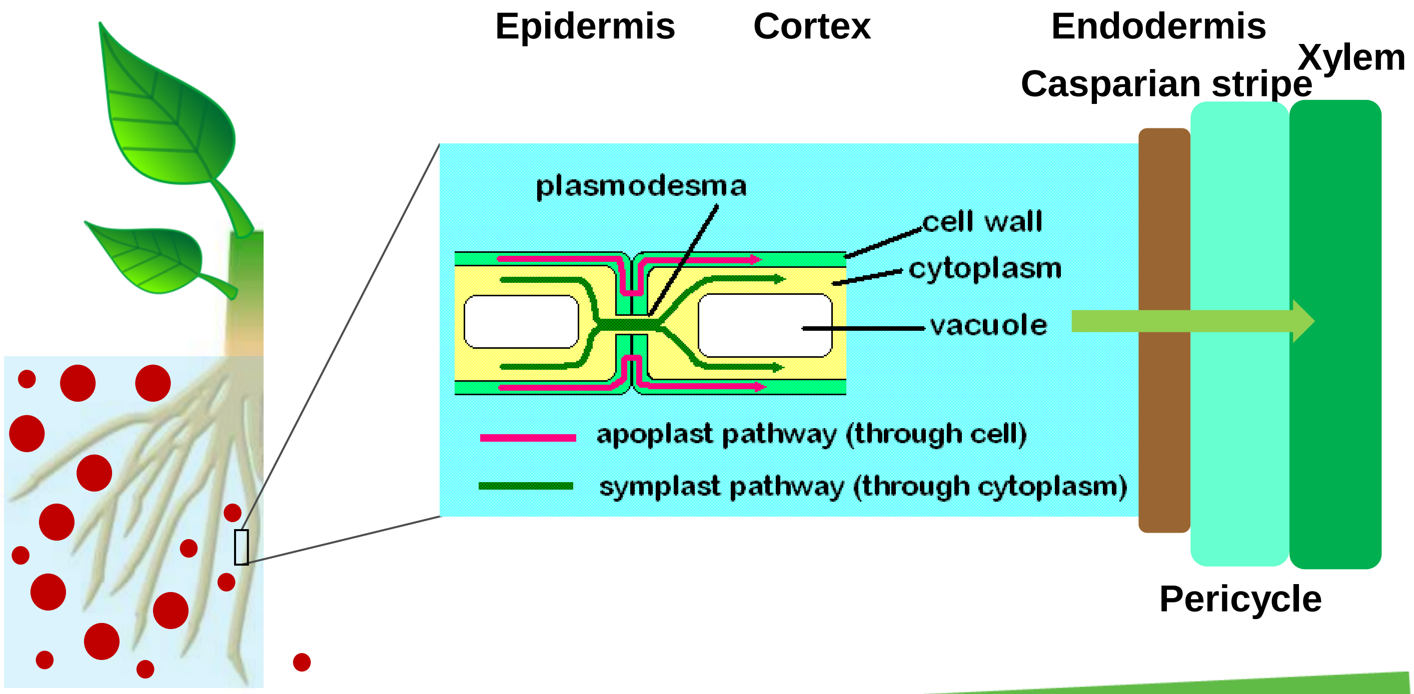
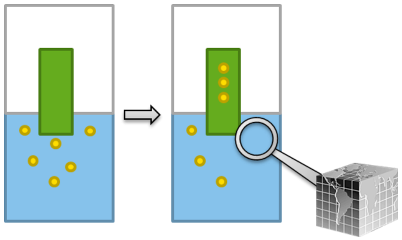


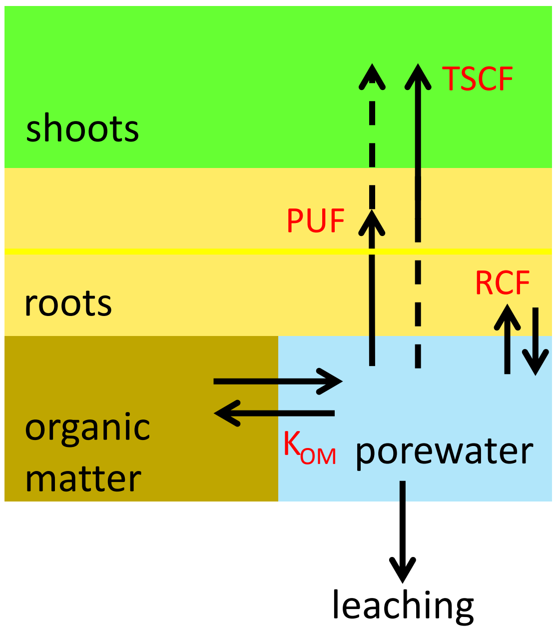
After entering the plant via the root hairs, a chemical can follow:
Apoplastic pathway via the cell walls
Symplastic pathway via the plasmodesma
Transcellular pathway from vacuole to vacuole

Introduction
Uptake Factors

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.



TSCF: * Transpiration Stream Concentration Factor
PUF: **Plant Uptake Factor
RCF: Root Concentration Factor
K_{OM}: Distribution Coefficient Soil Organic Matter and Porewater

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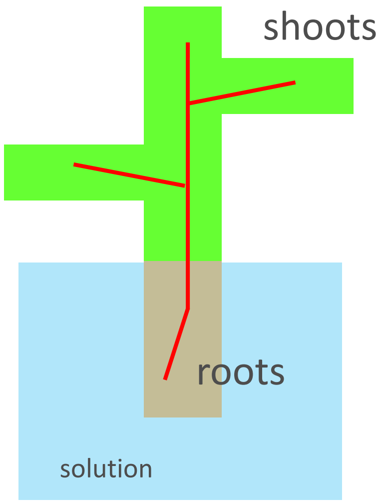
Introduction
Calculation of Uptake Factors

Uptake in aerial part (shoots)

$$TSCF = \frac{\ln\left(1 - \frac{m_{shoots}}{m_{shoots} + m_{sol\text{-}8}}\right)}{\ln\left(\frac{V_{sol\text{-}8}}{V_{sol\text{-}0}}\right)}$$

Uptake in whole plant (roots & shoots)

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



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

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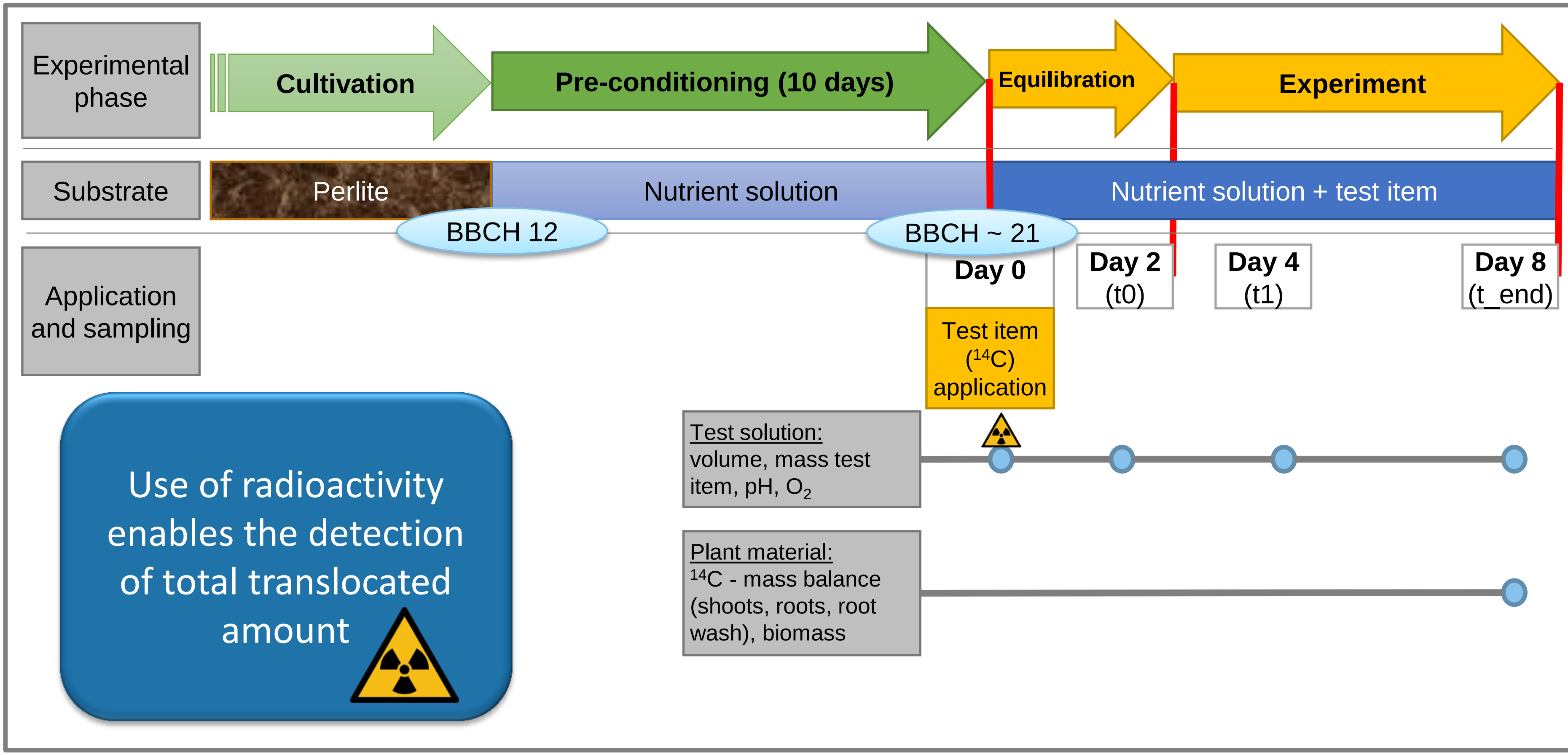
Plant uptake: study design



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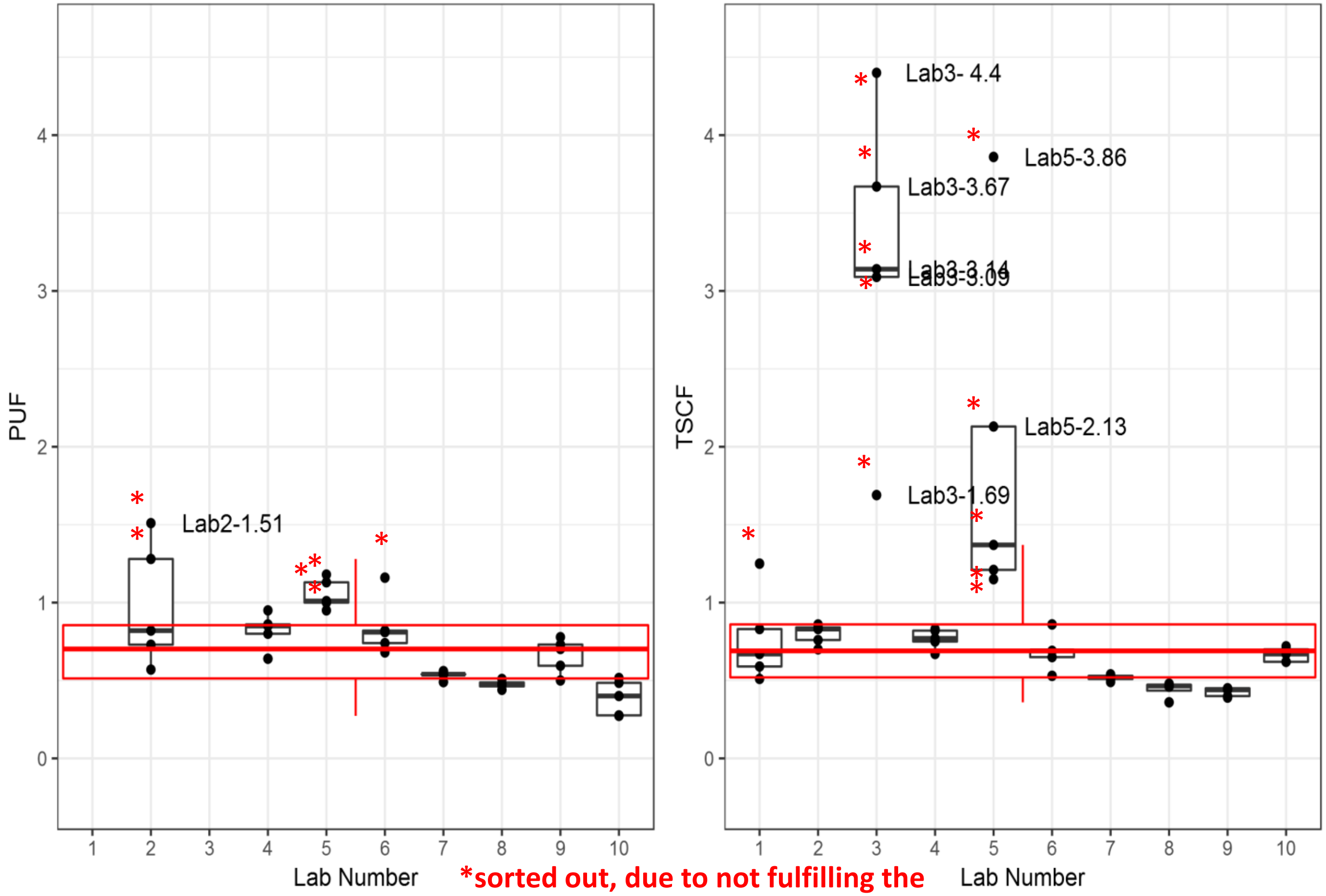
Results from ring test with 1,2,4-triazole in wheat



*sorted out, due to not fulfilling the quality criteria

*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)

	Mean	Confidence Interval (95%)
PUF		
PUF (n=39) without quality check	0.73	(0.64 - 0.82)
PUF (n=33) with quality check "biomass" (biomass factor >= 1.739 OR biomass factor < 1.739 and initial biomass > 1.55)	<u>0.65</u>	(0.57 - 0.73)
TSCF		
TSCF (n=49) without quality check	1.03	(0.76 - 1.3)
TSCF (n=39) with quality check "biomass" (only replicates with biomass increase of > 0.67 g over 8 d)	<u>0.64</u>	(0.58 - 0.70)

Conclusion: PUF≈TSCF, narrow confidence interval

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Plant uptake: study design

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

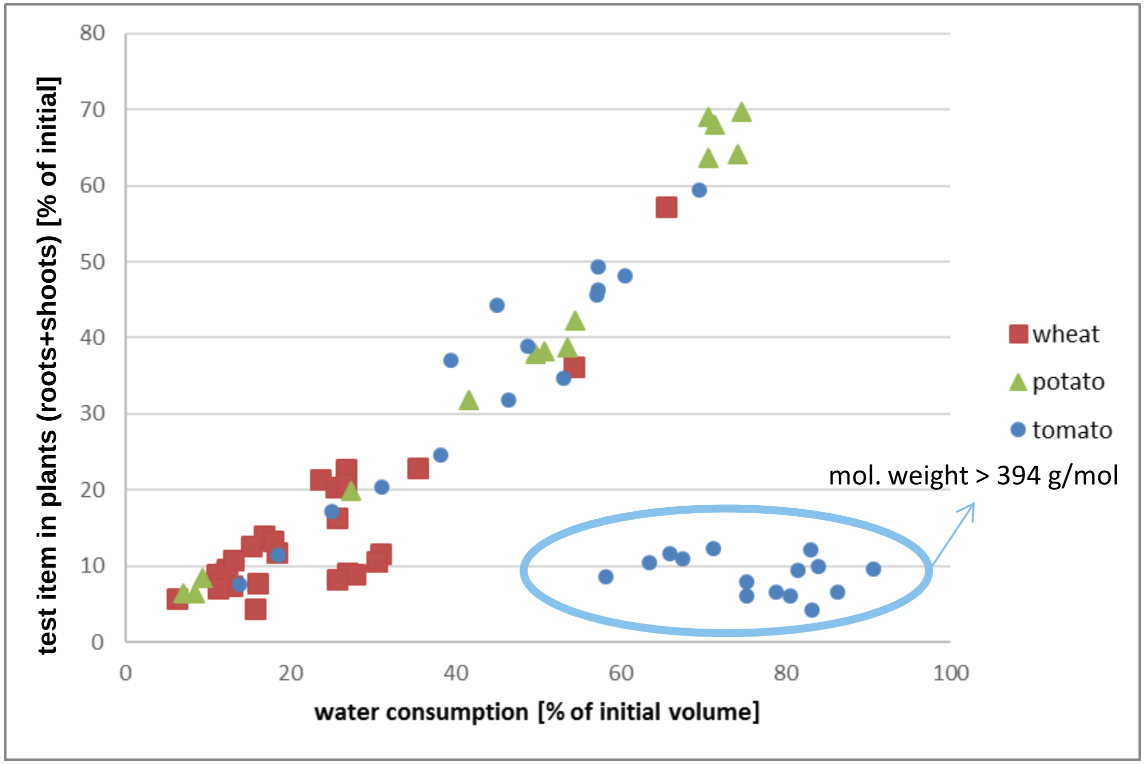
	1	2	3	4	5	6	7	8	9	10	11
Substance	A	B	C	D*	E	F	G	H	I	J	K
Potato	X	X	X								
Wheat				X	X	X	X	X			
Tomato					X	X	X		X	X	X

* 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

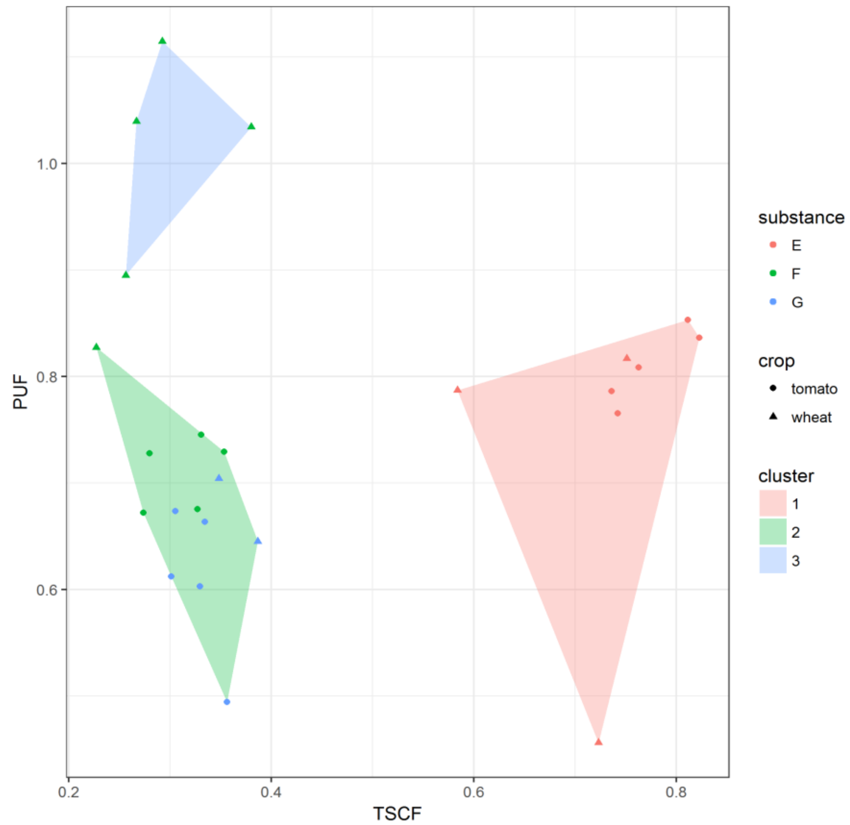
Uptake studies with various crops/compounds combinations

- Uptake is correlated with transpiration (mol. weight ≤ 363 g/mol)
- Uptake decreases when mol. weight > 394 g/mol



Uptake studies with various crops/compounds combinations

- **PUF and TSCF: 3 of 4 replicate clusters consistent with 3 tested substances**



Hypothesis:
If plants are comparable (size, growth, transpiration), then species per se does not play a major role.

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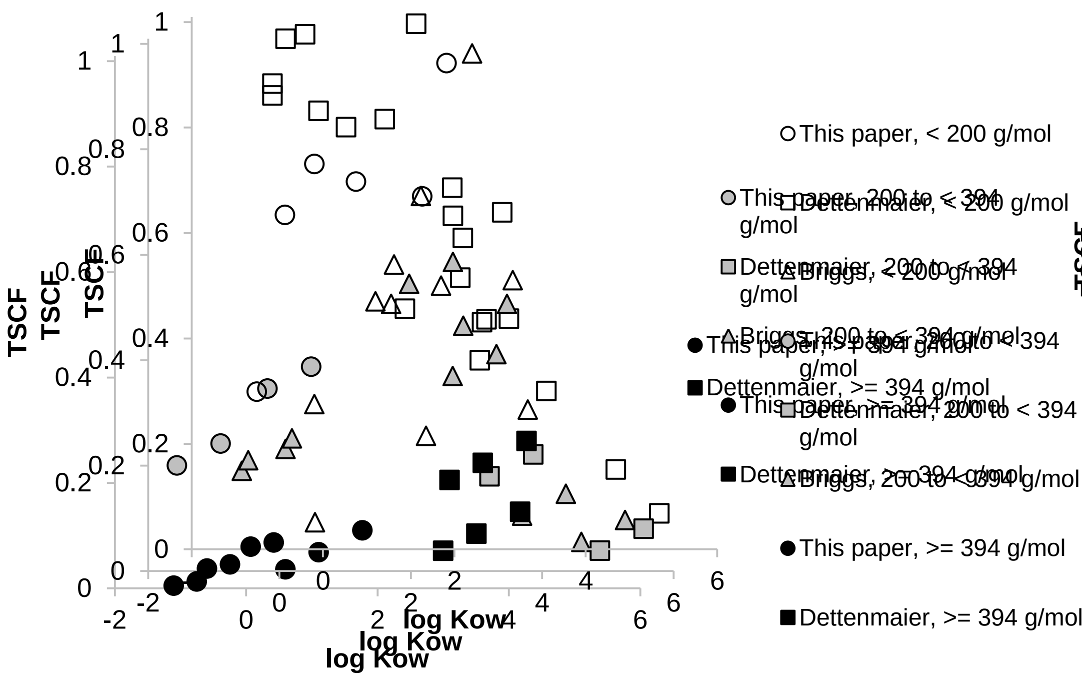
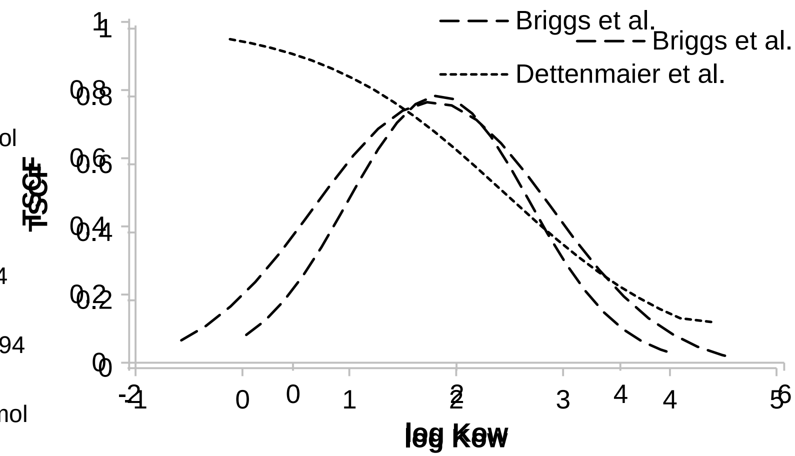
Summary of study results

Sub-stance	Plant	MW[(1)] [g/mol]	Log Kow	PUF (± SD)	Confidence Interval (95%)	TSCF (± SD)	Confidence Interval (95%)	Radioactive recovery [%]	WUE [g/L]
A	potato	114	0.50	0.67 ± 0.20	(0.48, 0.86)	0.70 ± 0.05	(0.66, 0.74)	95.8	97.3
B	potato	169	1.51	0.68 ± 0.02	(0.65,0.69)	0.67 ± 0.01	(0.65,0.69)	99.8	30.5
C	potato	183	1.88	0.95 ± 0.04	(0.91,0.99)	0.92 ± 0.03	(0.89,0.95)	96.0	63.4
D	wheat	69	-0.58	0.64 ± 0.19	(0.57,0.71)	0.67 ± 0.18	(0.61, 0.73)	98.0	35.9
E	wheat	141	-0.13	0.69 ± 0.16	(0.51,0.87)	0.69 ± 0.06	(0.64,0.73)	99.7	54.9
F	wheat	142	-1.01	0.98 ± 0.12	(0.88,1.08)	0.28 ± 0.05	(0.24, 0.32)	97.5	31.6
G	wheat	217	-0.18	0.65 ± 0.12	(0.55,0.75)	0.37 ± 0.03	(0.34,0.4)	96.4	14.9
H	wheat	369	-1.54	0.31 ± 0.07	(0.25,0.37)	0.2 ± 0.02	(0.18,0.22)	98.3	30.9
E	tomato	141	-0.13	0.81 ± 0.04	(0.77,0.84)	0.78 ± 0.04	(0.74,0.82)	96.2	54.9
F	tomato	142	-1.01	0.71± 0.03	(0.68,0.74)	0.31 ± 0.03	(0.28,0.34)	99.9	51.6
G	tomato	217	-0.18	0.60 ± 0.07	(0.55,0.67)	0.33 ± 0.02	(0.31,0.35)	96.1	35.3
I	tomato	394	0.60	0.13 ± 0.01	(0.12,0.14)	0.04± 0.00	(0.03,0.05)	96.5	47.0
J	tomato	549	-1.10	0.02 ± 0.03	(0.0,0.04)	0.01 ± 0.00	(0.00,0.01)	109.1	37.0
K	tomato	563	-0.75	0.09 ± 0.04	(0.05,0.13)	0.01 ± 0.00	(0.01,0.01)	95.7	40.5

- 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





- ❖ Briggs: small plants/short time (48h)
- ❖ Dettenmaier: detopped plants

- **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**

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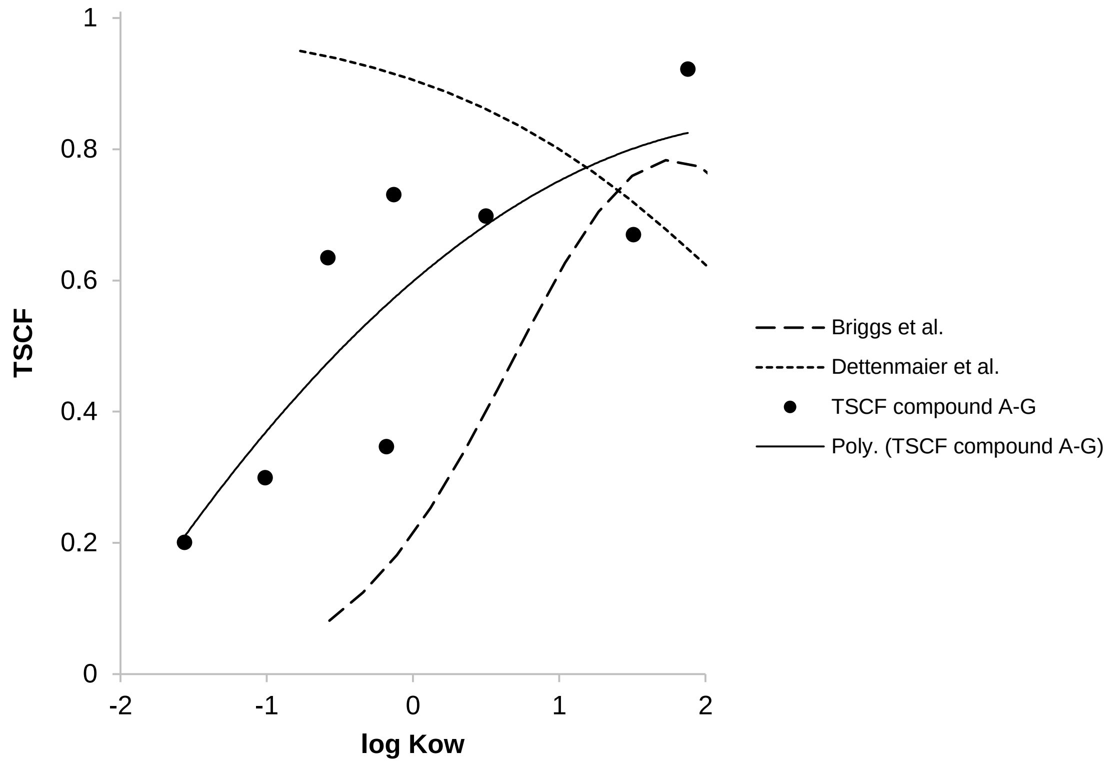
Conclusion on TSCF predictability



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)?

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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)

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Thank You!

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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**?

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

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