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

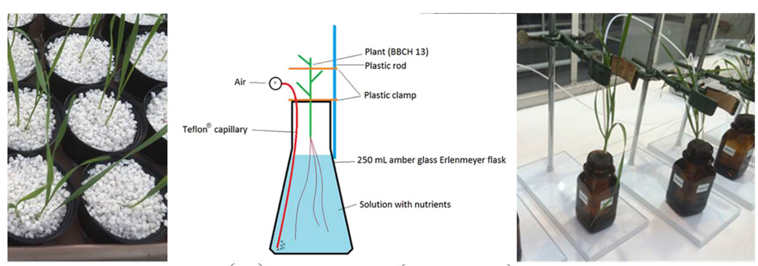
Marc Lamshoeft [a,*], Zhenglei Gao [a], Herbert Resseler [b], Carola Schriever [c], Robin Sur [a], Paul Sweeney [d], Sarah Webb [d], Birgit Zillgens [e], Marco U. Reitz [f]

[a] *Bayer AG, Monheim, Germany*
[b] *Syngenta Agro GmbH, Maintal, Germany*
[c] *BASF SE, Limburgerhof, Germany*
[d] *Syngenta Ltd., Bracknell, United Kingdom*
[e] *DuPont GmbH, Neu-Isenburg, Germany*
[f] *Industrieverband Agrar e. V. (IVA, German Crop Protection, Pest Control and Fertilizer Association), Frankfurt am Main, Germany*

HIGHLIGHTS

- Novel study design to determine the uptake of chemicals by plant roots
- Clear correlation of water uptake and compound uptake as well as TSCF and log Kow
- Limited uptake for substances with high molecular weight (>390)
- Mathematical derivation and proposal for calculation of uptake factors
- Statistic evaluations on uptake factors show robustness and reproducibility of the new test design

GRAPHICAL ABSTRACT



$$PUF = \frac{\ln\left(\frac{m}{M_0}\right)}{\ln\left(\frac{v}{V_0}\right)} \qquad TSCF = \frac{\ln\left(1 - \frac{m_{shoots}}{m_{shoots} + m_{sol}}\right)}{\ln\left(\frac{v_{sol}}{V_0}\right)}$$

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ABSTRACT

A new hydroponic study design to determine uptake of chemicals by plant roots was tested by (i) investigating uptake of [^{14}C]-1,2,4-triazole by wheat plants in a ring test with ten laboratory organizations and (ii) studying uptake of ten other radiolabelled chemicals by potato, tomato or wheat plants in two laboratories. Replicate data from the ring test were used to calculate plant uptake factor (PUF) values (uptake into roots and shoots) and transpiration stream concentration factor (TSCF) values (uptake into shoots). Average PUF for 1,2,4-triazole was 0.73 ($n = 39$, 95% confidence interval (CI): 0.64, 0.82) and the corresponding TSCF value was 1.03 ($n = 49$, 95% CI: 0.76, 1.3). Boxplots and subsequent classification tree analysis of PUF and TSCF values showed that potential outlier values were >1.38 and were observed for PUF replicates with low biomass increase (ratio of final to initial biomass ≤1.739) and small initial biomass (≤1.55 g) and for TSCF replicates with an increase in biomass of <0.67 g over a period of eight days. Considering only valid replicate data, average values of PUF and TSCF were 0.65 ($n = 33$, 95% CI: 0.57, 0.73) and 0.64 ($n = 39$, 95% CI: 0.58, 0.70). The additional experiments with ten chemicals and three plant species showed that uptake was low for polar substances of high molecular weight (≥394 g/mol) and that TSCF values increased with log K_{ow} values of the tested chemicals ranging from −1.54 to 1.88 (polynomial equation with $R^2 = 0.64$). A cluster analysis for three of the compounds that were tested on

* Corresponding author.
E-mail address: marc.lamshoeft@bayer.com (M. Lamshoeft).

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wheat and tomato indicated that the plant uptake was mainly determined by the substance. Overall, the findings show that the hydroponic study design allows for reliable quantification of plant uptake over a range of compound/crop combinations.

1. Introduction

The fraction of plant protection products (PPP) taken up by plants from soil water determines the quantity of PPP and their metabolites unavailable for leaching. The plant uptake factor (PUF) or the transpiration stream concentration factor (TSCF) account for this process in selected leaching models commonly used for regulatory risk assessment in the EU. However, an approved protocol for a standardised test design is not yet available.

The uptake of chemicals by plants is a complex biological process. The plant uptake of non-essential metals and neutral xenobiotics by roots was found to be mainly passive with the substances moving into the plant in proportion to the amount of water transpired (Sheets, 1961; Shone and Wood, 1972, 1974; Chen et al., 2009). This is also subject to influences such as humidity, sunlight, and exposure duration (Taiz and Zeiger, 1998; Marschner, 1995). Many different approaches were investigated to determine the translocated amount of chemicals from soil water into plants and resulted in information on the uptake of substances by whole plants (Chen et al., 2009) or on concentrations in parts of the studied plants (e.g., the transpiration stream, shoot parts, roots). The TSCF was introduced as the ratio of the concentration of a chemical in the xylem sap to that in the soil solution and has been widely used as a descriptor of compound uptake by roots into the upper plant parts (Shone and Wood, 1974; Briggs et al., 1982, 1983; Hsu et al., 1990; Burken and Schnoor, 1998; Trapp, 2000; Dettenmaier et al., 2009; Garvin et al., 2015).

The TSCF has also been used as surrogate value for describing compound removal from the soil pore water in regulatory environmental fate models (e.g. FOCUS PEARL, FOCUS PELMO and MACRO) (Carsel et al., 1998; FOCUS, 2000, 2009; Klein, 2012; Leistra et al., 2000). In these models, plant uptake is effectively a "sink" process or loss mechanism for chemicals in soil and the eventual fate of compounds within the plant is not accounted for. If a volume V of water is simulated to be lost from a soil layer containing a concentration of chemical in soil pore water of C, then the mass lost from the soil pore water if the chemical is taken up passively is C x V. If the uptake factor is 1, 100% of the mass contained within the soil pore water of volume V is lost from the soil system in that volume. If the uptake factor is 0.5, only 50% of the chemical in soil pore water will be lost from the water/soil system. Therefore, if the uptake factor differs from 1, then the concentration of the chemical in the water surrounding the roots must change over time. This dynamic is not explicitly taken into account in the environmental fate models, when using a TSCF, because storage in the root is then not considered.

For these reasons the PUF is proposed to be a more robust measure for parameterizing environmental fate models. This factor is defined as a descriptor of change in concentration in soil solution due to uptake of water and compound by the plant roots and determines the mass removed from soil into roots and shoots in total. However, the TSCF relating only to the mass that is transported into the shoots is preferred to parameterise the respective models, since this will lead to an underestimation of the compound mass removed from soil. Thus, regulatory decisions will be based on a more conservative figure per se. For modelling purposes, uptake parameter values were often estimated based upon the TSCF equation proposed by Briggs et al. (1982), because until now no standard protocol or guidance on determination of root uptake has been available that exactly matches the concept of root uptake implemented in the regulatory environmental fate models. Dettenmaier et al. (2009) conducted plant uptake studies and found that experimental data did not always reflect the expected compound behaviour according to Briggs et al. (e.g. sulfolane (log $K_{ow} = -0.77$): $TSCF_{exp} = 0.88$, $TSCF_{Briggs} = 0.05$; MTBE (log $K_{ow} = 0.94$): $TSCF_{exp} = 0.82$, $TSCF_{Briggs} = 0.59$) (Doucette et al., 2005; Rubin and Ramaswami, 2001). In addition, a large set of reported TSCF values was reviewed (191 average TSCF for 115 compounds from 30 peer-reviewed publications), but Dettenmaier et al. found no apparent relationship between average TSCF and log K_{ow} of compounds. Moreover, large variations were observed for compounds with more than one average TSCF which was attributed to differences in experimental approaches and operational variables. The difference between measured and calculated TSCF values, as well as the high range of variability in experimentally determined values indicates that a standardised study design with good reproducibility is needed.

Due to these facts, there is a great interest to set up a simple but reliable test system to measure root uptake by plants in order to be able to parameterize regulatory environmental fate models to simulate uptake of chemicals from soil solution by plants (EFSA, 2013). Therefore a proposal for a novel test design to derive uptake parameters from simple laboratory studies was developed considering suggestions from academia and regulatory authorities during a workshop held in York (UK) in September 2013. The workshop was conducted to establish an up to date understanding of plant uptake science and its implementation in the leaching models.

The present article describes a novel experimental method for quantifying plant uptake of chemicals via the root system that has its first origin at the workshop in York. In addition, we provide a novel mathematical derivation of PUF and TSCF to calculate these input parameters for regulatory environmental fate models. Root uptake is measured in a hydroponic environment where plants are exposed to a root-zone chemical concentration. The hydroponic environment is generally used for ease in measuring and controlling the exposure concentration (Aryal and Reinhold, 2013; Gent et al., 2007; Inui et al., 2011; Murano et al., 2010). Furthermore, the hydroponic environment facilitates attribution of changes in exposure concentration to plant uptake, since adsorption like in soil-bound systems is excluded in this set-up. PUF values can be calculated based on measured chemical concentration in the solution and measured solution depletion due to transpiration over the course of the experiment. This method is therefore analogous to how regulatory models of environmental fate simulate this process. Additionally, determination of chemical in root and shoot tissue at the end of the experiment allows for calculating TSCF values. The purpose of the novel study design is to obtain clear evidence of the extent to which molecules can be taken up by plant roots. The study design is not intended for investigating the dynamic of uptake (and distribution) processes during various developmental stages of the plant. Sorption and degradation processes of chemicals in soils which affect (reduce) uptake of PPP are also excluded. The novel study design was tested in a tiered approach: In the first stage of investigations, ten laboratory organizations with different levels of experience with uptake testing participated in a round robin test and studied uptake of [^{14}C]-1,2,4-triazole by wheat plants. In a second phase the study design was used to investigate uptake of ten additional radiolabelled chemicals by potato, tomato or wheat plants in two laboratories. The experimental data were used to calculate uptake parameters PUF and TSCF and statistical analysis were performed to conclude about the repeatability and reproducibility associated with this study design.