DEVELOPMENT AND APPLICATION OF HYDROPONIC LIKE TEST SYSTEMS FOR THE DETERMINATION OF PLANT UPTAKE FACTORS (PUF) OF XENOBIOTICS TO BE USED AS PARAMETER IN ENVIRONMENTAL FATE MODELS

by

Victor Gourlay
from Cherbourg (France)

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Thesis examiners:
Prof. Dr. Gabriele E. Schaumann, University Koblenz-Landau
Prof. Dr. Roland Kubiak, RLP AgroScience GmbH

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ABSTRACT

Environmental monitoring and bioremediation programs require the description of the environmental fate of specific compounds. In the context of modern agricultural treatments, mathematical models are used to describe the environmental exposure, distribution and accumulation. These are established upon the interaction between "sink" compartments. In this study, the *plant* compartment was more specifically investigated, via the plant uptake. The aim of the study, as there is presently no universally accepted experimental approach to estimate this uptake, was to develop a test protocol which could be standardized for the investigation of extended ranges of compounds, crops properties and soil pH conditions.

The plant uptake was estimated in terms of Plant Uptake Factor (i.e. PUF), which can be used as parameter for environmental models. This factor was calculated based on the compounds depletion in an artificial soil solution which was available for root uptake. In this contest, both the concentration and the transpiration volume were investigated. The test system consisted of intact plants incubated in treated solutions. Two successive versions of the protocol were investigated, mainly diverging in terms of plant cultivation and properties of the solution.

The range of investigation consisted of different crop types (tomato, wheat and oilseed rape), pH levels (5.5, 6.5 and 7.5) and compounds (×9, $\log K_{ow}$ = {-3.2 to 3.9}). First of all, despite some senescence, all crop species developed new tissue and led to sufficient transpiration. Overall, the uptake behaviour differed significantly between crop types. Secondly, the target pH conditions were achieved using adapted buffers. There was however no significant influence of the pH level on the uptake. Finally, most of the chosen compounds were adapted for investigation. On the one hand, the depletion of compound in solution led to a mean plant uptake factor value of 1.01 ($n = 189$, $SD < 25$ %), corresponding to a relative unrestricted passive uptake within the transpiration stream, independent from the lipophilicity of the compounds. One the other hand, when restricted to the shoots, the plants uptake was inversely linear with the lipophilicity.

Six experimental recommendations could finally be given for the establishment of an adapted standardized protocol. First of all, (1) the presented test protocol is primarily adapted for the investigation of water soluble and low sorbing compounds. However, it can be adapted for the investigation of high sorbing compounds with (2) the implementation of a relevant equilibration period with the treated solution. Furthermore, (3) it is recommended to use non-buffered nutrient solutions to ensure optimal plant conditions. Yet, for dissociating compounds, simplified and controlled solutions (e.g. defined nutrients, buffer systems) can eventually be used. Moreover, (4) it is recommended to cultivate the plants under hydroponics conditions. Notwithstanding, if the use of soil media cannot be avoid, the root integrity should be ensured before treatment. Additionally, (5) for representative uptake results, the duration of the treated incubation period should be primarily chosen regarding coherent water consumption volumes. Finally, (6) it is recommended to use a continuous aeration in solutions in order to ensure optimal plant cultivation conditions.

KEYWORDS: Plant uptake factor, Lipophilicity, Experimental recommendations.

EXTENDED ABSTRACT

Introduction

In the context of an efficient environmental contamination monitoring, and the suitable design of bioremediation programs, the description and the prediction of the environmental fate of xenobiotic compounds are nowadays particularly relevant. For a given compound, several physical and chemical processes may occur, depending not only on its properties but also on its environmental location and the surrounding conditions. Ultimately, these processes can lead to the accumulation of this compound in environmentally relevant compartments, such as plant material and groundwater.

Special interest was here given to the application of synthetic compounds for modern agricultural treatments. In this context, several environmental mathematical models are available, allowing for example the description of soil leaching flows and groundwater situation. These models are implemented with environmental data sets and compound properties, and take into account the interaction between various “sink” compartments, such as the adsorption on soil particles, the hydrolysis in the aqueous flows and the plant uptake.

Concerning the estimation of the plant uptake, there is currently a lack of universally accepted experimental approaches. The experimental state of the art corresponds to several experimental test protocols that have been developed over the years, mainly diverting in terms of calculations and system characteristics. As a consequence, the comparison between the various experimental results cannot be straightforward.

Purpose of the study

The purpose of the study was to develop a new standardized test protocol for the experimental determination of the plant uptake. The protocol was aimed to be adapted for the investigation of a large range of compounds, in terms of lipophilicity, adsorption and eventually dissociation properties. It was therefore important to ensure that the measurements were independent from expected parallel processes occurring in soil, such as particle adsorption and degradation.

Furthermore, it was intended to describe the plant uptake for representative field culture crops, within their development cycle, with sufficient water transpiration. In this context, it was important to ensure that the estimation of the plant uptake was independent from any eventual side events, such as evaporation losses.

Material and Methods

The quantification of the plant uptake was described in terms of plant uptake factor and was performed via the monitoring of both the concentration of compound available for uptake and the cumulative transpiration. The corresponding mass depletion of the compound in the solution was a result of the plant uptake.

The description and discussion of the final test protocol resulted from the investigation of two successive test protocols. A standard test protocol was initially proposed, followed by its improvement. Both consisted of intact plants with free-floating roots, incubated in treated solutions for a period of at least eight days, and under typical greenhouse conditions. These protocols nevertheless diverged in terms of cultivation method used for the plants, as well as properties of the test solutions.

On the one hand, the *standard* test protocol consisted of soil-cultivated plants, rinsed from soil and incubated in buffered calcium chloride solutions (see Figure I). One the other hand, the *improved* test protocol consisted of hydroponics-grown plants cultivated in pots hovering over aerated nutrient solutions (see Figure III).

The range of investigation comprehended three representative crop types: tomato, wheat and oilseed rape; three soil-pH conditions: 5.5, 6.5 and 7.5; and a set of nine compounds taken on a wide range of lipophilicity, with $\log K_{ow}$ between {-3.2 and 3.9}.

Validation of the test systems

First of all, the test system with intact plants was established for most of the given set of compounds and corresponding properties, allowing the plant uptake factor values to be determined. However, based on some outlier results, the test system appeared to be limited for the investigation of compounds with very high sorption abilities.

Furthermore, the investigated pH conditions for the incubation solutions were successfully established by using specific environmental buffer systems. On the one hand it was shown that, when actively controlled, the pH level in solution did not significantly impact the resulting plant uptake factor. On the other hand, the presence of buffers in the solutions caused some phyto-toxicity. It was therefore acquired that the use of buffer in solutions should be only considered in accordance with the compound's properties.

Additionally, the soil-less characteristics of the test system allowed estimating the plant uptake factor values independently from the given soil specific processes. Furthermore, the expected parallel processes could be estimated and corrections could be implemented in the plant uptake factor calculations. This was particularly important when an active aeration was used in the solutions, thus enhancing evaporation losses. Finally, the amount of solution available for uptake was always adapted compared to the plant development stage and the corresponding transpiration rate, allowing the achievement of coherent uptake volumes.

Moreover, the differentiation between the three crop species was possible, revealing a significant impact on the plant uptake factor values. The sufficient health of the plant during the incubation, required for the natural water transpiration, could be ensured. More importantly, despite the senescence of the older leaves, the development of new plant tissue was always observed. However, when the hydroponics cultivation in hovering pots was used, as part of the improved test protocol, a significant enhancement of the general plant health and development could be observed, together with higher water consumption rates, indicating a better adaptation of the plants to the test system.

Finally, independently from the test system, the incubation protocol gave the opportunity to eventually adapt the plant uptake factor calculation to specific properties or behaviours of the compounds, such as a strong initial adsorption on the roots. It also gave the possibility to restrict the calculation to chosen plant fractions, such as the shoots, as confirmation of the eventual translocation as well as possible comparison with previous studies.

Plant uptake factor results

On the one hand, when the depletion of compound in solution was considered, the lipophilicity of the compounds did not greatly influence the plant uptake factor values. The overall mean value for all compounds, crop types and pH levels was determined to be 1.01 ($n = 189$, $SD < 25$ %), as illustrated in Figure II with each crop type. This corresponded to a relative unrestricted passive uptake within the transpiration flow. Furthermore, the consideration of the radioactive equivalents in the plant material on termination mostly confirmed the concentration measurements and the resulting plant uptake factor results. Finally, when an equilibration phase with the treated solutions was implemented prior to calculation, only compounds with the highest lipophilic properties were affected, resulting in slightly lower plant uptake values.

On the other hand, when the plant uptake factor calculation was restricted to the residues in shoots, similar to the transpiration stream concentration factor, the uptake results were found lower, but always indicated a potential ability for xylem transport. Furthermore, as illustrated in Figure IV on the higher lipophilicity range, there was a linear correlation between the decreasing uptake values and the increasing lipophilicity of the investigated compounds. Inversely, independently from the test protocol used, the investigated strongly polar compound was seldom translocated to the shoots, indicating an accumulation in the roots.

Conclusions and recommendations

The investigation of both the standard test protocol and its improvements on the range of compound properties, crop types and plant incubation conditions allowed drawing a list of six recommendations. These can be taken into account for the establishment of the most adapted test protocol for the description of the plant uptake behaviour and the corresponding factors.

Recommendation 1: The test protocol is primarily adapted for the investigation of compounds with adequate water solubility, sufficient biotic and abiotic stability in the uptake solution and low adsorption properties. It can nevertheless be further adapted to the investigation of compounds for which adsorption properties are found significant.

Recommendation 2: An equilibration period with the treated solution prior to calculation should be implemented only when found relevant, considering for example the properties of the compound and the initial incubation results.

Recommendation 3: It is recommended to perform the incubation in non-buffered nutrient solutions. Nevertheless, if the pH level is pertinent, with dissociating compounds or specific

soil properties for example, additional buffer systems can be used in the solutions in order to investigate environmentally specific pH ranges.

Recommendation 4: It is recommended to cultivate the test plants under adapted hydroponics conditions, with free roots, in order to ensure optimal plant development and transfer. However, if cultivation in soil cannot be avoided, the integrity of the roots should be ensured before treatment.

Recommendation 5: The duration of the treated incubation period should be primarily chosen in order to ensure coherent water consumption volumes, and therefore representative uptake results.

Recommendation 6: It is recommended to use a continuous aeration in solutions in order to ensure optimal plant cultivation conditions and to avoid non-realistic anaerobic conditions. Furthermore, as enhanced evaporation and volatilisation losses can be expected, these should be investigated in parallel.

Figure I Standard test system.

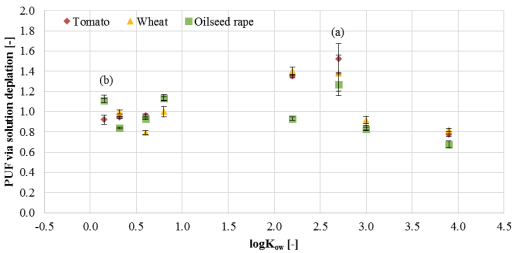

Figure II Plant uptake factor (PUF) as a function of lipophilicity. Mean, ± SE, n = {9, 6 (a) or 3 (b)}.

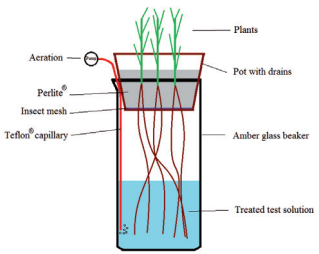

Figure III Improved test system.

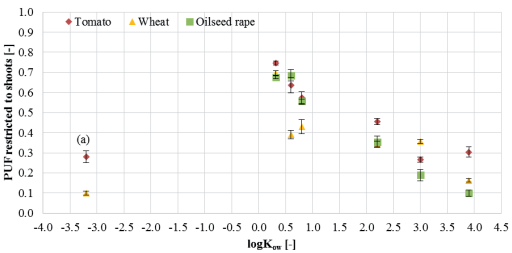

Figure IV Plant uptake factor restricted to shoots as a function of lipophilicity. Mean, ± SE, n = {9 or 3 (a)}.

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LIST OF ABREVIATIONS

A	ANOVA	**AN**alysis **O**f **Va**riance
	AR	**A**pplied **R**adioactivity
B	BBCH	**B**iologische **B**undesanstalt, Bundessortenamt und **CH**emische Industrie i.e. phenological plant development stages (Meier, 2001, [37])
C	$Ca(NO_3)_2$	Calcium nitrate
	CV	**C**oefficient of **V**ariation
D	DAT	**D**ays **A**fter **T**reatment
E	EPA (US)	**E**nvironmental **P**rotection **A**gency (**U**nited **S**tates)
F	FOCUS	**FO**rum for **C**o-ordination of pesticide fate models and their **US**e
G	Gly	**Gly**phosate
H	HPLC	**H**igh **P**erformance **L**iquid **C**hromatography
K	K_a	Equilibrium constant for a chemical **A**cid / Base reaction
	K_{oc}	Soil **O**rganic **C**arbon / Water partitioning coefficient
	K_{ow}	**O**ctanol / **W**ater partition coefficient
	K_{sw}	Bulk **S**oil / pore **W**ater distribution coefficient
L	LC-MS/MS	**L**iquid **C**hromatography coupled with tandem **M**ass **S**pectrometers
	LSC	**L**iquid **S**cintillation **C**ounting
M	MES	2-(N-**M**orpholino)**e**thane**s**ulfonic acid
	MUP	Cumulative **M**ass **UP**take of compound

O	OECD	**O**rganisation for **E**conomic **C**ooperation and **D**evelopment
N	n	Number of replicates
P	pK_a	**A**cid dissociation constant
	PUF	**P**lant **U**ptake **F**actor
Q	QSAR	**Q**uantitative **S**tructure–**A**ctivity **R**elationship
R	RCF	**R**oot **C**oncentration **F**actor
	ρ	Mass density
	ROI	**R**egion **O**f **I**nterest
T	TI	**T**est **I**tem (i.e. compound)
	TLC	**T**hin **L**ayer **C**hromatography
	Tris•HCl	**Tris**(hydroxymethyl)aminomethane **hydrochloride**
	TSCF	**T**ranspiration **S**tream **C**oncentration **F**actor
V	VUP	Cumulative **V**olume **UP**take of solution