University of Reading

# Quantifying and understanding the uptake of plant protection products from soil into plants

**Doctor of Philosophy**

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# Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged

Benjamin M. Jones

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# Glossary of Terms

TSCF – Transpiration Stream Concentration Factor

RCF – Root Concentration Factor

PUF – Plant Uptake Factor

Log $K_{OW}$ – Octanol Water partition coefficient coefficient

PEARL – Pesticide Emission Assessment at Regional and Local Scale, environmental fate model

PELMO – Pesticide Leaching Model, environmental fate model

PRZM – Pesticide Root Zone Model, environmental fate model

MACRO – Environmental fate assessment model

PESTRAS – Pesticide Transport Assessment, environmental fate model

PESTLA – Pesticide Leaching and Accumulation, environmental fate model

SWAP – Soil Water Atmosphere Plant model

pKa – Acid dissociation constant

$K_{OC}$ – Partition coefficient between water and organic carbon

$PEC_{GW}$ – Predicted Environmental Concentration in Groundwater

PPPs – Plant Protection Products

1,2,4-Triazole –

LSC – Liquid Scintillation Counting

TLC – Thin Layer Chromatography

BBCH – Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie

FOCUS – FOrum for the Co- ordination of pesticide fate models and their Use

DT50 – Time taken for the compound to degrade to half the original concentration

$C_{1m}$ – The concentration of pesticide in the liquid phase at 1 metre depth

$T_{MAX}$ – Daily maximum temperature

$T_{MIN}$ – Daily minimum temperature

$ET_{REF}$ – Potential evapotranspiration

MARS – Monitoring Agricultural Resources, an EU Joint Research Council group used for developing methods, tools and systems for agricultural monitoring

Agri4cast – European Commission Joint Research Council database providing gridded meteorological data for use in scientific community

EFSA – European Food Standards Agency

# Introduction to Thesis

## Rationale

Plant protection products (PPPs) is a term that has a very similar definition to pesticides, however pesticides can be used for wider applications such as biocides and other chemicals harmful to fauna. PPPs specifically refer to chemicals used to guard and increase the yield of profitable or desirable crops. This term originated from the Plant Protection Products directive (91/414/EEC) which pertains to the registration of products on the market (Jess *et al.*, 2014). Regulator interest in the safety of pesticides began with the development of synthetic organic chemicals and the creation of DDT. The publication of Silent Spring by Rachel Carson in 1962 brought the mal effects of synthetic pesticides to the forefront of public thinking and proposed that Springtime will be devoid of birdsong if they continue to be used as they are (Zadoks and Waibel, 2000; Beard, 2006). This preceded the setup of the Environment Protection Agency (EPA) in 1970. Within Europe the environmental policy has undergone many iterations since this, with the current strategy for regulation of pesticides within Europe now governed by the European Union (EU) and the individual states within a tiered setup. The first tier is that the producer must provide a dossier to the EU specifying that the product is safe for use within their framework. This is then assessed by the 'rapporteur member state (Regulation No. 1107/2009). As a result of the increased regulatory interest and environmental impact, agrochemical companies began to produce their products to not only have deleterious effects on the target organism but also to have less impact on the environment. This means that pesticides are often designed to reduce the risk to non-target organisms. Methods of doing so include: limiting the transport of the compound from the site of application; reducing the persistence of the compound within the soil profile; and ensuring that the mode of action and toxicity is as organism specific as possible. Despite this, the assessment of the producer needs to be verified by the rapporteur member state and a full risk assessment must be conducted to identify potential hazards, risk of exposure, and determine the likelihood of risk to the environment and non-target organisms.

The second tier is then for the product to be approved for use within the individual member states. Following the approval by the member states, EFSA are then responsible for conducting a peer-review of the risk assessment (EFSA, 2014).

Due to increasing demand and agricultural intensification, production of naturally occurring and synthetic plant protection products (PPPs) has increased, causing a rise in regulatory interest of the use and potential application of these compounds to the soil. To protect human and environmental health, it is imperative to be able to predict how the compound will behave in the soil profile prior to releasing it into the environment.

Environmental fate is the study of the behaviour of compounds once they enter the soil profile and analyses the likelihood that they will be exposed to living organisms. One such pathway is through leaching down the soil profile into potable water supplies (Arias-Estévez et al., 2008). Plant uptake has been proposed as a sink pathway that could help reduce the concentration of PPPs being transported (Lamshoeft et al., 2018). As it cannot be known how much a newly developed product would leach from the soil, it cannot be released to the environment prior to assessment. To conduct these studies, environmental fate models have been developed to simulate example locations and indicate how likely it is that the underlying groundwater will be polluted. A plant uptake value was inputted into these models based on a statistical relationship derived from laboratory experiments. Previously, within these models the statistical relationship was used to determine how much of the compound was taken up by the plants. However, a lack of agreement and reproducibility of results within the scientific community has led to this approach being abandoned, with research calling for a new method to be established. This thesis aims to test the main assumptions used within the current laboratory framework and improve the realism of the test system. This work will then be linked with modelling experiments to determine the effects of plant uptake on the leaching output on simulated soil profiles.

## Aims and Objectives

1. Test whether the main assumptions within the laboratory methodology framework hold true for PUF. Namely whether PUF can remain stable over time.
2. Test the suitability of the hydroponic system as a surrogate for the soil profile by increasing realism.
3. Test the effect that plant uptake has on the leaching output within environmental fate models
4. Test the scenarios where plant uptake may become more significant.

# Chapter Breakdown

Chapter 1 is a literature review of the main methodologies and models that are used within the study of environmental fate, with a focus on the regulatory aspect of plant uptake. Transpiration Stream Concentration Factor (TSCF) and Plant Uptake Factor (PUF) are introduced, with a discussion of the proposed statistical relationships for the octanol-water partition coefficient (log $K_{OW}$) against TSCF relationships. PEARL, PELMO, PRZM and MACRO are the key environmental fate models used within the European regulatory framework. Plant uptake within these will be discussed with a view to how the outputs can be used to measure leachability.

Chapter 2 is a hydroponic experiment designed to test the assumption that PUF is linear over time. This study was conducted using a key metabolite of the azole fungicides, 1,2,4-Triazole. To conduct the study over 21 days, the existing methodologies were adapted to allow for the solution to be topped up daily. Results from this experiment showed that PUF does have a linear relationship over time. Showing a strong linear regression when sampling the solution and the plant material. When conducting these assessments, the composition of the compounds in solution is considerably important as each compound has its own PUF value and degradation can affect the results of PUF.

The aim of chapter 3 was to improve the realism of the current laboratory method by measuring PUF using a sand column and hydroponic solution. The presence of sand provided an inert material that created air filled pores, rather than having the roots fully submerged in hydroponic solution. The methodology in this chapter closely resembles that of Chapter 2 to allow for comparison between the datasets. Unfortunately, fast degradation of the 1,2,4-Triazole meant that a linear relationship could not be determined, but recommendations for future studies have been discussed in detail.

Chapter 4 is the first modelling experiment and was setup using the FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe) locations that are currently used by regulators across Europe. Within this chapter, 3 example compounds were used to give variability in mobility and persistence. The PUF value was then varied between 0-1 and applied to 9 locations using two regulatory models (PEARL and PELMO). The results from this study showed that there was no significant difference between the leaching outputs of both models, however the plant uptake did have a significantly different effect on the leaching output of one compound between the models. This suggests that the model selection may be important when modelling high $K_{OM}$ and long DT50 compounds.

Two experiments are included within chapter 5, aimed to test the effects of climatic inputs on the plant uptake and leaching outputs. The first experiment tests the effects of the climate file on the plant uptake and showed that both potential evapotranspiration and maximum temperature had the strongest linear relationships. The second experiment used a more recent dataset to the original FOCUS files and examined whether the effect of plant uptake was different between the two. Results showed that the effect of plant uptake was not significantly different. However, the leaching levels were lower using the most recent dataset, suggesting that the FOCUS files may need periodic updating.

Chapter 6 aimed to define new 'typical' scenarios using harvested produce and pesticide sales data across Europe, compared to the FOCUS scenarios which are defined as 'realistic worst-case'. Results showed that there was no significant difference between the effect of plant uptake in the 'typical' and FOCUS scenarios.

The implications of the results reported within the chapters are discussed within chapter 7, with recommendations for future research. This chapter also includes a short section reflecting on personal development during this PhD to acknowledge the research training and progress made during the completion of this programme.

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# Chapter 1 – Measuring and modelling the plant uptake and accumulation of synthetic organic chemicals with a focus on pesticides

## 1.1 Abstract

Plant uptake of synthetic organic chemicals involves the transport of a xenobiotic into the plant cells via the roots or shoots. Once the compound enters the plant there are several routes that it can take, which can lead to translocation to the shoots or accumulation within the roots. Modelling is an important aspect of plant uptake as it allows for the prior assessment of risks to human health. There is currently a significant amount of debate within the research community as to the preferred way for quantifying uptake and most appropriate experimental method for measuring uptake. The use of Transpiration Stream Concentration Factor (TSCF) predicted by the octanol-water partition coefficient (log $K_{OW}$) has long been the dominant model, however, recent research has suggested a move away from this predictive relationship. Many studies have been conducted with approximately 200 data points being reported in the peer-reviewed literature; however, statistical analysis has shown that we are no closer to establishing a definitive algorithm to predict plant uptake of organic chemicals. While log $K_{OW}$ could still be an important predictor, other physical-chemical properties, such as molecular weight and hydrogen bond donors could also play a role. Currently, there is ongoing debate as to whether TSCF is the most suitable measure of plant uptake as it only considers the fraction of the compound that has been translocated to the above-ground plant parts. The Plant Uptake Factor (PUF) considers uptake into the whole plant by measuring the change in concentration of the compound in the uptake solution against the change in volume and may provide a more accurate uptake value as a result. Despite this, both the use of further physical-chemical properties and PUF are relatively new and require rigorous testing by researchers to establish their suitability.

## 1.2 Introduction

Human population growth, increasing industrial production, intensification of agriculture, medical development and chemical advances all lead to the production of synthetic organic compounds potentially increasing our exposure to toxic chemicals (Beard, 2006; Kim et al., 2011; Miller et al., 2015). Increased industrial production of naturally occurring and synthetic organic compounds has increased regulatory interest in the use and potential application of these compounds to the soil profiles (Sass and Colangelo, 2006). To protect human and environmental health, it is imperative to be able to predict how the compound will behave in the soil profile prior to releasing it to the environment. Plant uptake is an important process when considering the environmental fate or risk assessment of potentially toxic organic compounds (Trapp, 2004).

Uptake of organic compounds is a process that involves the transport of a xenobiotic substance into the plant physiological system. It is important to clarify that sorption of a xenobiotic to the surface of plant cells is not considered uptake, the compound must cross the cell wall and into the cell structure. Whilst root uptake is the main pathway for transport into plants for most organic compounds, it is also important to note that this is not the only pathway for entering the plant's cells, with the soil-air-plant pathway presenting an alternative (Collins et al., 2006). Compounds that enter the leaf by this route are susceptible to volatilisation from the soil and are transferred to the air before then being deposited onto the leaf surface. This is likely to be an important pathway for high log $K_{OW}$ compounds, which are virtually insoluble in water and tightly bound to carbonaceous material in the soil. The low solubility of these compounds and neutral state suggest they are unlikely to be subsequently transported within the plants xylem and phloem and will remain at the point of deposition (Collins and Finnegan, 2010). Transfer of chemicals post application to soil is one of the dominant routes of environmental contamination. Environmental fate models such as PEARL, PRZM, PELMO and MACRO which are used by manufacturers and regulators to assess each compound within a range of agricultural scenarios to quantify the potential for it to leach into groundwater (Carsel et al., 1985; Klein et al., 1997; Larsbo and Jarvis, 2003; Leistra et al., 2001). Plant uptake is assumed to be a passive process (although there are a few examples of compounds being taken up actively) within these models with values bound between 0 (none) and 1 (complete) (Collins et al., 2006; Zhan et al., 2010). The higher the uptake the more compound is removed from the soil profile, therefore reducing leaching and potential aquifer contamination (Lamshoeft et al., 2018).

This chapter aims to review the elements of plant uptake and discuss their application. The various pathways of plant uptake will be reviewed alongside the methods used to quantify and better understand them. The quantification of uptake will then be discussed with the various contexts for plant uptake.

## 1.3 Plant uptake of xenobiotic compounds

### 1.3.1 Plant uptake via the root pathway

Plant uptake is the transport of organic chemicals dissolved in water into the cortex by transpiration. Small compounds (Molecular weight = <500) can enter the root via the root hairs which vastly increase the surface area (Miller et al., 2015). There are three main pathways for the root uptake of solutes: Apoplastic, Symplastic, and Transmembrane (Figure 1.1). Apoplastic is the extra-cellular transport of the compound, via movement through the cell walls and outside the plasma membrane. Symplastic transport involves intra-cellular movement, through the plasmodesmata; gaps in cell walls creating a thread of cytoplasm and allowing the transfer of solutes. Transmembrane transport is the intra-cellular movement of a compound dissolved in solution, passing in and out of the cell through the plasma membrane each time (Taiz and Zeiger, 2010).

In order to cross the cell membranes and enter the symplastic pathway, the compound needs to cross the lipid bilayer within the cell membrane. A compound needs to be lipid-soluble to pass through the hydrophobic core and permeability is very low to polar or ionic compounds (Sterling, 1994). Compounds traversing the symplastic and transmembrane pathways are translocated more readily because of the Casparian strip (Tanoue et al., 2012). Prior to reaching the xylem, the Casparian strip is a collection of suberised cell walls, which block the movement of compounds via the apoplastic transport pathway. Therefore, compounds taken up solely by the apoplastic route will not be translocated, unless they can cross the plasma membrane of the surrounding cells (Miller et al., 2015; Sicbaldi et al., 1997).

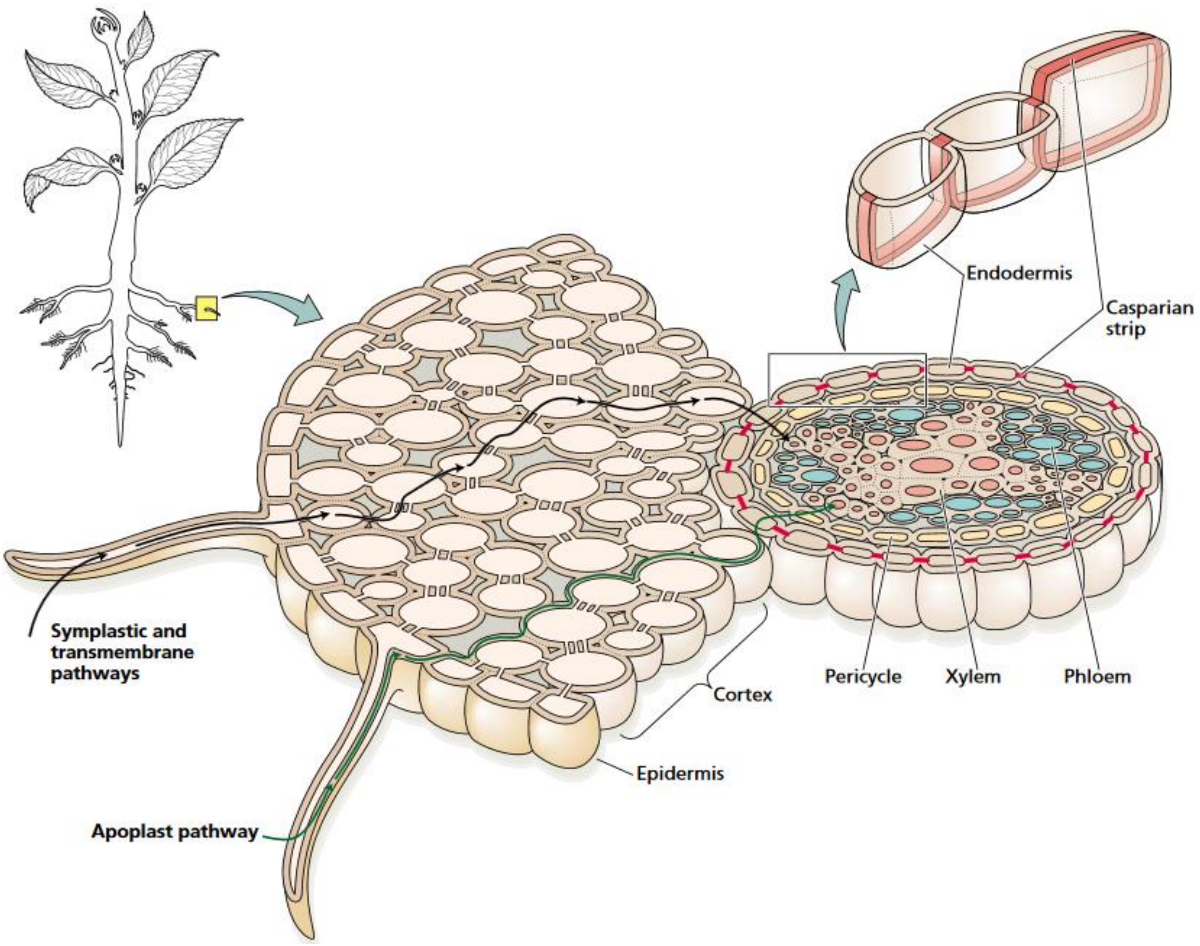

**Figure 1.1 Schematic diagram showing the three main root uptake pathways: Symplastic, Transmembrane and Apoplastic. Also highlighted is the direction of flow once entering the root, the Casparian strip which blocks the apoplastic flow of compounds and the Xylem where compounds are translocated to the above-ground compartment of plants. (from Taiz and Zeiger, 2010)**

Crossing the cell plasma membrane can also lead to a phenomenon called ion trapping which can lead to accumulation within the cells. This occurs due to the different pH that is seen inside the cell membrane (usually around pH 7-7.5), compared to outside the cell which can vary much more substantially. It is possible for a compound to be neutral outside the cell but become ionised in the cytoplasm, thus accumulating inside, as ions are unable to cross the cell membrane (Trapp, 2000). This is dependent on the acid dissociation constant (pKa) of the compound, which helps you to predict how a compound will behave at a specific pH (Sicbaldi et al., 1997). In practice, acids are increasingly neutral when dissolved in solution where the pH level is below the pKa of the compound and bases are increasingly neutral when the solution pH is above the pKa. As the pH gradient between the outside and the inside of the cell increases, the ion trap effect becomes stronger. Reviews of the literature show that pH can affect that bioaccumulation of organic compounds and this effect is highest when the ion trap mechanism occurs. As the interaction between the pH and the pKa value is the most important factor behind this, a relationship is observed when the pKa is subtracted from the pH. Therefore, the effect of pH is greatest when pH – pKa is in the range of -1 to 3 for acids and from -3 to 1 for bases (Rendal et al., 2011).

The plant uptake of solute is strongly coupled with the leaching to groundwater (Legind et al., 2012). Currently, no standardised methodology for calculating plant uptake values has been agreed and a lack of consensus within the scientific community has led to further confusion within the literature (Hoke et al., 2016). The following sections discuss many of the existing equations and derived relationships for measuring the plant uptake of pesticides. This will help to highlight an apparent lack of scientific consensus on the modelling of experimentally derived plant uptake values and help highlight potential avenues for future research.

# 1.4 Measurement of plant uptake

## 1.4.1 Equations and transfer factors for measuring the accumulation of organic compounds

There many approaches for measuring plant uptake experimentally and determining where the compound has accumulated in the plant tissue. Although there has been evidence of some active uptake, it is a widely-held view that the uptake of pesticides and other synthetic organic compounds is mostly a passive process (Collins et al., 2006; Zhan et al., 2010). Compounds taken up by plant roots are dissolved in the soil solution and are absorbed during transpiration (Collins et al., 2006). Therefore, it is possible to assert that the amount of compound available to the plant for uptake is closely related to the concentration in the solution (Shone and Wood, 1974). In general transfer factors (comparison of the concentration within two compartments within a system) can be used to compare any combination of accumulation within a compartment within a plant. In the following subsections those transfer factors used within the measurement of plant uptake in experiments and models are discussed.

### 1.4.1.1 Transpiration Stream Concentration Factor (TSCF)

TSCF is the calculation of the concentration of the compound within the xylem divided by the concentration in solution surrounding the roots (TSCF, Equation 1.1). This calculation allows for the establishment of a fraction of the translocation to the shoots compared to the amount available to the plant roots. All compounds that are taken up passively have a value between 0 – 1, giving a relatively simple concept of uptake. A value of 1 means that all compound that is passively taken up by the roots during transpiration becomes translocated to the shoots. A value of 0.5 means that exactly half of the compound taken up by the roots becomes translocated into the shoots (Russell and Shorrocks, 1959; Shone and Wood, 1974).

$$TSCF = \frac{C_{xylem}}{C_{solution}} \tag{1.1}$$

Although, compounds that are taken up passively can only have results of 0-1, values greater than 1 are seen if the plant is actively taking up the compound (Briggs et al., 1982). Another method for deriving a TSCF value is to analyse the concentration of the target compound within the shoots of a plant and normalise this by the amount of water transpired during this period.

$$TSCF = \frac{[C_{shoots} * \ W_{shoots}]}{C_{solution} * water\ transpired} \tag{1.2}$$

$$TSCF = \frac{C_{shoots}/V_{transpiration}}{C_{solution}} \tag{1.3}$$

$$TSCF = \frac{ln\left(1 - \frac{m_{shoots}}{m_{shoots} + \ m_{sol-t}}\right)}{ln\left(\frac{V_{sol-t}}{V0}\right)} \tag{1.4}$$

Equation( 1.2 )measures the concentration of the compound in the shoots and multiplies the weight of the shoots against this. A similar method is seen in the lower half of the equation, using the solution transpired by the plant multiplied against the concentration in solution (Felizeter et al., 2014). Equation 1.3 uses a similar approach to Equation( 1.2, except the concentration in the shoots is divided by the transpiration volume. This is then divided by the concentration in solution to compare how much has been translocated (Namiki et al., 2015). Equation 1.4 takes the natural log of mass of the chemical within the shoots over the mass within the shoots and the solution, this is then divided by the natural log of the change in volume during the test period (Lamshoeft et al., 2018). Unless specifically stated that a correction has been applied for each study, all the equations listed above work on the assumption that the compound is not phytovolatilised or metabolised after uptake and is therefore present within the plant following sampling (Dettenmaier et al., 2009; Felizeter et al., 2014). All equations are still bound between the values of 0 and 1 for passive uptake and could be used interchangeably with directly measured TSCF datasets.

### 1.4.1.2 Plant Uptake Factor (PUF)

Suggested as an alternative to TSCF the PUF considers uptake into the whole plant rather than the above-ground elements of the plants. Originally it was defined as a simple transfer factor between the plant material and the soil solution (Equation 1.5, Chen et al., 2009). However, more recently an alternative has been suggested, that was derived from the model description of plant uptake. (Equation 1.6, Lamshoeft et al., 2018).

$$PUF = \frac{C_{plant}}{C_{solution}} \tag{1.5}$$

$$PUF = \frac{\ln\left(\frac{m_{sol-t}}{m_{sol-0}}\right)}{\ln\left(\frac{V_{sol-t}}{V_{sol-0}}\right)} \tag{1.6}$$

Where C = Concentration of compound, m = mass of compound, V = volume of solution. The new definition of PUF assumes the plant (roots and shoots), is a ‘black box’ with the roots being surrounded by a solution containing the measured compound (Lamshoeft et al., 2018). By measuring the change in the mass within the solution and the change in the volume over the same period you can determine the fraction of the mass that is lost for the fraction of volume.

## 1.4.2 Laboratory methods of measuring plant uptake

There appears are two methodological approaches to measuring plant uptake in the laboratory. Both have strengths and weaknesses, and these are discussed below.

### 1.4.2.1 Intact plant

The original method devised by Briggs et al. (1982) was conducted using 10-day old whole plants, exposed to their test chemical for 24 hours then shoots chopped off for quantification of the exposure chemical. More recent methods have used this same approach of taking a young plant and measuring the amount of chemical that is taken up into the shoots with minor alterations (Briggs et al., 1982; Burken and Schnoor, 1998; Lamshoeft et al., 2018). The majority of the TSCF values within peer-reviewed literature have been conducted using this method (Doucette et al., 2018).

#### 1.4.2.2 De-topped plant

The alternative to this method is to 'de-top' the plant and maintain the flow of the xylem using a pressure differential. This method was first reported by Hsu et al. (1990), where plants were cut below the first cotyledonary node and the roots submerged in half-strength Hoagland's solution. The first internode above the root system was debarked and trimmed before being sealed within a 1 ml disposable pipette tip. A rubber stopper was then fitted to the top of the pipette tip with a tube to extract the xylem. Compressed air then generated a pressure of (0.27-0.45 MPa) to draw the xylem out through the transpiration stream (Hsu et al., 1990). The method was later used by Dettenmaier et al. (2009) to develop their TSCF vs log $K_{OW}$ relationship.

The reported benefits of this method are that the transpiration stream is directly sampled rather than all shoot material with a subsequent estimate of the uptake based on the transpiration (Dettenmaier et al., 2009). With other methodologies, the plants are incubated for a set period of time, if the plant metabolises the test compound during the exposure period, it is very difficult to tell whether the parent or the metabolite was subject to root uptake. It is possible to correct the uptake value if the rate of metabolism or volatilisation are known, however, they are difficult to determine and such measurements are rarely conducted by researchers (Dettenmaier et al., 2009).

#### 1.4.2.3 Future method development

Current studies are conducted in hydroponic solution, this is suggested as a surrogate for the soil system as it is easier to set up in the laboratory. Firstly, it allows for the transfer of an individual that has been grown in 'clean' hydroponic solution into 'treated' hydroponic solution (Briggs et al., 1982; Dettenmaier et al., 2009). This allows for easier control of the concentration when the plant is exposed to the 'test item' (Doucette et al., 2018). When measuring plant uptake from a soil profile in the laboratory, it is very difficult to transfer the plant and therefore the compound would need to be applied directly to the native profile. This will cause problems with the mixing of the compound within the profile, although this may be more realistic when compared with applications to the environment. It has also been suggested that plants grown in hydroponic solution do not always have the same physiology to those grown in soil, with hydroponic roots showing lower rates of root growth and less development of the Casparian strip (Miller et al., 2015; Perumalla and Peterson, 1986).

TSCF has long been established for measuring the uptake of pesticides and other organic compounds. However, recent scientific debate has centred around whether belowground uptake should be considered, something that TSCF does not incorporate. When considering environmental fate, plant uptake becomes a sink process that removes the pesticide from the soil pore water and transfers it to the biomass. Hence, TSCF is likely an underestimation of the true uptake from soil (Lamshoeft et al., 2018).

Much of the experiments of TSCF and PUF have been between the reproducibility of the data and how well each method performs in comparison with previous (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990; Lamshoeft et al., 2018). Not many tests have been conducted into the consistency of these calculations and how stable their measurements are over time. Adding to this, questions remain about the effect of concentration of the compound and the age of the plant on the uptake. For example, the experiments that have currently been published are conducted almost exclusively on young plants (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990; Lamshoeft et al., 2018). This is likely due to the shorter lead up time for this type of experiment, compared to an experiment that would require a plant that has been grown to maturity. Mature regions of roots are known to develop an exodermis that becomes relatively impermeable to water and solutes (Miller et al., 2015). It is, therefore, possible that younger plants take up the compounds differently to older plants due to the age of the roots.

## 1.5 Physical-chemical relationships used in the modelling of plant uptake

### 1.5.1 Octanol-water partition coefficient (log $K_{OW}$) and TSCF

Log $K_{OW}$ is defined as the concentration in octanol, divided by the concentration in water within a two-phase system (Braekevelt et al., 2003). Log $K_{OW}$ can be considered a measure of the hydrophobicity of a compound and is useful within the application of environmental fate studies due to the observed relationship between a log $K_{OW}$ value and bio-concentration values (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990). Studies of cellular absorption of chemicals across the plasma membrane have shown that compounds with low log $K_{OW}$ values <1.5 can easily absorb through the cell wall but then struggle to pass the cell membrane due to its hydrophobic nature. Alternatively, compounds with very high log $K_{OW}$ values >4.5 become trapped within the cell structure but due to their high lipophilicity (Sicbaldi et al., 1997). The optimum log $K_{OW}$ for cell uptake is between log $K_{OW}$ 2-4, where compounds become more readily absorbed by the cell and are transported more easily (Briggs et al., 1982; Burken and Schnoor, 1998; Hsu et al., 1990; Sicbaldi et al., 1997).

Briggs et al., (1982), used a suite of compounds with a range of log $K_{OW}$ values, plant roots were exposed to the compounds for a 24-hour period, with equilibrium assumed to be reached. Their derived relationship was a Gaussian shape curve with an optimal TSCF at approximately 1.5-2 (Figure 1.2). The pragmatic approach of Briggs et al., (1982) and the simplicity of the relationship allowed for a seamless transfer into pesticide leaching models, with the uptake being derived from the log $K_{OW}$ value. This was later removed due to a lack of agreement and reproducibility in later datasets (EFSA, 2013). Further studies into the relationship of TSCF and log $K_{OW}$, have shown similar relationships but exhibit a variation in the point of maximum uptake and slopes of the curve leading to questions over the use of a universal equation within plant leaching models (Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990). Lamshoeft et al. (2018) restricted their relationship between -2 and 2, due to the compounds they tested being focussed on the risk of leaching into the groundwater (Lamshoeft et al., 2018).

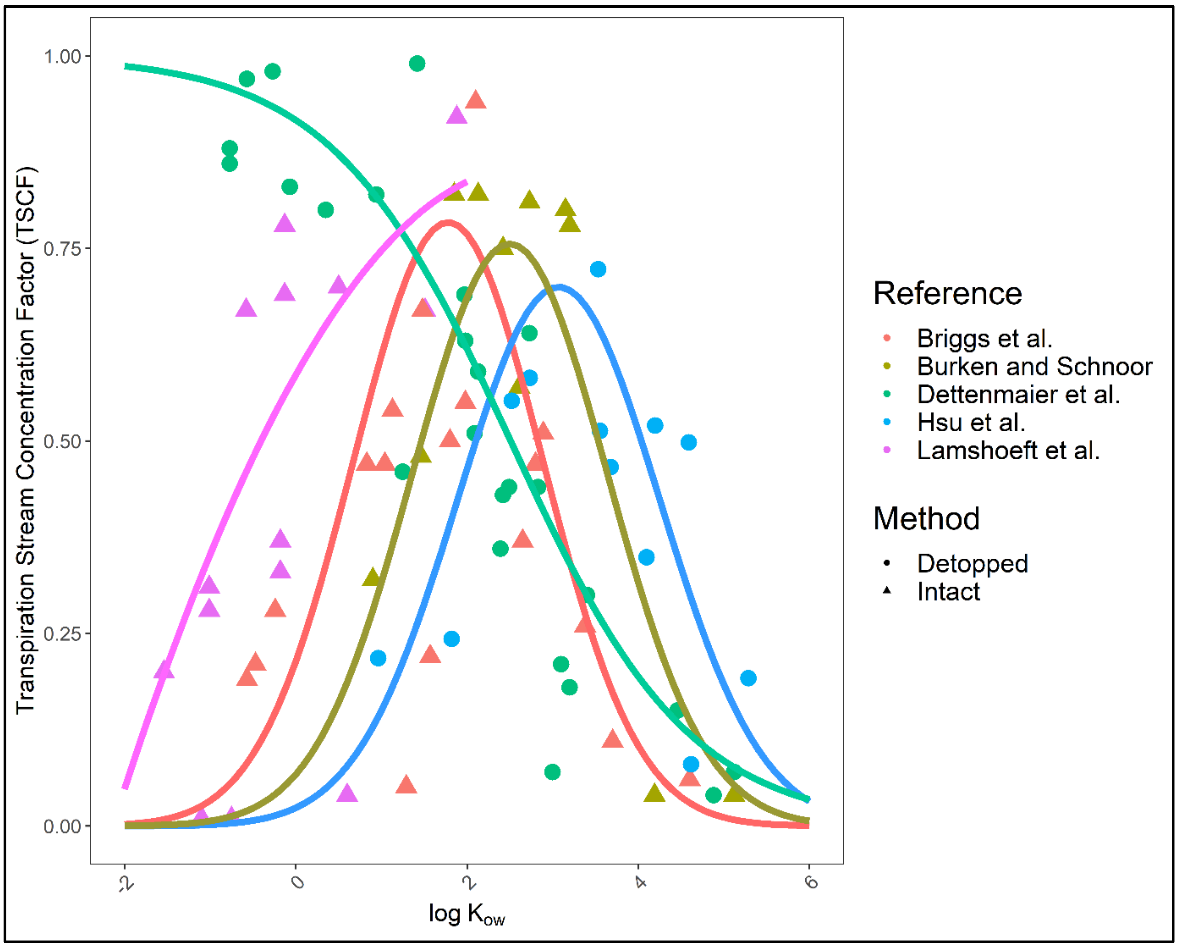

**Figure 1.2 Modelled relationships for log $K_{OW}$ against TSCF. Briggs et al., (1982), (Red); F C Hsu et al., 1990, (Blue); Burken and Schnoor, 1998, (Gold); Dettenmaier et al., 2009, (Green); Lamshoeft et al., 2018, (Pink).**

Briggs et al., (1982)

$$TSCF = 0.784 \exp - [(\log K_{ow} - 1.78)^2/2.44] \tag{1.7}$$

Burken and Schnoor (1998)

$$TSCF = 0.756 \exp - [(\log K_{ow} - 2.50)^2]/2.58 \tag{1.8}$$

Dettenmaier et al. (2009)

$$TSCF = \frac{11}{11 + 2.6^{\log K_{ow}}} \tag{1.9}$$

Hsu et al. (1990)

$$TSCF = 0.7 \exp - [(\log K_{ow} - 3.07)^2]/2.78 \tag{1.10}$$

Lamshoeft et al., (2018) $$TSCF = -0.0359\,(log\ K_{ow})^2 + 0.1972 \log K_{ow} + 0.5859 \quad (1.11)$$

The variations in relationships proposed could be a function of several things, one of these being the plant species used, with barley, poplar, soybean and tomato all being utilised (Briggs et al., 1982; Hsu et al., 1990; Burken and Schnoor, 1998; Dettenmaier et al., 2009). Another source of variation is the use of different methods; Briggs et al., (1982), Burken and Schnoor (1998) used the intact method, whereas Dettenmaier et al. (2009) and Hsu et al (1990) used the de-topped method, both approaches have been discussed earlier in this chapter.

Many years of experiments conducted using TSCF has led to a large dataset seemingly showing that there is no apparent relationship between TSCF and log $K_{OW}$ (Figure 1.2) (Dettenmaier et al., 2009). This is likely due to the variations in methods and operators as the dataset contains 196 individual TSCF measurements, 110 unique compounds and 21 plant genera. Recent work has attempted to discover new trends within the dataset using Lipinski's 'rule of five'. The rule of five was developed for the administering of oral medicine to determine absorption by the human intestine if it has 5 or fewer hydrogen bond donors, 10 or fewer hydrogen bond acceptors, molecular weight < 500 Da and a log $K_{OW}$ of < 5 (Limmer and Burken, 2014; Lipinski, 2000). Bagheri et al., (2019), using a neural network model, integrated the 'rule of five' and created a predictive relationship of TSCF (R = 0.802), suggesting that log $K_{OW}$ is an important indicator in plant uptake, however, molecular weight, hydrogen bond donors and rotatable bonds should be considered alongside this.

## 1.6 Modelling plant uptake for environmental fate predictions

One such pathway for humans to be exposed to a xenobiotic is through the leaching of a compound down the soil profile and into potable water supplies in the groundwater (Arias-Estévez et al., 2008). The study of the behaviour of an organic chemical within the soil profile is termed environmental fate. This area of study is often applied to pesticides because the compounds are regularly applied and it has been shown that approximately 0.1% reaches the target pest (Pimentel and Levitan, 1982). Statistics like this have served to strengthen the drive for regulation of this practice and often models are used to assess the leachability of potential plant protection products (Wheeler, 2002;

Zadoks, 2003). Within environmental fate modelling, there are currently four main regulatory accepted models currently used in the prediction of leaching of a pesticide from the soil profile: PEARL, PELMO, PRZM and MACRO. All models follow a similar approach for measuring plant root uptake of plant protection products (Equation 1.12), however they take differing approaches to other elements of the model, such as the hydrology. These varying approaches can result in significant differences in the model outputs when sensitivity and uncertainty analyses are conducted (Dubus et al., 2003).

$$M_U = R_L \, PUF \qquad C_L \tag{1.12}$$

Where $M_U$ is pesticide uptake (kg $m^{-3}$); $R_L$ is the water uptake ($m^3$ $m^{-3}$); PUF is Transpiration Stream Concentration Factor (TSCF) or Plant Uptake Factor (PUF) (-); $C_L$ is the concentration of the pesticide in solution (kg $m^3$).

PEARL (Pesticide Emission Assessment at Regional and Local scales) was developed specifically for use in the pesticide registration process to replace it's forbearers, PESTRAS (PESticide TRansport ASsessment) and PESTLA (PESTicide Leaching and Accumulation) due to their contrasting results observed in certain scenarios (Tiktak et al., 2000). PEARL is a one-dimensional, dynamic, multi-layer model which is coupled with SWAP (Soil Water Atmosphere Plant model) (Leistra et al., 2001). SWAP uses a finite-difference method to solve Richard's equation, a combination of Darcy's law and the continuity equation for soil water (van Dam et al., 1999). Within PEARL, the pesticide is assumed to be taken up passively into the roots and subsequently translocated to the shoots.

MACRO is a one-dimensional, non-steady state model of water, heat and solute transport in a variably saturated layered soil profile. MACRO is a dual-permeability model whereby soil porosity is classified into micropores and macropores. Micropore water flow is described by Richard's equation and macropore water flow being described using gravity flow (Stenemo and Jarvis, 2010). This is a similar approach to PEARL, with both plant uptake equations being comparable (Stenemo and Jarvis, 2010).

PRZM (Pesticide Root Zone Model) is a one-dimensional, dynamic compartmental model designed for simulating chemical movement in unsaturated soil systems within and immediately below the plant root zone (Carsel et al., 1985). The original PRZM model was released in 1985, however, it was shown that this was not adequate for describing the movement of water and solutes below the crop root zone, and therefore a second compartment, VADOFT, was designed. VADOFT simulates the hydrological and chemical processes in the Vadose Zone, this coupled with PRZM, gives the full model PRZM-3 (Suarez, 2005). The equation reported in the manual for PRZM adds some extra elements compared to PEARL and MACRO. This is the depth and the cross-sectional area and whilst this is slightly different to the one given above (Equation 1.12), it was not deemed distinct enough to present as a separate equation here.

PELMO (Pesticide Leaching Model) is based on the US-EPA's PRZM model, it is however modified so that it better aligns with the process used by the German authorities for the registration of pesticides. This means that both models are very similar; PELMO also uses equation 1.12, with the addition of cross-sectional area and depth like PRZM, and takes a plant uptake value between 0 and 1 (Klein, 2012).

### 1.6.1 Plant uptake within current environmental fate models

As detailed in the previous sections, all models have a similar approach to simulating the plant uptake of pesticides in environmental fate models. Within all models, a plant concentration factor is required, which for passive uptake is restrained between 0 and 1. Previously, if no relevant laboratory data could be found, this value was calculated from the TSCF against log $K_{OW}$ relationship (Briggs et al., 1982; FOCUS, 2000). This relationship has long been contested within the scientific literature, with many authors proposing new statistical relationships and demonstrating that some compounds do not exhibit the expected behaviour (Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990). The discussion and lack of reproducible results within the scientific literature have led to a loss of confidence in the ability of the Briggs equation to predict the TSCF of organic compounds and therefore this advice has been removed. The current procedure for the setting of a TSCF value is to supply 0 for most pesticides and 0.5 for systemic pesticides which are known to be taken up (EFSA, 2013).

To the authors' knowledge, there has only been a small amount of work published which discusses the effect that plant uptake has on the leaching of compounds (EFSA, 2013). This was conducted using PEARL and centres on the predicted environmental concentration in the groundwater ($PEC_{GW}$). $PEC_{GW}$ is a measure taken from the model outputs and is defined as the 80th percentile of the mean concentration at 1 metre depth. This allows for a quick assessment of the risk of a compound leaching down the profile, compounds are rejected that show value over the threshold of 0.1 μg/L (FOCUS, 2009). Results from this work found that leaching concentration reduced by 24% - 43% when uptake was set as 1 (EFSA, 2013). This work suggests that there is a significant effect of plant uptake on the leaching behaviour of certain compounds and it could theoretically reduce a $PEC_{GW}$ value below the threshold.

## 1.7 Conclusion

Plant uptake has been discussed scientifically for a long period and can be defined in relatively simple terms. However, when it comes to quantifying and defining within environmental fate models it is an understudied area of research. Since the definition of the original relationship (TSCF vs log $K_{OW}$), there has been little agreement on the multipliers in the equation. This means that progression in this field has been slow, to the point that the current advice remains that plant uptake should be set to 0 for most pesticides and 0.5 for systemic pesticides. Organic compounds are complex and there are several different factors that affect uptake, recent suggestions point to the Lipinski 'rule of five' being more suitable to explain the uptake behaviour of organic compounds than just a TSCF against Log $K_{OW}$ relationship. Within this approach, log $K_{OW}$ would still be used but alongside other physical-chemical properties such as hydrogen bond donors, molecular weight and rotable bonds. Whilst there remains reasonable doubt and the more recent suggestions have not been put under proper scientific scrutiny, we should define the plant uptake based on default values of 0 and 0.5 or individual experimental results. It would also be important to consider further sources of variation within the experimental data, such as the use of different plant species, length of exposure and the age of the plants.

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# Chapter 2 –Understanding the plant root uptake of [$^{14}$C]-1, 2, 4-Triazole into Wheat plants in a near-constant hydroponic experimental system

## 2.1 Abstract

The leaching of Plant Protection Products (PPPs) can severely pollute groundwater supplies. Crop uptake of PPPs post application has been proposed as a significant factor in reducing their transport down the soil profile. There is significant uncertainty surrounding the use of the derived plant uptake values currently used within environmental fate models and there is a strong drive within the research and regulatory communities to establish a reliable and reproducible methodology. A recent method for measuring Plant Uptake Factor (PUF) was published which showed that it could be used to determine plant uptake in a similar way to Transpiration Stream Concentration Factor (TSCF). This approach was then adapted to allow for a longer incubation period with the applied compound to test whether PUF was reproducible and stable over time. This differs from many previous studies where a single plant uptake value was produced over a set period. Many previous studies devised methodologies that requires the plant to be grown in a solution where the volume of solution becomes depleted over time. This presents the issue of the roots not being completely submerged throughout incubation. Using a new 'topping-up' method allowed for the roots to be completely submerged until harvest and meant that the length of exposure could be extended whilst keeping all individuals in the same size of vessel. Two approaches to determining PUF were tested with the results from each compared. Results show that this 'topping up' method does provide reproducible and reliable estimates of PUF with both approaches producing significant linear relationships. Depending on the degradation of the compound within the solution then the optimal length of exposure can be determined using this method. If there is potential for degradation or phytovolatilisation then it is more suitable to use shorter exposure times such as 48 hours.

## 2.2 Introduction

Improved understanding of the processes and parameters that affect leaching of Plant Protection Products (PPPs) down the soil profile is a matter of great importance as PPPs can pollute potable water supplies, many of which are being placed under greater demand due to reduced availability and increasing population (Arias-Estévez et al., 2008; Stavenhagen et al., 2018). Plant root uptake is a process that has been proposed to have a significant effect on the environmental fate of PPPs within the soil profile, potentially reducing the contamination of the groundwater water and interconnected surface water bodies (Lamshoeft et al., 2018). Regulator interest in the adherence of PPP producers to human and environmental safety means a registration process is required prior to the release of a potential compound. An important part of this process is the assessment of the leaching potential of PPPs through the use process simulation models, also called environmental fate models; within Europe the most commonly used models are FOCUS PEARL, FOCUS PELMO, MACRO and PRZM (Dubus et al., 2003a). The need to produce accurate estimates of PPP leaching potential has led to investigations of the uncertainty of various parameters and variables used within these models (Boesten, 2004; Dubus et al., 2003b). One of these variables is the Transpiration Stream Concentration Factor (TSCF) or Plant Uptake Factor (PUF) which is a compound specific property to quantify the transport of pesticides into crop biomass; it is currently derived from short-term laboratory experiments. Uptake by the plants is assumed to be a passive process that occurs as the plant is extracting water from the soil profile (Briggs et al., 1982; Shone and Wood, 1974). Within the environmental fate models, it is considered a loss mechanism from the soil profile and is calculated through the relationship between the amount transpired by the plants; the concentration of the compound in the solution and the TSCF or PUF (Carsel et al., 1985; Lamshoeft et al., 2018; Tiktak et al., 2000). PUF has a range of 0-1; if set to 1 then all the compound within the solution that is extracted will enter the plant root (Lamshoeft et al., 2018) Uncertainty within models can potentially lead to inaccurate predictions of the behaviour of PPPs within the environment. Previous analysis has highlighted the uncertainty of laboratory derived PUF/TSCF and sensitivity of the environmental fate models to the applied PUF value (Severinsen and Tjalling, 1998). Polder et al. (1995) found that although overall the experimental data agreed with the Briggs et al. (1982) the variation of measured TSCF within this is deemed to be ‘large’.

Plant uptake is a complex process and in addition to this, organic synthetic organic compounds such as pesticides can be many and varied in their chemistry (Taiz and Zeiger, 2010). There are three main routes of uptake into a plant root, apoplastic (along cell walls), symplastic (through plasmodesmata) and transmembrane (through cells via the membrane) (Miller et al., 2015). For a compound to move to the above-ground parts of a plant, the compound must pass through the Casparian strip, a 'barrier' of cell wall type material deposited on the outer layer of the endodermis that prevents the compounds from entering the vascular system via the apoplastic pathway (Grebe, 2011). This ensures that all water and solutes entering the pericycle have passed through the cytoplasm of the endodermal cells i.e. by the symplastic or transmembrane of the cells.

A seminal paper by Briggs et al., (1982) sparked much discussion around this subject, as he proposed a Gaussian relationship between the TSCF and the octanol-water partition coefficient (log $K_{ow}$) of a compound. TSCF had been used, however Briggs *et al.* was the first to link this parameter with the log $K_{ow}$ (Briggs et al., 1982; Russell and Shorrocks, 1959; Shone and Wood, 1974). Following this, there have been several relationships proposed to determine a TSCF value for use within environmental fate models based on laboratory experiments. These relationships were derived using a variety of plant species, compounds and methods. However, researchers have struggled to agree on the true position of the relationship and until recently Briggs et al. was used as the default predictor for plant uptake (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990; Lamshoeft et al., 2018). Even with further reviews and meta analyses into the dataset, a statistical relationship between log $K_{OW}$ and TSCF has yet to be found (Bagheri et al., 2019; Dettenmaier et al., 2009; Lamshoeft et al., 2018; Limmer and Burken, 2014). Therefore, scientific opinion then stated that a move away from the Briggs equation would be preferred. For example the European Food Safety Authority (EFSA) recommend a PUF value of 0 set for the majority of pesticides and a value of 0.5 set for systemic pesticides that are known to be taken up (EFSA, 2013).

PUF considers the amount of compound in the whole plants as 'uptake', where previously TSCF only considered the concentration of the PPP that has been translocated to the above-ground parts of the plant. PUF then allows for compounds that are not translocated to be considered within environmental fate assessments. This is also much closer to how the models consider plant uptake of compounds from the soil, with the plant being treated as a loss mechanism for pesticide from the soil profile (Lamshoeft et al., 2018). Many previous studies have been conducted over short exposure times and often they have been conducted to provide one TSCF or PUF value over a single time period (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990; Lamshoeft et al., 2018). There are a few concerns with the current laboratory methodologies used to determine PUF, with regards to how applicable a short-term exposure in hydroponic solution is to the season-long uptake of a growing plant in a soil profile. This study aims to use destructive harvesting of samples over multiple time points to test whether PUF remains stable over time and can be tested for up to 21 days. This will test whether PUF is reproducible over time and performs as expected with the compound being taken up as a passive process when abstracting solution. 1,2,4-Triazole was selected as a suitable compound for this study as it a known metabolite of a large group of PPPs, the triazole fungicides.

## 2.3 Methodology

### 2.3.1 Crops

Wheat (*Triticum aestivum*, L. Variety "TYBALT") seeds were germinated in sharp sand until BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) growth stage 11 was reached. Plants were then transferred to a conditioning stage during which the plants were grown in 50% strength Hoagland's solution in a 250ml glass amber jar vessel. The plants were held upright within the vessel by a polyurethane bung which had a slit at the full length to make it easier to load the plant and adjust if needed. Care was taken to ensure that the shoots were clear of the top of the bung and are not likely to grow into the foam, the roots were completely covered by the solution and the bung did not meet the solution. The bung also helped to reduce evaporation losses from the vessel.

The plants were maintained within the glasshouse at the following conditions: 20°C (+/- 2°C) for 16 hours during the day and then 16C (+/- 2°C) overnight for 8 hours. The humidity was kept at 50% (+/- 1%) throughout. 75 plants were taken to the conditioning phase, this provided enough plants to go forward into the test period. The conditioning phase lasted for a week and allowed the plants to grow to BBCH 12-13 approximately. Following this the plants were transferred to 100% Hoagland's solution and treated with [$^{14}$C]-1,2,4-triazole. Pre-experiment assessments were conducted (data unpublished) in the growing conditions to determine whether the plants would be healthy growing for 21 days after exposure. From this it was decided that air being bubbled through the solutions was not required and that the required oxygenation of the solution would come from the additional solution added when topping up and the air trapped within the roots when taken out for the topping up procedure. It was also important to determine whether removing the plant from the solution every day would place them under stress and adversely affect their growth.

### 2.3.2 Treatment solution

The [$^{14}$C]-1,2,4-triazole was prepared in a solution of methanol and diluted in the nutrient solution to achieve a concentration of 100µg/L. 1,2,4-Triazole has a solubility in water of 730,000 mgL$^{-1}$ at 20°C and so was expected to become dissolved in the water phase following the addition of the co-solvent (Saraiva Soares et al., 2013). Co-solvent levels remained <0.01% throughout. To ensure thorough mixing into the solution, an hour prior to application, the appropriate volume of [$^{14}$C]-1,2,4-triazole in methanol was pipetted into a vessel filled with 1.3L of 100% Hoagland's solution, enough solution for 5 replicates of each time point and 2 x 1 ml aliquots for liquid scintillation counting (LSC). This ensured that the solution was well mixed and that each replicate has been dosed with the same amount of test item ([$^{14}$C]-1,2,4-Triazole). A 1ml aliquot was taken from each batch to test for homogeneity and to calculate the dosing concentration.

250ml of 100% Hoagland's solution was added to 39 vessels (5 for each time point, 5 controls, 3 degradation vessels and 1 evaporation). To 33 of these vessels, 100µg/L [14C]-1,2,4-Triazole was added. This volume was achieved by noting down the weight of the empty vessel, zeroing the balance and adding 250ml of solution by weight. The vessel was then removed, the balance zeroed again, and the total weight of the vessel containing solution measured; this became the target weight for the sample when topping up. The plant was then secured in the bung and placed in the vessel. As the weight of the vessel, solution and the bung are known, the difference between all these values and the total weight was used to indirectly determine the plant weight throughout the measurement period. The 3 degradation samples were dosed with the same concentration of [$^{14}$C]-1,2,4-Triazole. This highlighted if there were any phytotoxic effects of the test item or co-solvent on the growth of the plants.

All samples were kept in the same conditions for a maximum of 21 days. Destructive sampling of the plants was conducted on Days 1, 2, 6, 10, 16 and 21. The plants were inspected regularly to detect any health issues with any individuals and each sample was topped up with 100% strength untreated Hoagland's solution every 24 hours. To top up the solution, the plant was fully removed from the solution, the solution topped up until the mass balance reached the target value set at the start of the experiment and then replaced back into the vessel.

### 2.3.3 Analysis

At each time point, 5 vessels were removed from the greenhouse for analysis. The plants and bung were carefully removed from the vessels and the excess solution from the roots allowed to drip back into solution. The roots and shoots were then separated and weighed. The treatment vessel containing the solution was placed onto the balance and the weight recorded. The solution was topped up to the target weight, a lid placed on the vessel and this was taken up to the lab for further analysis.

Once the samples were returned to the lab, they were agitated to ensure that they were thoroughly mixed. Duplicate aliquots of 1ml were taken by pipette and mixed with 15ml of the scintillation cocktail ProSafe+ and run on the Liquid Scintillation Counter (LSC, Tri-Carb 4910TR liquid scintillation counter). At this point an additional 1ml aliquot was taken from one sample to determine the compounds present within the solution by Thin Layer Chromatography (TLC).

The roots were washed in 25ml of 4:1 acetonitrile:ultra-pure water solution in a 50ml centrifuge tube. The solution was swirled around the roots for approximately 20 seconds, with manual shaking ensuring that the roots are fully covered and there was thorough mixing with the roots. 2ml samples were removed by pipette and taken in duplicate for LSC quantification. The washed roots were then left in the fume cupboard to dry before further analysis.

The plant material samples were analysed using a Sample Oxidiser (Perkin Elmer Model 307), whereas the washed roots and shoots were cut into appropriately sized portions to fit into the Combusto-cone ready for combustion. A combusto-pad was placed on top, and 3-4 drops of ultra-pure water and 3 drops of Combustaid were added to each sample, prior to combustion. Once the sample had been combusted the resulting aliquot of Carbosorb E was mixed with 15ml of the scintillation cocktail PermaFluor E+. The roots and shoots were analysed separately to allow for quantification of the amount of test compound that had been translocated to the above-ground parts of the plant. Once the radioactivity had been determined for the treatment solution, root wash, roots and shoots, the recovery of radioactivity was calculated.

## 2.3.4 Plant uptake factor

The plant uptake in this system was from a solution that is being topped up daily, therefore the concentration is calculated by equation 2.1.

$$C = C_0 e^{\frac{-\propto v}{V_0}} \tag{2.1}$$

In this equation, C = Concentration in the solution, $C_0$ is the initial concentration in the solution, e = the exponent of the fraction where α = the PUF, v = volume transpired at the measurement interval and $V_0$ = the initial volume. Taking alpha as the PUF, this can be calculated using two methods detailed in the equations below (Equation 2.2 and 2.3). The first equation focusses on the concentration in the solution and how much has been lost in relation to the volume transpired. The second focussing on how much mass remains within the plant material in relation to the volume being transpired.

$$PUF = \frac{\ln \frac{C_0}{C}}{\frac{v}{V_0}} \tag{2.2}$$

$$PUF = \frac{\ln \frac{M_0}{M_0 - m}}{\frac{v}{V_0}} \tag{2.3}$$

Where $M_0$ = the mass in the initial concentration, m = the total mass of radiolabelled substance within the plant material, v = the total volume of water transpired by the plant, $V_0$ = the top-up volume. The PUF of the whole experimental period will be deduced as the slope of the linear relationship between the top half of the equation ($\ln(C_0/C)$ for Equation 2.2) and the bottom half of the equation ($v/V_0$ for equation 2.2).

## 2.3.5 Statistical analysis

Linear regression analysis was conducted on the PUF calculations over time, with the p-value, $R^2$ value and the equation for the linear model being reported. A linear mixed effects model was run for the plant weight data to determine whether there were any negative effects on the growth of the plants following the application of the test item.

# 2.4 Results and Discussion

## 2.4.1 Degradation and recovery of [$^{14}$C]-1,2,4-Triazole

Over time, some compounds are broken down in solution or after uptake by the plant, this is referred to as degradation or phytovolatilisation and can alter the PUF value as metabolites often do not show the same uptake (Bonmatin et al., 2015). Therefore, recovery of total radioactivity and relative concentration of the compounds in solution are vitally important for determining a Plant Uptake Factor (PUF). The threshold value for recovery of total radioactivity was set at 95%. This value was selected as it is the value that is often required within radiolabelled assessments for regulatory purposes. Figure 2.1 shows the total recovery of radioactivity, it should be noted that the variation in points the x-axis is due to the style of the plot and is designed as such so that each individual point can be as easily distinguished as possible.

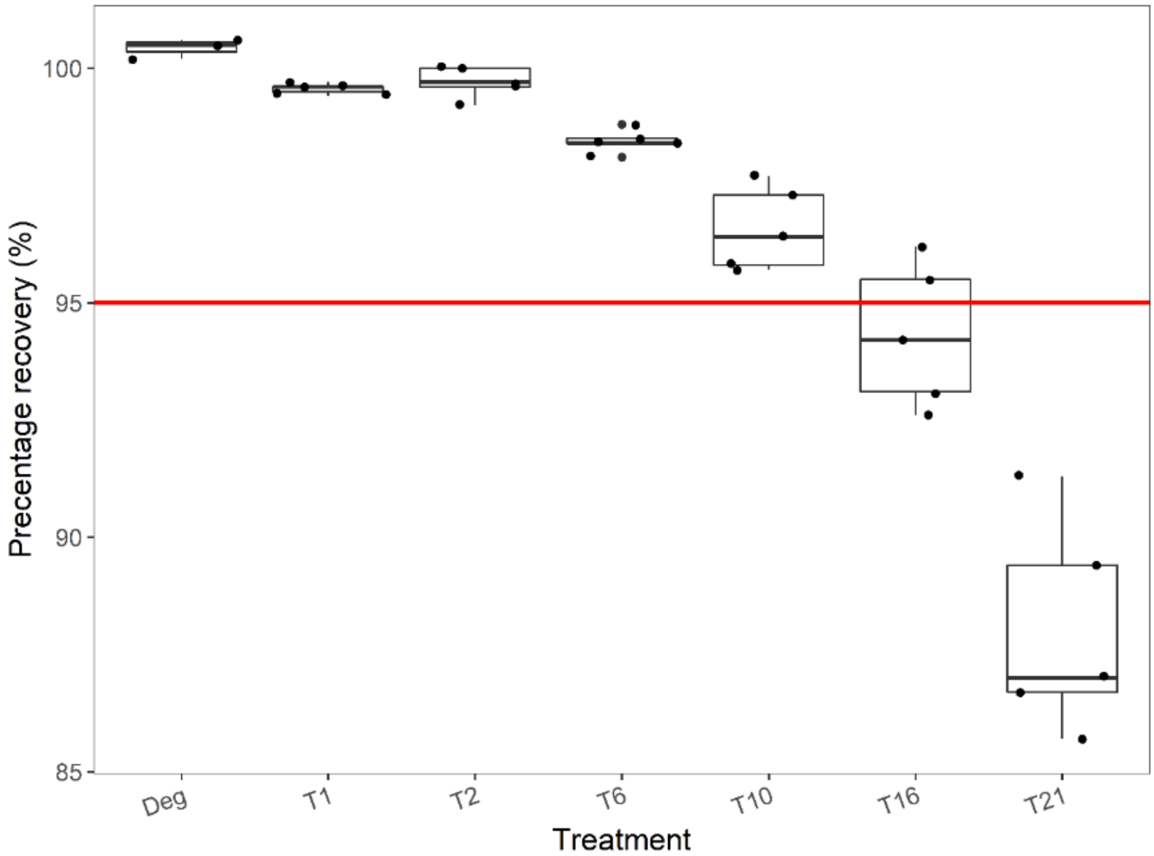

**Figure 2.1. Boxplots of percentage recovery of total radioactivity (n = 33). The red line at 95% represents the threshold set at the start of the experiment. The labels on the x-axis show the day that the sample was harvested on, for example T16 was harvested on day 16. Deg represents the degradation samples.**

From 1 to 10 days, all the measured samples recovered over this threshold (Figure 2.1), day 16 had only two samples above this value with mean recovery of 94.3%. By day 21 all samples were below the threshold. The degradation sample, treated with the test item but without a plant inserted to the bottle, was harvested at the same time as the day 21 samples and showed very high levels of recovery and low production of metabolites in comparison. Figure 2.2 shows an image of a radiolabelled Thin Layer Chromatography.

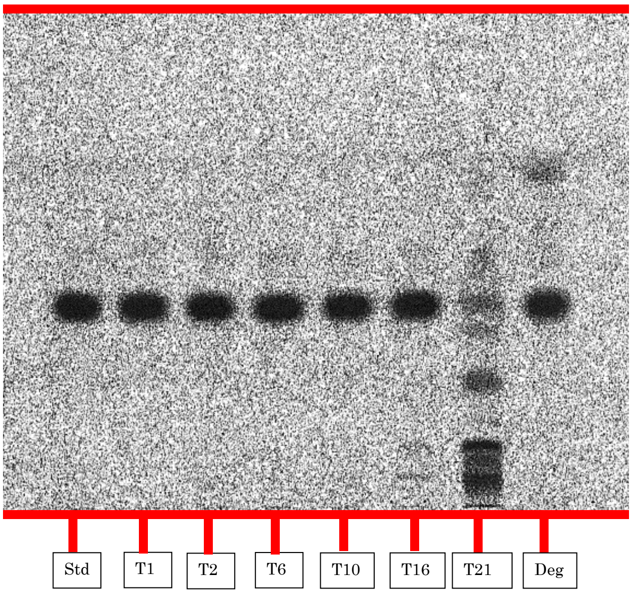

**Figure 2.2. Thin Layer Chromatography of of a sample of the hydroponic solution taken following the destructive sampling. Each sample was applied to be at the same bequerel level and the colouring is relative based on the radioactivity of each spot.**

As the recovery was above 95% and the degradation was < 5% for the samples from T1 to T10, it was deemed that the PUF value measured over this period was the true value of PUF from 1,2,4-Triazole. It is important to note that although the optimum period of uptake being between 1-10 days for this experiment, this is not suggested to be uniform across all compounds and test systems. Other compounds that are not degraded in solution or phytovolatilised by the plant could be measured for a further extended period. As the temperature that the plants were grown in were much higher than would be observed within a field experiment in Europe, it could be supposed that the degradation processes observed within this experiment occurred more rapidly than in the outdoor environment.  There are a number of processes that could have metabolised the triazole including microbial degradation, phytodegradation and phytovolatilisation (Issa and Wood, 1999; Limmer and Burken, 2016; Liu et al., 2019).

## 2.4.2 PUF from solution and plants

### 2.4.2.1 PUF from Solution

As mentioned previously, the optimum period for measuring plant uptake of 1,2,4-Triazole in this experiment was selected as being between day 1-10. Therefore, the plots presented within the following sections show only the data from this period. The results in this section show PUF when calculated from solution (Equation 2.2). Figure 2.3 shows the bottom half of the equation ($v/V_0$) as the ‘volume fraction’ and the top half of the equation ($\ln(C_0/C)$ as the ‘Concentration fraction’. The uptake was consistent across the whole experimental period ($r^2 = 0.997$) with a calculated Plant Uptake Factor of 0.67. This value is consistent with those previously reported for the PUF of 1,2,4-Triazole with a value of 0.64 reported by Lamshoeft et al. (2018) also using wheat.

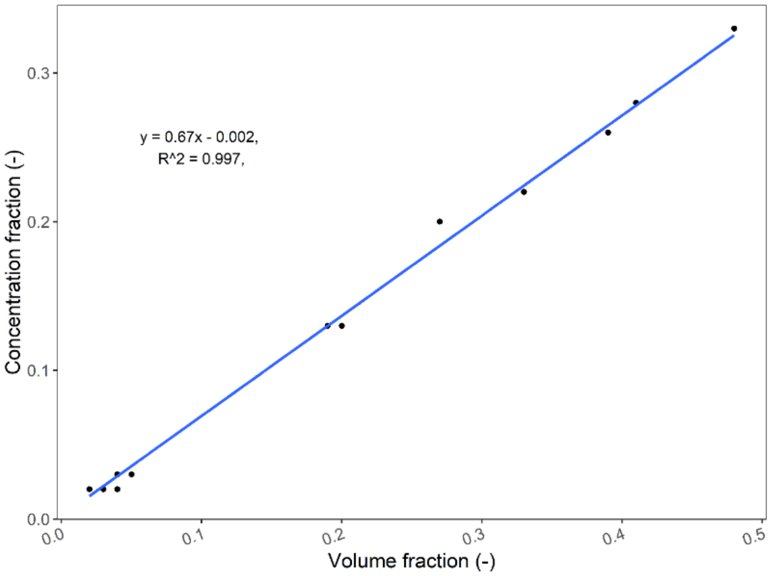

**Figure 2.3. Scatter plot for the PUF from the concentration of radioactivity in the solution. The blue line represents the derived linear model.**

Figure 2.4 shows the PUF as a boxplot at each harvest time point. The red line indicates the slope of the linear regression from Figure 2.3. As shown the slope is a good fit across all time points barring the T1 time point. Overall the recovery of the radioactivity was not low enough for a glass wash to be conducted (Figure 2.1). The results of T2, T6 and T10 fall on the red line of the slope and show a very good relationship. To the author's knowledge, the ability to measure stable plant uptake (PUF or TSCF) has not been tested and this is a very important finding when moving forward with PUF. Another study Lamshoeft et al., (2018) found that mentioned interim measurements being taken, however no PUF values were published from this.

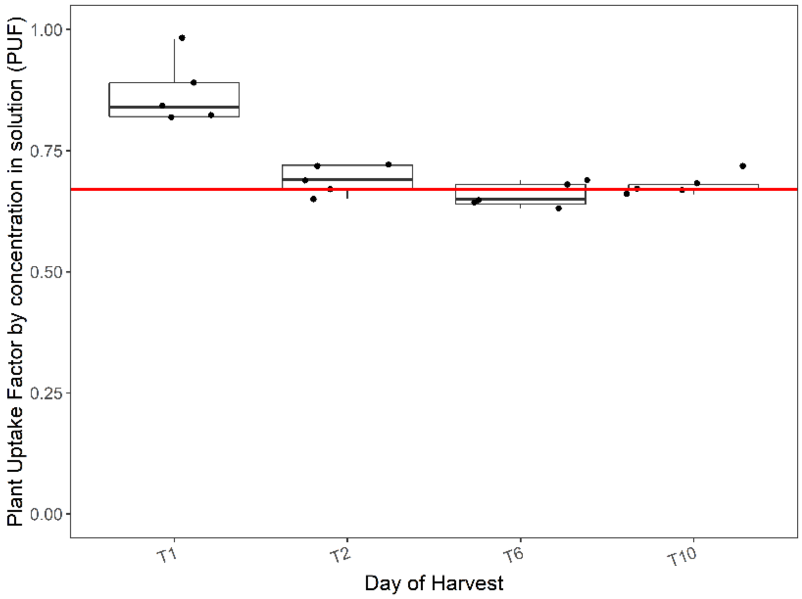

**Figure 2.4. Box plots of the PUF by concentration in solution for each harvest date. The red line denotes the slope of the linear equation (Figure 2.3) to show the fit of this model to the individual results.**

One major area of uncertainty with measuring PUF from the concentration of the solution surrounding the plant roots is that you must be certain that the measured loss from solution is uptake by the plant. Within solution there is a lot of evidence of processes that degrade compounds over time, care needs to be taken to ensure that these are not affecting the result (Issa and Wood, 1999; Limmer and Burken, 2016; Liu et al., 2019). Although this experiment was conducted in the laboratory and care was taken to reduce or eliminate the presence of microbes where possible, there are still likely to be microbes present within the solution which could contribute to the degradation of the test item. The only feasible way to reduce the effect of degradation on the PUF is to monitor the degradation and recovery as has been done in this experiment.

Some uncertainty within this topic has surrounded the concentration effect of plant uptake and whether a higher concentration of solution will lead to higher levels of uptake and vice versa. Although the concentration was not altered during this experiment, the plants were exposed to varying concentrations throughout due to the amount of compound removed as uptake (Figure 2.5). Although this figure cannot conclusively prove that concentration doesn't have an effect, it shows that despite the dropping concentration (due to the topping up of solution), there was not a drop in the PUF as a result.

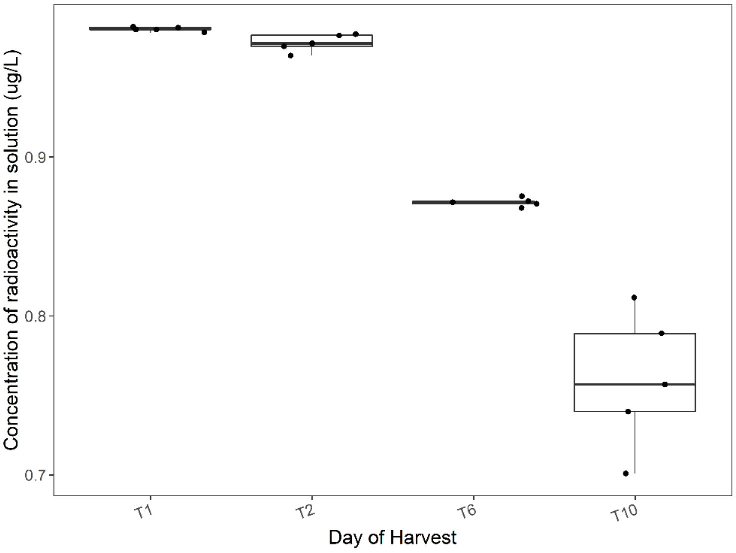

**Figure 2.5. Boxplots showing the concentration of the total radioactivity in the solution over time.**

#### 2.4.2.2 PUF from plant material

Figure 2.6 shows the linear relationship of the mass and volume fraction calculated from the mass of the compound within the plant material (Equation 2.3). As with the PUF calculated from the concentration in solution, the linear relationship is very strong (p-value = <0.001 and $R^2 = 0.997$). The derived PUF value is 0.56 compared to 0.67 in Figure 2.3. One potential reason for the lower slope value from this method is that, once a compound starts to be metabolised by the plants and respired as $CO_2$ in the process of phytovolatilisation, it is no longer considered within the PUF equation. This is because the calculation of the concentration within the plant material is conducted following the harvest. If some of the compound has become phytovolatilised, then it would not be considered within the plant using this equation, however it has still been taken up. As a result, if the compound is likely to be metabolised then it would be preferable to conduct an assessment over a shorter timescale. Although the recovery and degradation analysis of the samples from T6 and T10 did not fall below the threshold of 95%, the general trend of the recovery (Figure 2.1**Error! Reference source not found.**) shows that it is possible that metabolism had started before this point.

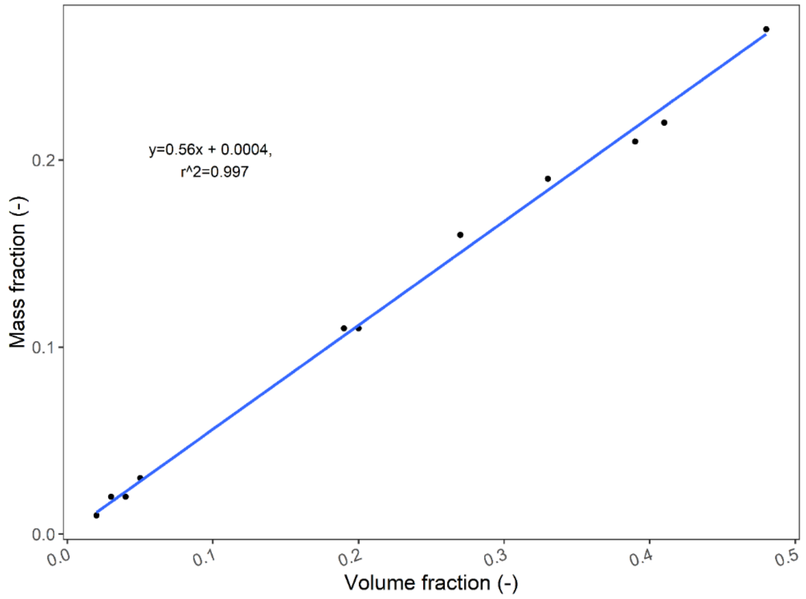

**Figure 2.6. Relationship between mass fraction and volume fraction calculated from the mass of radioactivity within the plants. The blue line represents the derived linear equation.**

The PUF by day of harvest (Figure 2.7) shows similar results to the linear regression. The slope shows a good representation of the general trend of the data, particularly for the T6 and T10 results.

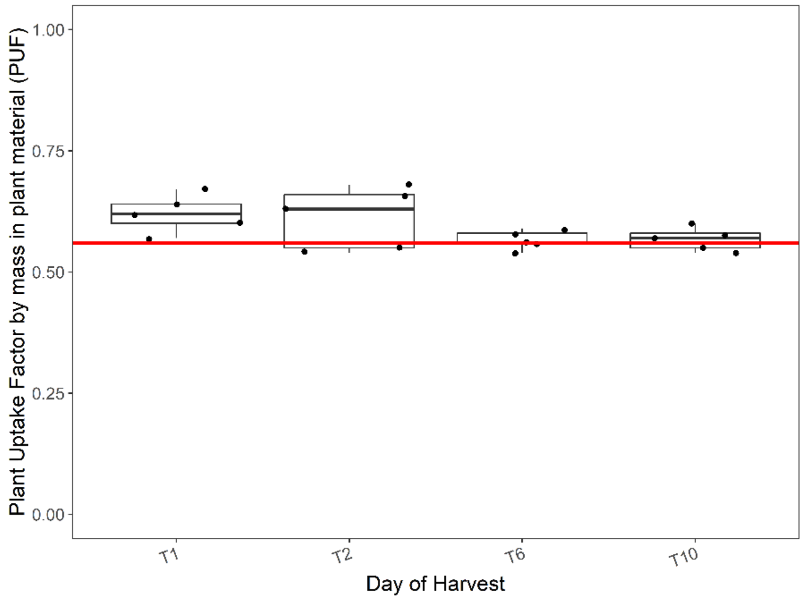

**Figure 2.7. Boxplot of PUF derived from the activity in the plant material. The red line denotes the slope of the linear equation (Figure 2.6) to show the fit of this model to the individual results.**

### 2.4.2.3 Comparison of PUF calculation methods

Calculating PUF from concentration in solution considers any loss from the solution to be taken up by the plants. This has potential to be an overestimate of uptake as there are other processes going on within the system that could remove radioactivity from the solution, such as degradation and volatilisation (Houbraken et al., 2016; Sørensen et al., 2003; Wang et al., 2012).

The results from the first harvest of samples (24 hours) show an elevated PUF when compared with the later harvests, this period is likely to be turbulent due to the fresh plant roots being added to the solution. This time point is also the one with the highest amount of variation in the dataset. This theory is reinforced by the fact that this effect is not seen in PUF by mass in plant material. PUF from the mass within the plant is based on radioactivity measured within the plant material but is confined by the fact that it can only measure what is contained within the plant at that point in time. There is no indication of how much the plant has respired out as $CO_2$. Therefore, this method is likely to be a slight underestimation of the true position of PUF. Within the context of measuring plant uptake for regulatory purposes, this would be preferable to potential overestimation of the PUF value that is possible by the solution measurement. As plant uptake is considered a loss mechanism with regards to the environmental fate of pesticides, a slight underestimation would mean that more pesticide is potentially available than observed in laboratory hydroponic studies. Meaning that the output model predictions are conservative, in line with the realistic worst-case scenarios that are modelled (FOCUS, 2000).

Both methods (Equation 2.2 and 2.3) when plotted as a linear regression show strong linear relationships with both having an $R^2$ value of 0.997. Both equations also pass very close to the intercept; it indeed would be expected for this relationship that zero mass would be lost for zero loss of volume. There is a slight disagreement in the calculated PUF, when measured from solution, PUF shows the line to be at 0.67, whereas PUF from plant material shows the slope to be at 0.56. As stated, for opposing reasons this can be explained by the degradation of the compound; if degradation occurs and the compound is phytovolatilised by the plant then it is likely that this divergence will increase over time. Therefore, it is of importance that the length of exposure is selected based on the degradation of the compound within solution over time. If a compound is likely to degrade then a shorter exposure time would be preferable for both methods.

### 2.4.3 Plant health assessment

The plant weight was measured indirectly each day through measuring the total weight and subtracting the flask filled with solution and the weight of the foam bung.

Throughout the experiment, there were no negative effects on the plant growth (Figure 2.8). As expected, the plants gained weight throughout the treatment period and showed a steady rise in growth rate. The linear mixed effects model run for the difference between the plant weights throughout the experiment showed a significant difference between the treated samples and controls, with the treated samples showing a higher plant weight than the controls. Further analysis of the difference between the linear relationships showed that this difference was observed from day 12-20. This period represents the time that degradation was observed in the treatment solution. Therefore, this suggests that there may have been a metabolite of the 1,2,4-Triaozle that promoted the growth of the plants. As no further analysis of the treatment solutions over this period was conducted, it is impossible to determine whether this statement is true.

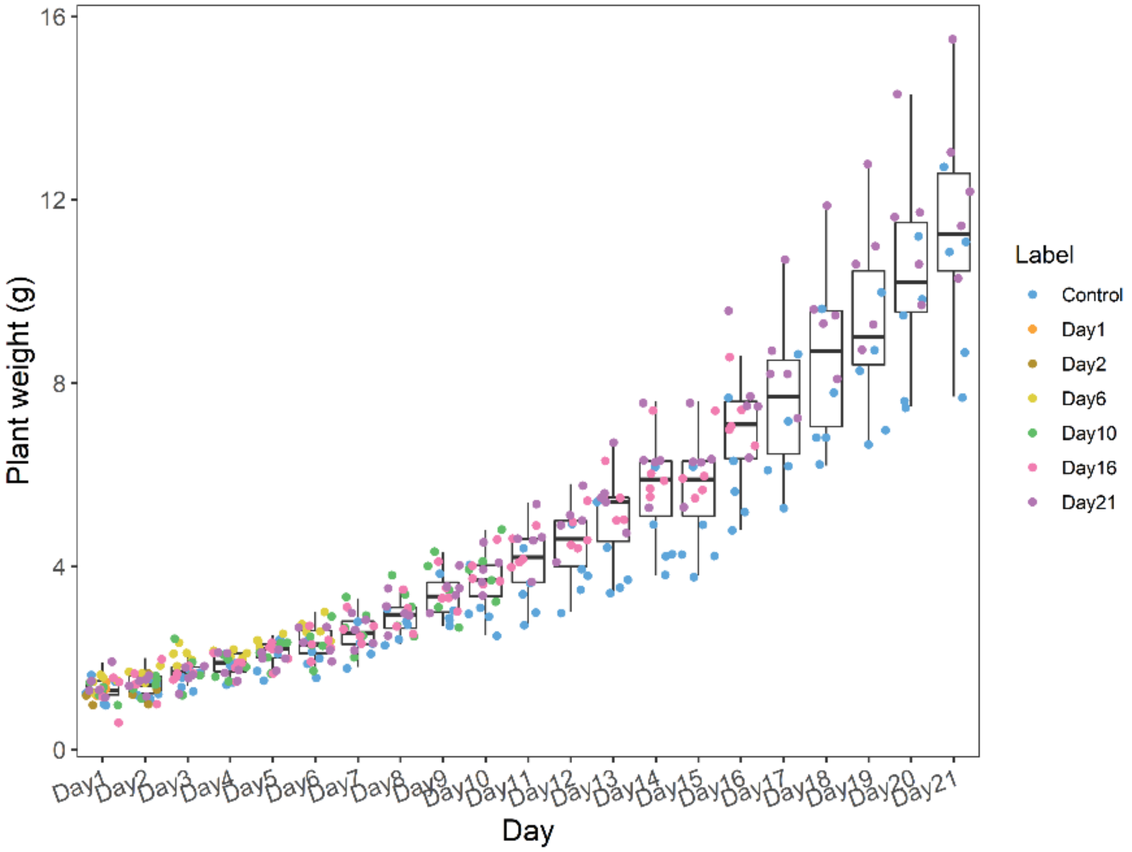

**Figure 2.8. Boxplots of plant weight data as a function of time, with the rate of change of plant weight denoting plant growth. The label and colour in the legend shows the day that the group was harvested on.**

The daily top-up gives a rough estimate of the daily transpiration of each plant (Figure 2.9) and showed a similar picture to the growth rate, with the plants steadily increasing over time. The pattern isn't quite so smooth, possibly owing to daily variation but there are no overall concerns. In the final week of the treatment period there are two outliers, both are likely down to the natural growth of plants, with the Day 21 outlier being the largest plant in the dataset so is likely to take up more water.

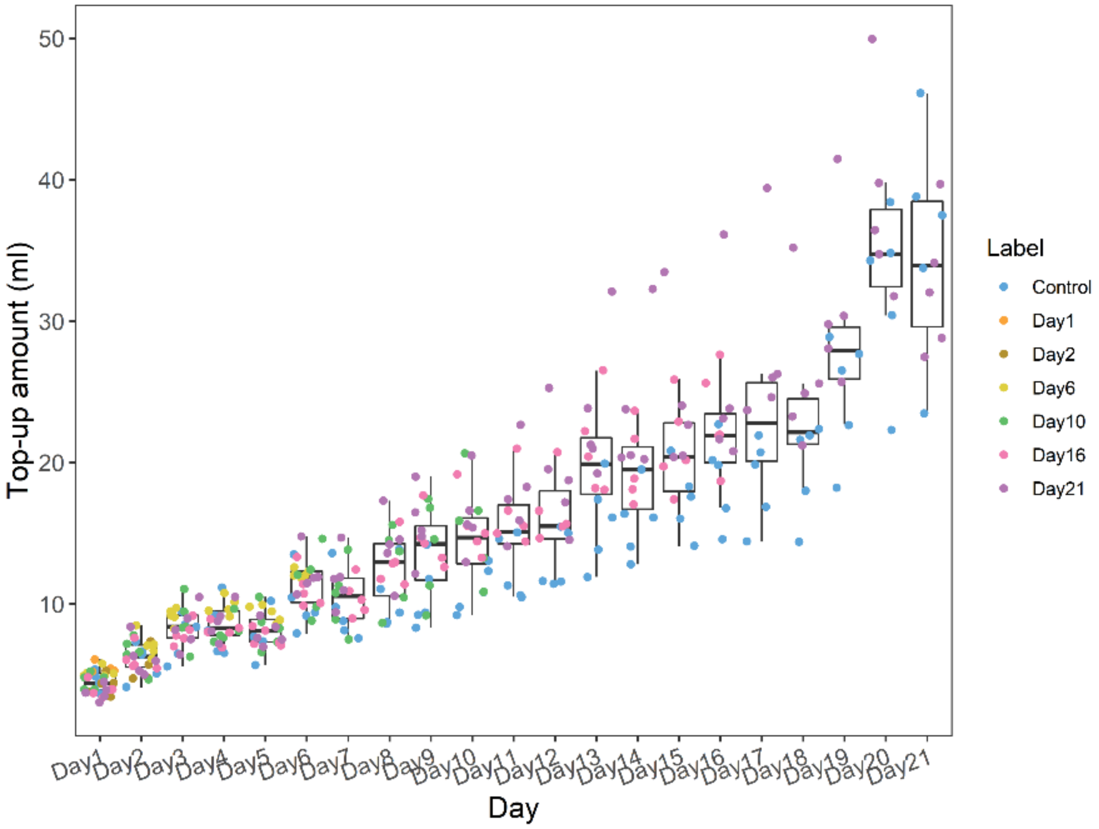

**Figure 2.9. Boxplot of top-up data for each sampleas a function of time , with top-up being a proxy for water loss by transpiration. The label and colour in the legend shows the day that the group was harvested on.**

## 2.5 Conclusion

The results in this paper show that an adapted approach to measuring PUF can produce a reliable and reproducible dataset when measured by destructive harvests over time, showing that plant uptake remains stable if the compound remains pure in solution. The linear relationships shown within this chapter show that the model assumption of plant uptake being stable over time is correct. The topping up approach also produced comparable results when compared to other PUF studies. Topping up of the solution has the advantage that the test can be run over a longer period but also that the whole plant remains submerged throughout.

A test period of between 1-10 days is recommended based on the data within this study, however, a longer test period could be used if the compound remains pure in the solution for longer. Once the compound starts to degrade in the solution, the PUF can change depending on how much the metabolite is taken up by the plant. If the test item starts to be converted to $CO_2$ and respired out of the system, then this is no longer considered within the PUF from mass in plant material and would produce a divergence between the two equations. In this case, it is most suitable to have a shorter exposure time to help reduce the potential inaccuracies created.

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# Chapter 3 –Increasing realism in the regulatory plant uptake assessment: Using a sand profile to test the uptake of [$^{14}$C]-1,2,4-triazole

## 3.1 Abstract

Plant uptake has become a key point of discussion within environmental fate assessments due to the uncertainty surrounding the current methodologies and a lack of reproducibility of results. Many of the previous studies that have derived a TSCF or PUF value have been conducted in hydroponic growth media, however, questions remain over the realism of this system. Use of hydroponics as a surrogate for soil makes it much easier to control the concentration of the test item that is dissolved in the solution available for plant uptake. The method used within this experiment contained a sand growing media with hydroponic solution providing the nutrients for the plant to grow. Within a sand system it is more difficult to control the concentration as the plant cannot be transferred to a new, fully mixed profile that has been treated. The profile was topped up daily with fresh solution and allowed to drain freely to prevent saturation. Degradation of the compound in the sand profile meant that it could not be determined how stable the uptake of 1,2,4-Triazole was over time. It is recommended that if any future experiments use this experimental system, another compound is selected to measure uptake and that this compound remains in solution and degradation remains < 5% for approximately 8 days to allow for comparative studies to be conducted in hydroponic solution. It should also be considered that application to the top of the profile may not allow for full mixing of the compound instantaneously and so measures should be taken to ensure full mixing of solution within the profile.

## 3.2 Introduction

Studies have estimated that less than 0.1% of pesticides applied to agricultural systems actually reach the target pest, the rest is proposed to enter the environment and be subject to further environmental processes (Arias-Estévez et al., 2008; Pimentel and Levitan, 1986). Plant uptake is a process within environmental fate that acts as a sink for the compound and binds it within the plant tissues (Lamshoeft et al., 2018). Plant uptake has recently become a key point of discussion in recent years due to the uncertainty surrounding the Transpiration Stream Concentration Factor (TSCF) against log $K_{OW}$ relationship which is used to quantify it. There are many potential reasons for this uncertainty e.g. there is no set methodology for measuring plant uptake (Hoke et al., 2016).

Currently, the majority of TSCF and Plant Uptake Factor (PUF) lab experiments are conducted in hydroponic growth media as a surrogate for the soil profile (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990; Lamshoeft et al., 2018). Growth of plants in hydroponic 'Hoagland's' solution mean that it is much easier to control the application of the compound to the plant. One of the reasons for this is that you can transplant an individual from a clean growth solution to a treated solution (Briggs et al., 1982; Dettenmaier et al., 2009). Within a sand or soil profile, this cannot be done and therefore the compound needs to be applied from above and allowed to filter down the profile. Physiological differences can also be seen between plants grown in hydroponic growth media to those grown in soil. In some species, the Casparian strip does not develop fully when grown in hydroponics compared to soil (Miller et al., 2015; Perumalla and Peterson, 1986). This is particularly important for measurements of TSCF which focus on the translocation of compound to the above ground parts of the plant (Shone and Wood, 1974). If the Casparian strip is underdeveloped, then it is possible that hydroponic studies would overestimate translocation.

Whilst hydroponic studies provide an easier method for measuring plant uptake and allow for greater control for the operator, they are not subjected to the same physical and chemical forces that would be found in a soil system (Totsche et al., 2010). Within a hydroponic system, the solution is constantly allowed to mix, and the plant roots can always theoretically access the whole reservoir (Doucette et al., 2005). Within a soil profile there are pore spaces that are created between the solid particles that require pressure to release the water inside them. These pores are are characterised by their size Transmission pores (> 50 μm), Storage pores (50 > x > 0.5 μm) and Residual pores (0.5 > x > 0.005 μm) (Greenland, 1981). Transmission pores require to smallest application of pressure to release the water within them but are generally for the movement of the water down the profile and are only filled when the soil is saturated. Storage pores are full when the soil is at field capacity and are the available water supply to a plant when the roots are abstracting water. The residual pores are the smallest and the water contained within these pores is unavailable for a plant root to access and require a high amount of pressure to empty (-1500 kPa) (Di Bonito et al., 2008). The presence of air-filled pore spaces in the profile can create gaps within the solution and reduce the hydraulic conductivity of the profile. This means the applied compound can only mix via a tortuous route or when the soil profile is saturated (Köhne et al., 2009). This can lead to heterogenous concentrations of pesticide, in contrast with the hydroponic system which is more homogenous due to full mixing.

Increased realism in the assessment of plant uptake is key to increasing confidence in the plant uptake values that are produced. This study aims to take a step closer to a soil profile that would be observed when applying a pesticide to the environment. Sand was chosen for this experiment as it is an inert media that provides pore space but does not provide as much chemical sorption as would be seen in a growth media containing organic carbon (Gevao et al., 2000). In this study the PUF is measured using two methods, the concentration remaining in the sand profile and the concentration in the plant material. This is a similar approach to that seen in Chapter 3 and was designed to allow for easy comparison between the two experimental set ups.

## 3.3 Methodology

### 3.3.1 Plant growth and treatment

Preliminary work has shown that the best growth of plants is in columns of size 20cm tall by 5cm diameter, such that plants can grow without becoming stressed during the experimental period. The crop selected for this study was Wheat (*Triticum aestivum*, L. Variety "TYBALT"). Prior to starting the growth of the seeds, sharp sand was sieved with a 3.0mm sieve. A tube and petri dish were labelled, and the column filled with sand 2cm from the top. A single wheat seed was then placed on top of the sand prior to adding an additional 1.5cm of sand. At this point, the sand column and petri dish were weighed. Water was slowly added to the sand column until saturation, and excess was dripping from the bottom of the column. All columns and were then allowed to stand, until no more water was released. At this point the column and petri dish were weighed again, this was the recorded weight. The difference between the dry weight and the saturated weight provided the solution volume in the profile. Plants were maintained within the glasshouse as follows: 20C (+/- 2°C) for 16 hours during the day and then 16C (+/- 2°C) overnight for 8 hours. The humidity was maintained at 50% (+/- 1%).

During the growth stage, each column was topped up with water daily until they reached BBCH 11. At this point they entered the conditioning phase, at which point they were topped up with 50% Hoagland's Solution. Plants were treated with [$^{14}$C]-1,2,4-Triazole in 100% Hoagland's solution at BBCH 13 to saturation weight.

The [$^{14}$C]-1,2,4-Triazole was prepared in a solution of methanol and applied to achieve an approximate concentration of 100µg/L. Co-solvent levels did not exceed 1%. For application of the 1,2,4-Triazole to the sand profiles, the test item was added to 175ml of 100% Hoagland's Solution. This concentration was set so that 5 x 1ml pipettes of solution could be applied to each plant sample, with remaining 2 x 1ml aliquots to be taken for analysis by LSC to ensure homogeneity.

In total there was 39 samples: 5 for each time point; 5 controls (with plant but no [$^{14}$C-1,2,4-Triazole; 3 degradation samples and 1 evaporation check. Of the total, 33 samples were dosed with [$^{14}$C]-1,2,4-Triazole. This was achieved by taking the total weight of the column and petri dish prior to the addition of solution. 5 x 1ml pipettes were dosed into the column at the 4 points of a compass and a final central point. If required, the column was then topped up to the target weight using untreated 100% Hoagland's solution. This step was repeated for every sample. Each sample was kept in the same conditions and was topped up to the target weight with 100% Hoagland's Solution every 24 hours. They were incubated for a maximum of 21 days and samples were destructively harvested on days 1, 2, 6, 9, 16, 21.

### 3.3.2 Analysis

At each time point, 5 samples were removed from the greenhouse. A sheet of BenchKote was laid out in a fume cupboard, this was used to catch any sand that fell from the column when handling the plant. The plant was then gently pulled from the column and roots placed in a 500ml Bel-Art wide mouth Mason jar, 100ml of water was then poured over the roots to remove excess sand from the outside. The roots and shoots were then cut and placed separately into pre-weighed centrifuge tubes. A root wash was conducted using 25ml of 4:1 Acetonitrile:Ultra-Pure Water solution to remove any of the compound that had become sorbed to the surface of the roots. The tube was manually shaken for approximately 20 seconds, ensuring the roots are fully covered, with the solution being poured off into a separate centrifuge tube. 2ml of this sample was then taken in duplicate and analysed by Liquid Scintillation Counting (LSC). The centrifuge tubes were then re-weighed and the difference between the initial weight and final weight taken weight of plant material.

The remaining sand in the column was then added into the Mason jar and the lid placed on. The jars were then transferred to an open-air platform shaker for 30 minutes to ensure full mixing of the solution and sand. The weight of each jar was recorded, and water was added, if needed, to balance the centrifuge. The centrifuge was run at 3000 Relative Centrifugal Force and 20 degrees centigrade for 10 minutes. Once the centrifuge had stopped, the supernatant was poured off into pre-weighed 250ml storage bottles. 100ml of water was then added to each mason jar and placed back onto the shaker. This process was repeated 3 times until 4 washes had been completed. 1ml of each sample was taken and analysed by LSC.

The plant material samples were combusted using a Sample Oxidiser (Perkin Elmer Model 307), the washed roots and shoots were cut into appropriately sized portions to fit into the Combusto-cone. A combusto-pad was placed on top, 3-4 drops of ultra-pure water and 3 drops of Combustaid added to each sample. The carbon dioxide arising from the combusted sample was trapped by Carbosorb E and dispensed inot scintillation vial followed by mixing with 15ml of the scintillation cocktail PermaFluor E+. The radioactivity was determined for treatment solution, root wash, roots and shoots, and the recovery of radioactivity was calculated. Prior to the experiment an ‘acceptable range’ for recovery of radioactivity was set at 95-105%. The threshold for degradation was set at 5% to allow for confidence that the plant was taking up 1,2,4-Triazole during the experiment. This value was also compared against the degradation samples to determine whether any degradation was due to the presence of the plant or biota in the sand. Further to the treated samples, on day 21 the control, evaporation and degradation samples were also removed from the greenhouse and destructively harvested. A note was made of how much solution had been lost from each sample and the degradation samples was topped up to the original weight. Degradation samples were transferred straight to the 500ml Mason Jars and put through 4 centrifuge washes as described previously.

### 3.3.3 Plant Uptake Factor

The plant uptake in this system was from a solution that is being topped up daily, therefore the concentration is calculated by equation 3.1.

$$C = C_0 e^{\frac{-\propto v}{V_0}} \tag{3.1}$$

In this equation, C is Concentration in the solution, $C_0$ is the original concentration in the solution, e is the exponent of the fraction where α is the plant uptake factor, v is volume transpired at the measurement interval and $V_0$ is the original volume. Taking alpha as the plant uptake factor, this can be calculated using two methods detailed in the equations below. Equation 3.2 focuses on the concentration in the solution and how much has been lost in relation to the volume transpired. Equation 3.3 focuses on how much mass remains within the plant material in relation to the volume being transpired.

$$PUF = \frac{\ln \frac{C_0}{C}}{\frac{v}{V_0}} \tag{3.2}$$

$$PUF = \frac{\ln \frac{M_0}{M_0 - m}}{\frac{v}{V_0}} \tag{3.3}$$

Where $M_0$ is the mass in the starting concentration, m is the total mass of radiolabelled substance within the plant material, v is the total volume of water transpired by the plant, $V_0$ is the top-up volume. Therefore, the individual PUF for each sample can be calculated by filling in and solving the equation. The mass balance will be reported within the results.

### 3.3.4 Statistical analysis

The PUF for the whole treatment period can be calculated by plotting ln $C_0/c$ or ln $(M_0/(M_0\text{-}m))$ against $v/V_0$, the slope provides the mean PUF over the treatment period. The plant health assessment, using the transpiration data as proxy, was tested using a repeated measures linear mixed model.

## 3.4 Results and discussion

### 3.4.1 Degradation and recovery of [$^{14}$C]-1,2,4-Triazole

Degradation of 1,2,4-Triazole (Figure 3.1), began after 24hrs (T1) unfortunately, this means that any plant uptake results calculated from this dataset cannot be accepted since there is no certainty as to which compound is being taken up. In later sections there is discussion of plant uptake results, but these should not be taken as PUF for [14C]-1,2,4-Triazole from sand but to provide discussion of how the method can be improved.

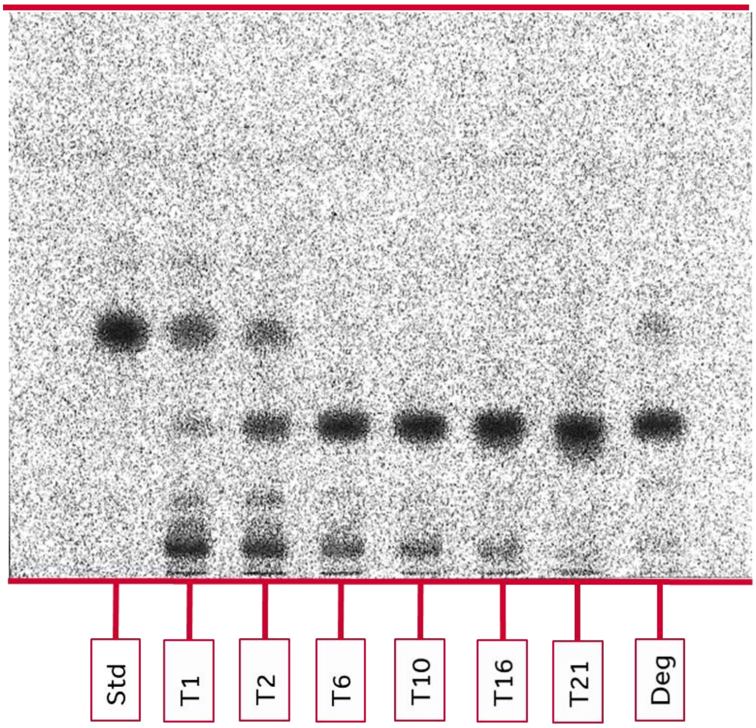

**Figure 3.1. Thin layer chromatography of samples taken following the harvest of the plants at each of their allotted harvest points. Std is the standard of the [$^{14}$C]-1,2,4-Triazole and Deg is degradation samples which were harvested on day 21 also.**

Faster degradation of 1,2,4-Triazole in the sand profile, compared the hydroponic study in Chapter 2, suggests that degradation is a big consideration when considering similar assessments of environmental fate in the future. 1,2,4-Triazole has been suggested for these assessments as it is a metabolite of a well-known and large group of pesticides the azole fungicides (Blondel et al., 2018). It has been shown to be taken up (PUF approximately 0.65) and translocated by plants (Lamshoeft et al., 2018). The DT50 of 1,2,4-Triazole in soil is approximately 10 days, it is clear from this experiment that DT50 values cannot be translated to this system as the compound has been almost fully removed by T6 (Blondel et al., 2018). Although no microbial assessment was conducted, within the sand and hydroponic solution system, there is a potential for this mechanism to be impacting the results shown here. In the previous experiment in hydroponic solution, degradation between 10-16 days (Chapter 3).

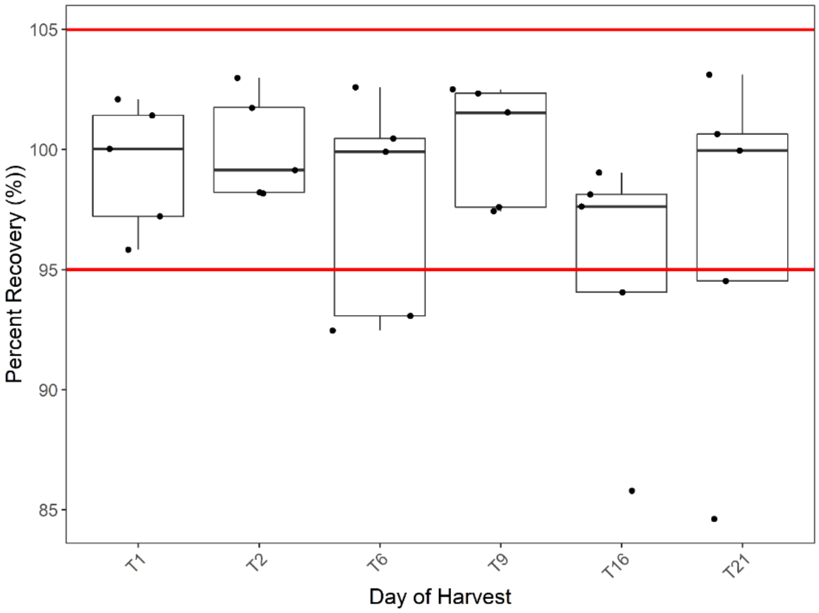

**Figure 3.2. The percentage recovery of total radioactivity after harvest. The two horizontal red lines represent the expected acceptable range for recovery determined at the start of the experiment.**

Percent recovery of the total radioactivity (Figure 3.2) showed that the devised method was suitable for extracting the radioactivity back out of the profile. This is particularly apparent for the first two harvests, where all data points come within the predefined 'acceptable range' for recovery. In total only 6 data points do not come within this acceptable range, leaving at least 3 data points remaining for each harvest point, allowing for a reasonable mean to be calculated.

## 3.4.2 Plant uptake Factor (PUF) results by time for both methods

As mentioned previously, the results presented in this section should not be taken as true PUF values for [14C]-1,2,4-Triazole from sand as the compound significantly degraded after 24 hours (Figure 3.1). PUF results derived using the concentration of the compound within the sand profile show much higher values than expected (Figure 3.3). As degradation had occurred, there was a cocktail of compounds within the solution, each compound has a different PUF value. It is unknown as to which [14C] compounds were present in the profile following the degradation, however, it has previously been shown that some organic compounds are taken up actively (Collins et al., 2006; Zhan et al., 2010). Therefore, it cannot be ruled out that active uptake would cause the PUF values to be higher than 1.

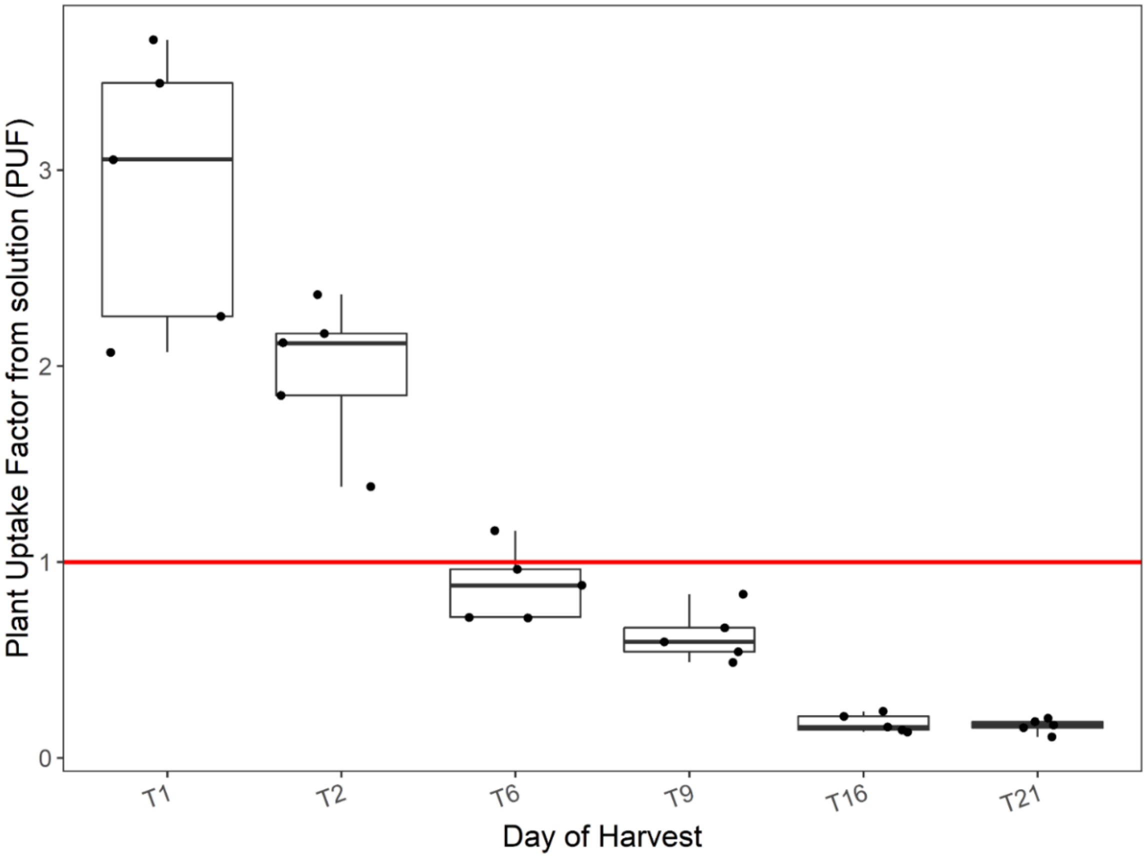

**Figure 3.3. Boxplots of PUF derieved from measurement of total radioactivity in the solution (equation 3.2). The horizontal red line denotes the maximum value expected for passive uptake when using PUF.**

It is also possible that the method of application lead to a concentrated area of [$^{14}$C]-1,2,4-Triazole in the top half of the sand column. This would mean that the starting concentration used within the PUF calculation would be an underestimation. This should be considered as a part of future planned experiments to ensure that the concentration is even throughout the column. This would ensure that the starting concentration is calculated correctly and would allow for measurement of a stable PUF.

The plant uptake calculated from the concentration within the plant material shows a similar pattern of uptake to PUF measured from the concentration in the solution (Figure 3.4). One of the main differences between the two methods is that the measurement from the concentration in the plants showed much higher values at each harvest point. It is unclear as to why this occurs as both results should produce similar values.

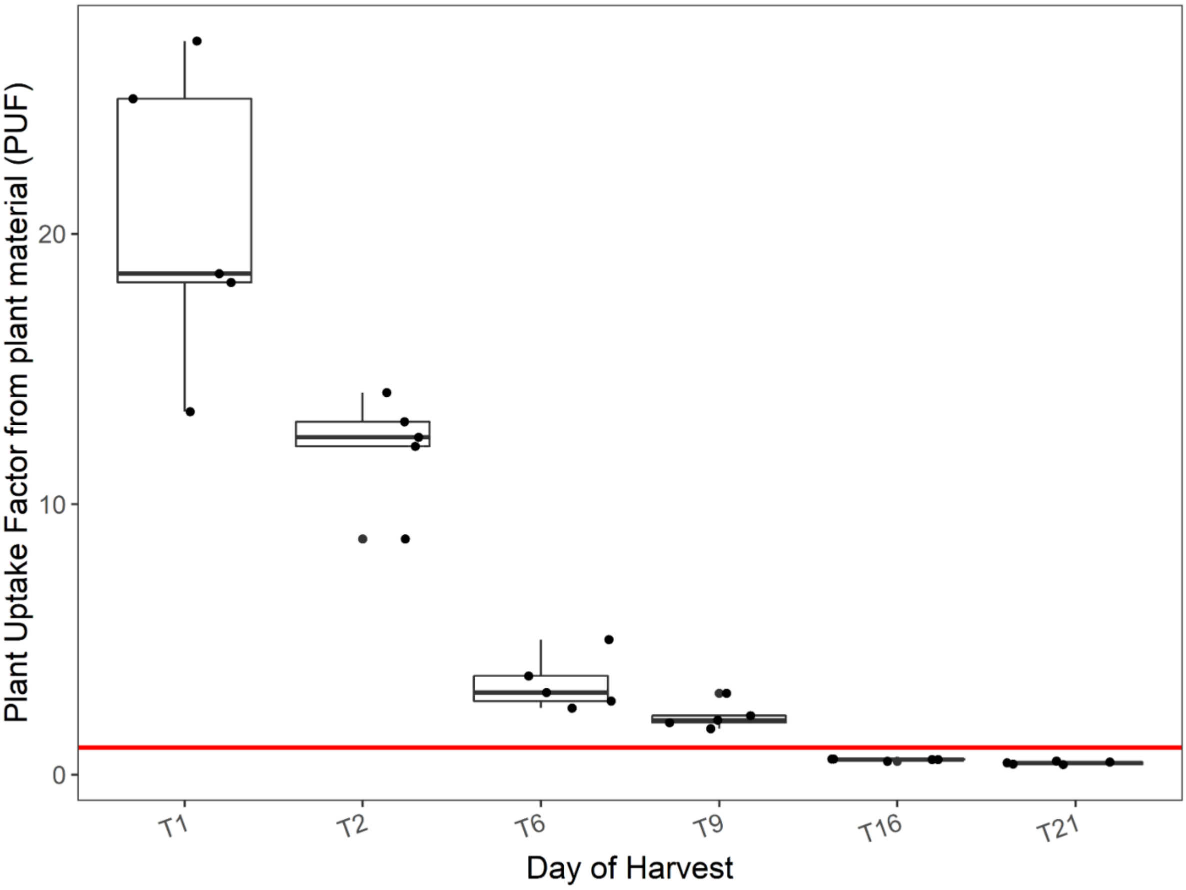

**Figure 3.4. Boxplots of PUF derieved from measurement of total radioactivity in the plant material (equation 3.3). The horizontal red line denotes the maximum value expected for passive uptake when using PUF.**

## 3.4.4 Plant health assessment

The plants could not be weighed during the experiment due to being enclosed within the sand profile. Therefore, the available measurement that could form part of a plant health assessment was transpiration. To calculate this, the average amount of solution lost from the evaporation samples was subtracted from the amount lost from the planted samples. Transpiration by plants is subject to a few environmental factors, however in young plants it is generally expected to increase with the age. While the general trend is positive, daily variation is seen. A linear mixed model was conducted on the transpiration data which showed that there was a significant difference between the control and treated samples (p-value = <0.0001). With the controls showing higher transpiration than the treated samples. Further investigations were conducted which showed that the significant difference was not across the whole period but on 9 out of 21 days. A significant difference was observed on days 2, 3, 4, 10, 11, 12, 13, 14 and 18.

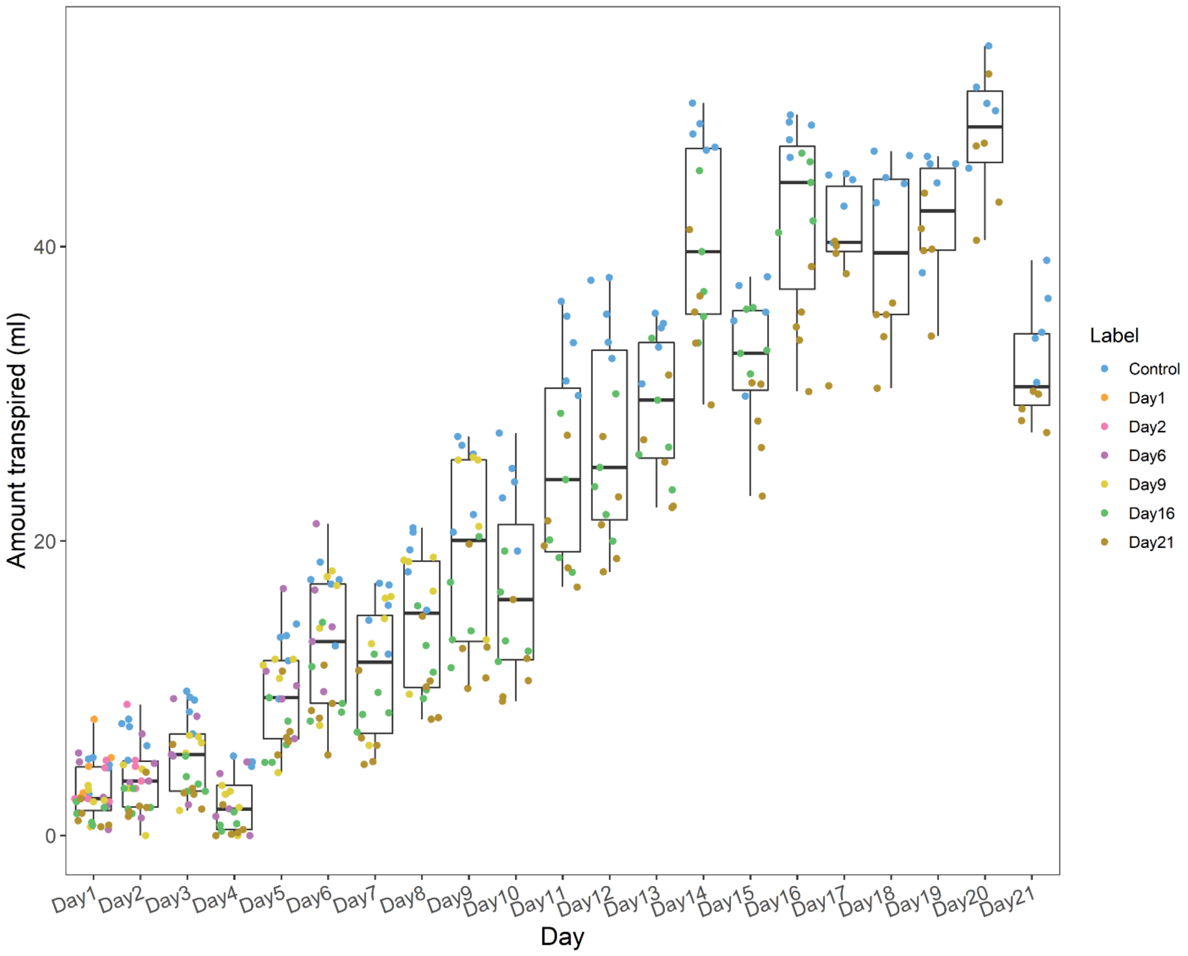

**Figure 3.5. The amount of water transpired each day by the plants. This value was derived from the amount of solution lost from the experiment, with the average value lost from the evaporation and the degradation samples removed.**

## 3.4.5 Considerations for future determination of PUF from sand

1,2,4-Triazole was selected as it is a key metabolite of the azole fungicides (Blondel et al., 2018). It has also been studied previously for plant uptake experiments and is known to be taken up by plant roots and translocated (Lamshoeft et al., 2018). The sand/Hoagland's solution profile was selected as it provided an inert mineral substrate with the nutrient base being provided by the solution. Based on the reported DT50 of 1,2,4-Triazole of 6-9 days in soil, it was unclear as to whether this would be reduced or increased in a hydroponic/sand profile (Blondel et al., 2018). The reduction in organic material meant that the microbiome would not be potentially large as in soil, but the provision of the Hoagland's solution means that the nutrients were more readily available for the microbes in the system. Results from the study show that degradation of the compound was much faster than observed within a fully hydroponic system (Chapter 3). For plant uptake to be studied by current methodologies, degradation needs to remain < 5% for approximately 8 days.

Another consideration for future experiments in sand or soil would be the concentration of the compound following application to the profile. Although the biggest explanatory factor behind this result is likely to be the degradation of the test item, a discrepancy between the expected concentration and the actual concentration could be a contributary factor. As the profile and solution cannot be mixed prior to the application of the compound, it would be required that it became fully mixed instantly following application for the concentration to be equal to the amount calculated at the beginning. The method of application was designed so that at application, the compound was mixed in as much solution as possible, allowing for a spread into multiple sites following several pipettes of the solution. The volume of solution added to the profile during treatment represented an average amount solution taken up by the plants each day, with extra untreated solution added if required to reach the target weight. Despite these measures, it is possible that it took time before the test item became fully mixed within the profile and the concentration became stable. Therefore, any of the test item that was taken up during this period was taken up at a higher concentration than factored in within the calculation. In this scenario, whilst some of the roots were taking up solution containing the compound at a higher concentration, other sections of the roots were taking up solution containing no compound or at a very low concentration. This imbalance would make it very difficult to determine a rate of uptake for the whole system and may help to explain some of the variation in PUF throughout this experiment (Figure 3.4 and Figure 3.6). It is also important to consider that uptake of solution is not uniform throughout the length of the plant root. Older parts of the roots start to become suberised making them almost impermeable to water, meaning that the new section of the roots take up most of the water (Taiz and Zeiger, 2010). If there are hotspots of concentration within the profile, uneven uptake by the roots may cause variable PUF results.

## 3.5 Conclusion

Degradation of the applied test item meant that the plant uptake factor measured in this study cannot be taken forward as a value used within environmental fate assessments. Firstly, the degradation of the 1,2,4-Triazole before the first harvest point meant that none of the time points would qualify to be used within regulatory based assessments. Despite the degradation of the test item within the profile, the percentage recovery of the total radioactivity remained at a good level. This shows that the method of extraction was good and could be used going forward. There are some key elements with regards to developing further methods with increased realism when compared to soil that need to be considered. Unless a new system can be derived that doesn't degrade the compound as quickly, 1,2,4-Triazole would not be suitable going forward for sand and Hoagland's solution profile due to not being stable for long enough. A compound with a longer DT50would however be suitable within this method and would allow for the experiment to be run for a long enough period to allow for comparison with comparable hydroponic studies. Future methods should also determine whether application of a compound from the top of the profile leads to a high concentration of the test item in the upper sections of the profile. Uneven concentration within the profile could adversely affect PUF results depending on where the concentration hotspot occurs and where the roots are situated in the profile.

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# Chapter 4 – Quantifying the effect of plant uptake on environmental fate using the FOCUS scenarios

## 4.1 Abstract

Environmental fate modelling is one of the preferred methods for assessing the potential of plant protection products (PPPs) to leach down the soil profile into potable water supplies. Assessing the risk prior to registration is key to ensuring that compounds do not enter the groundwater, which is of increasing importance as global populations increase, hence increasing demand for water. Plant uptake acts as a sink for a pesticide that is applied to the soil profile and reduces the concentration of the pesticide within the soil pore water, therefore reducing the amount that has the potential to become leached. There are a variety of environmental fate models that are used for this assessment. Here, PEARL and PELMO have been selected to compare the effect that plant uptake has on the leaching of 3 example compounds, for several locations across Europe, with different soil types and climates. The example compounds have been selected to give a variety of mobility and persistence. Results show that overall the predicted environmental concentration in the groundwater ($PEC_{GW}$) can be affected in the range of 0-85%, with a total mean of 46.8%. Compound C ($K_{OC}$ = 500 and DT50 = 500 days)) was the only compound where plant uptake affected leaching for both models, with PELMO predicting a higher percentage change than PEARL. There were marked differences between plant uptake and leaching during the growing season as simulated by PEARL and PELMO, even though both models employ very similar approaches to calculating amount uptake. Despite the differences in the models' soil hydrological approach, the leaching patterns throughout the year were very similar. Results from this study show that plant uptake is an important consideration when looking at the leaching of a compound from the soil profile, but that this is also highly dependent on the compound in question and the conditions at the location being modelled.

## 4.2 Introduction

Environmental fate modelling remains the main method for assessing potential plant protection products (PPPs) prior to their release into the environment. The focus of this assessment is to measure the amount of pesticide that is leached through the soil profile and into the groundwater (FOCUS, 2009). Due to the varying regulatory requirements, several models have been developed to assess pesticide leaching across the world. The main regulatory fate models within the EU are PEARL ver. 4.4.4 (Tiktak et al., 2000), PELMO ver. 5.5.3 (Klein, 2012), MACRO ver. 5.5.4 (Stenemo and Jarvis, 2010) and PRZM ver. 5 (Carsel et al., 1985). PELMO was originally based on PRZM ver. 1 but has been adapted to make it of greater use to regulators in Europe (Klein et al., 1997). As a result, PELMO sees greater use within the EU regulatory framework, although, PRZM is also used sparingly. Largely due to the EU working group, FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe), much research and development has gone into these models over the years since their release. Part of their work consisted of providing a framework for the assessment of leaching by creating a dataset consisting of model parameters and deriving data for 9 locations with 12 to 16 crop scenarios (FOCUS, 2014). Following the release of the environmental fate models, much analysis has been conducted on the sensitivity and uncertainty of the model output to certain parameters (Dubus et al., 2003; Farlin et al., 2013; Klein et al., 2000; Köhne et al., 2009).

Plant uptake is one of the parameters that has been proposed to have a significant effect on the amount of pesticide leaching from the profile. Through this mechanism, it has been suggested that a $PEC_{GW}$ (Predicted Environmental Concentration in the Groundwater) that is close to the tolerable level of 0.1 ug/L could be reduced under the threshold (EFSA, 2013). Plant uptake is a sink for any applied pesticide (Lamshoeft et al., 2018). The way that environmental fate models deal with the input of plant uptake is by applying a singular value to the model that represents the fraction of the compound that is taken up based on the concentration by the plant for the fraction of the total volume that is taken up (Klein, 2012; Tiktak et al., 2000). This value is termed the Plant Uptake Factor (PUF) or Transpiration Stream Concentration Factor (TSCF) and is derived from laboratory experimental exposure of a plant to a compound in hydroponic solution (Briggs et al., 1982; Lamshoeft et al., 2018). Previously, where no compound specific laboratory-derived TSCF value was available, a statistical relationship derived by Briggs et al., (1982) would be used to estimate plant uptake based on the octanol-water partition coefficient (log $K_{ow}$) value. A lack of agreement in the scientific community on the true position of the relationship meant that a move away from Briggs et al., (1982) was suggested with a value of PUF = 0 suggested for the majority of pesticides and a value of PUF = 0.5 suggested for systemic pesticides with known uptake behaviour (FOCUS, 2000). Although this is known to be incorrect, it is the most risk-averse strategy and does not produce a plant sink of pesticide based on a scientifically debated statistical relationship which could provide a lot of uncertainty in leaching outputs.

Previous work in this area consisted of a small modelling exercise completed by EFSA that found that in PEARL, when Plant Uptake Factor (PUF) is varied between 0 and 1, $PEC_{GW}$ values decrease between 24-43% (EFSA, 2013). The current study aims to take an in-depth look at the effect of PUF on the leaching output of each model, across a range of locations, using one crop calendar. This modelling study uses three example compounds that were designed to give a range of mobility and persistence within the soil profile.

# 4.3 Methodology

## 4.3.1 FOCUS scenarios

### 4.3.1.1 Locations

The FOCUS locations were designed to represent nine realistic worst-case scenarios to allow for the assessment of environmental fate across a range of soil profiles and climatic conditions. The locations were selected based on three conditions: they represent the major agricultural regions in the European Union (EU); span a range of temperature and rainfall (Table 4.1) occurring under EU arable agriculture; they are distributed across the EU with no more than one location per member state (FOCUS, 2014). As shown in Figure 4. this covers the main climatic zones within the EU, ranging from temperate to Mediterranean. Each scenario is set to model the key soil processes and variables over a depth of 1m, which is designed to allow for easy comparison between locations.

**Table 4.1. Summary climate statistics for each FOCUS location.**

| Location | Average maximum temperature (°C) | Average yearly rainfall (mm) |
|---|---|---|
| *Châteaudun* | 15.5 | 620 |
| *Hamburg* | 12.5 | 796 |
| *Jokioinen* | 7.75 | 636 |

| | | |
|---|---|---|
| *Kremsmünster* | 13.0 | 932 |
| *Okehampton* | 13.7 | 1036 |
| *Piacenza* | 18.1 | 836 |
| *Porto* | 19.3 | 1151 |
| *Sevilla* | 24.0 | 496 |
| *Thiva* | 21.7 | 478 |

**Figure 4.1. Map of the 9 FOCUS locations modelling within this study, figure created using the latitude and longitude values provided by FOCUS.**

#### 4.3.1.2 Soils

Based on set criteria, each location was selected by the FOCUS experts to be a typical soil that you would expect to find in each location but with the intent that the soil profile would be more vulnerable to leaching than average soil profiles in this area. Each location within the FOCUS datafiles has been provided to give a soil profile of > 3m depth to allow for modelling of further depth if required within the scope of a project. However, by the FOCUS guidelines an assessment of leaching is only required to go to 1m depth and therefore, the following discussion of the soil composition and leaching will be to 1m. Figure 4.2a shows that there is a good range of soil textures, with some very sandy locations (Jokioinen and Hamburg), whereas Châteaudun is a high silt location and the locations with the largest clay fraction are Châteaudun and Thiva. The soil textures found within the locations are silty clay loam, sandy loam, loamy sand, loam/silt loam, loam and silt loam.

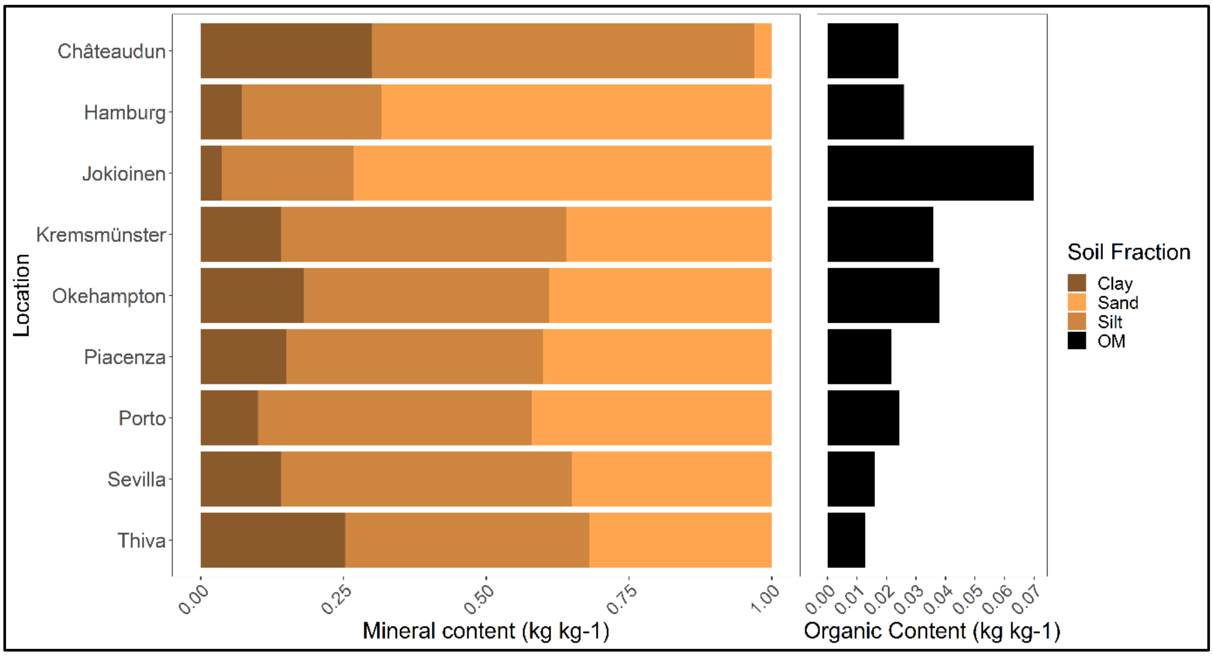

**Figure 4.2. The mineral (a) and organic (b) content of each FOCUS location used within this study**

Soil organic carbon (Figure 4.2b) is an important input to consider when modelling environmental fate as it can lead to the sorption of the target compound within the profile. The higher the carbon content, the more likely that the compound will become bound within the profile and not move down into the groundwater. Therefore, as expected, the organic carbon is relatively low within all scenarios apart from Jokioinen which has a value that is almost double that of the rest.

The soil water retention curve (Figure 4.3) is calculated using the van Genuchten equation (Equation 4.1) and shows the soil water content as a function of the soil water pressure.

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{[1 + |\, \alpha h \,|^n]^m} \qquad (4.1)$$

Here, θ is the soil moisture content (cm$^3$ cm$^{-3}$), $\theta_r$ is the residual soil moisture content (cm$^3$ cm$^{-3}$), $\theta_s$ is the saturated soil moisture content (cm$^3$ cm$^{-3}$), α is a scale parameter inversely proportional to mean pore diameter (cm$^{-1}$), h is the soil water (matric) pressure (kPa), n is a shape parameter of soil water content, and m = 1-1/n. Figure 4.3, shows that the high sand content locations (Hamburg and Jokioinen) have the lowest soil water content throughout the soil water pressures calculated. The location where the soil moisture changes the least over the range of *h* values is Châteaudun, this is also the location where the clay and silt content are highest.

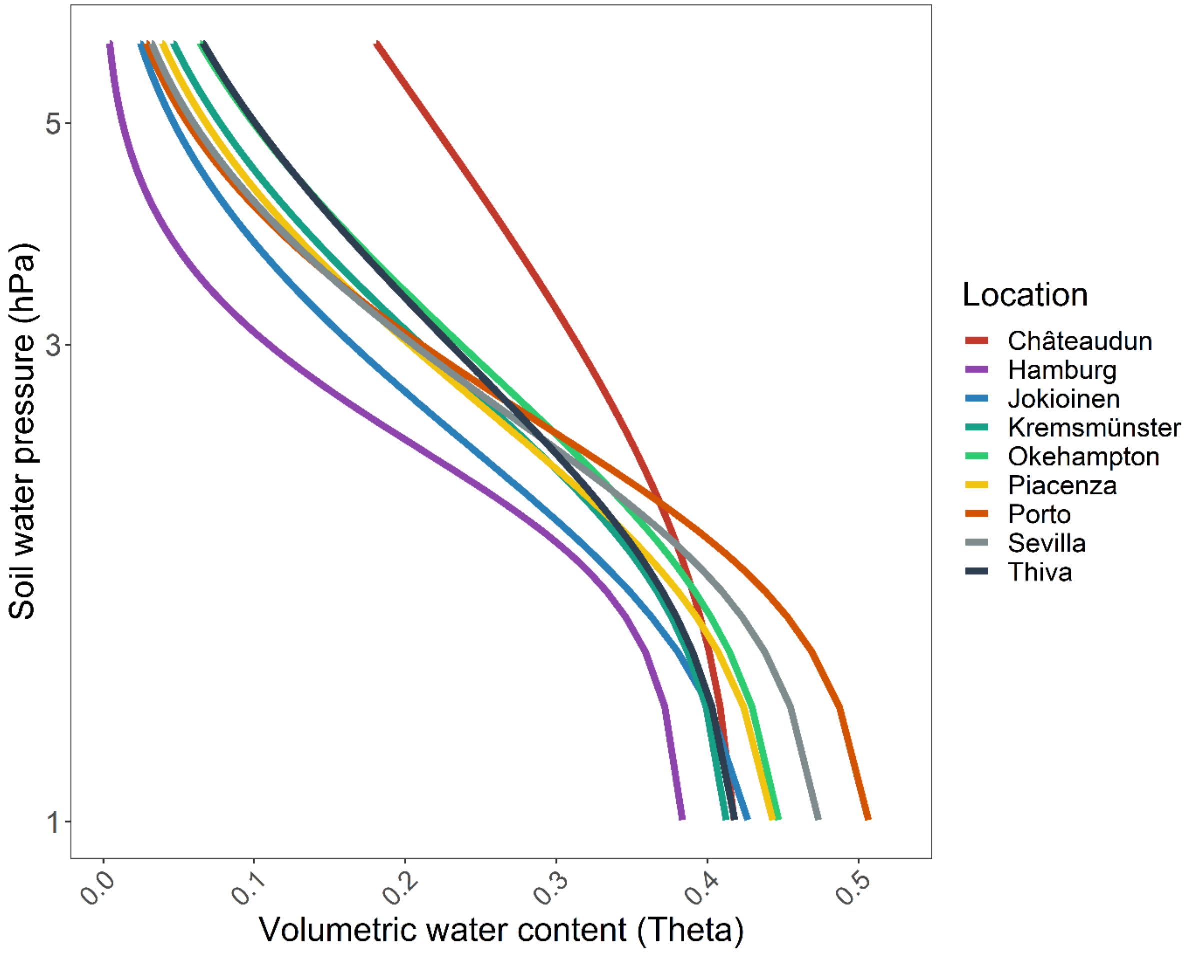

**Figure 4.3. The soil water retention curves for each FOCUS location used within this study. The soil water retention was calculated using the van Genuchten model (Equation 4.1) (van Genuchten, 1980).**

#### 4.3.1.3 Crop

Wheat is one of the most produced agricultural crops within the European Union. The length of the growth season of winter wheat means that there is a long sedentary period (approximately 4 months). Across the EU emergence dates (Table 4.2) range from 17th October to 1st December and the range of harvest dates is 31st May to 15th August (Table 4.). Maximum rooting depth is modelled alongside the crop development and ranges from 0.4 – 1m depth, depending on the location being modelled. Of this range, Sevilla shows a significantly lower rooting depth (0.4 m) compared to the others, with the remaining locations falling within 0.8-1 m.

**Table 4.2. Crop calendars for winter wheat at the FOCUS locations**

| Location | Emergence date | Harvest date |
|---|---|---|
| *Châteaudun* | 26th October | 15th July |
| *Hamburg* | 1st November | 10th August |
| *Jokioinen* | 20th September | 15th August |
| *Kremsmünster* | 5th November | 10th August |
| *Okehampton* | 17th October | 1st August |
| *Piacenza* | 1st December | 1st July |
| *Porto* | 30th November | 30th June |
| *Sevilla* | 30th November | 31st May |
| *Thiva* | 30th November | 30th June |

#### 4.3.1.4 Example compounds

Three example compounds (Table 4.3Table ) were used in the model, as distinguished by 2 physio-chemical parameters: $K_{OC}$ (the partition coefficient between organic carbon and water) and DT50 (the time taken to for the compound to degrade to half the original concentration):

**Table 4.3. Example compounds and the physical-chemical properties that were varied to give different mobility and persistence behaviour within the soil profile**

| Compound | $K_{OC}$ (L/kg) | DT50 (days) |
|---|---|---|
| *CA* | 10 | 100 |
| *CB* | 100 | 40 |
| *CC* | 500 | 500 |

For each compound the plant uptake was varied, with uptake scenarios for PUF = 0, 0.5 and 1.0. These compounds were also assigned other standard parameters to represent their behaviour within the soil profile. Further details of the parameterisation of both models can be found in Appendix A (PEARL) and Appendix B (PELMO).

## 4.3.2 Model Methodologies

### 4.3.2.1 PEARL 4.4.4

PEARL (Pesticide Emission Assessment at Regional and Local scales) was developed for use in the pesticide registration process to aid with the prediction of the mobility of a pesticide and the tendency for it to leach from the profile. Prior to this, PEARL's predecessors PESTLA (Pesticide Leaching and Accumulation) and PESTRAS (Pesticide Transport Assessment) were used in conjunction, however small but significant differences in their outputs in specific scenarios, led to a combination of both models by their creators to make them more suitable for regulatory processes (Tiktak et al., 2000).

PEARL is a one-dimensional, dynamic, multi-layer model which is coupled with SWAP (Soil Water Atmosphere Plant model). SWAP is used to describe the hydrological processes within the soil profile and uses a finite-difference method to solve the Richard's equation (Equation 4.4). In 1997 this equation was adapted to allow for the simulation of shallow groundwater. The Richard's equation is a combination of the Darcy's equation (Equation 4.2) for one-dimensional vertical flow and the continuity equation for soil water of an infinitely small soil volume (Equation 4.3, (Kroes et al., 2009).

$$q = -K(h)\,\frac{\delta\,(h+z)}{\delta z} \tag{4.2}$$

$$\frac{\delta\theta}{\delta t} = -\,\frac{\delta q}{\delta z} - S(h) \tag{4.3}$$

$$\frac{\delta\theta}{\delta t} = C(h)\frac{\delta h}{\delta t} = \frac{\delta\left[K(h)\,\left(\frac{\delta h}{\delta z}+1\right)\right]}{\delta z} - S(h) \tag{4.4}$$

Where q is soil water flux density (positive upwards) (cm $d^{-1}$), K is hydraulic conductivity (cm $d^{-1}$), h is soil water pressure (cm), z is the vertical coordinate (positive upward) (cm), $\theta$ is volumetric water content ($cm^3$ $cm^{-3}$), t is time (d), S is soil water extraction rate by plant roots ($cm^3$ $cm^{-3}$ $d^{-1}$) and C is the water capacity ($\delta\theta/\delta h$) ($cm^{-1}$). By treating the soil profile as one continuum of soil, air and water, Equation 4.4 is then solved numerically with the relationships of K, h and Θ being input from direct soil profile measurements or a pre-defined dataset (as in the FOCUS scenarios).

Plant uptake of water is modelled by solving the following equation (Equation 4.5) for the potential root water extraction rate at a given depth ($S_p(z)$) ($d^{-1}$).

$$S_p(z) = \frac{I_{root}\,(z)}{\int_{-D_{root}}^{0} I_{root}\,(z)\delta z}\,T_p, \tag{4.5}$$

Where $I_{root}$ (z) is the root length density at depth z (cm $cm^{-3}$), expressed as a fraction of the integrated root length density where $D_{root}$ is the root layer thickness (m) and $T_p$ is the potential transpiration rate (cm $day^{-1}$). As in practice the data of this distribution is often not available, SWAP will often assume a uniform root length density distribution, resulting in Equations 4.6**Error! Reference source not found.** and 4.7 below, where both equations are derived from the original Equation( 4.5 (Kroes et al., 2009).

$$\frac{I_{root}\ (z)}{\int_{-D_{root}}^{0} I_{root}\ (z)\delta z} = \frac{1}{D_{root}} \tag{4.6}$$

$$S_p(z) = \frac{T_p}{D_{root}} \tag{4.7}$$

Water uptake ($R_{u,L,p}$ ($m^3m^{-3}$ $d^{-1}$), equation 4.8) by plants roots is related to the potential transpiration rate ($T_p$, ($m^3m^{-2}d^{-1}$)) and volumic root length ($L_{root}(z)$, m $m^{-3}$).

$$R_{u,L,p}(z) = \frac{L_{root}(z)}{L_{root}(z)dz}\,T_p \tag{4.8}$$

Description of the environmental fate of pesticide within the soil system is described by the two following mass balance, equations 4.9 and 4.10.

$$\frac{c_{eq}}{t} = -R_s - \frac{J_{p,L}}{z} - \frac{J_{p,g}}{z} - R_t + R_f - R_u - R_d \tag{4.9}$$

$$\frac{c_{ne}}{t} = R_s \tag{4.10}$$

Where $c_{eq}$ is the pesticide concentration in the equilibrium domain of the soil profile (kg $m^{-3}$), $R_s$ is the volumic rate of pesticide sorption (kg $m^{-3}$ $d^{-1}$), $J_{p,L}$ is the mass flux of pesticide in the liquid phase (kg $m^{-2}$ $d^{-1}$), $J_{p,g}$ is the mass flux of pesticide sorption in the gas phase(kg $m^{-2}$ $d^{-1}$), $R_t$ is the rate of transformation of the compound (kg $m^{-3}$ $d^{-1}$), $R_f$ is the rate of formation (kg $m^{-3}$ $d^{-1}$), $R_u$ is the rate of pesticide uptake by plant roots (kg $m^{-3}$ $d^{-1}$) and $R_d$ is the rate of lateral discharge ($m^3$ $m^{-3}$ $d^{-1}$). Transport of pesticide within PEARL is described by convection, dispersion and diffusion (Tiktak et al., 2000).

$$J_{p,T} = qc_T - D_{dis,L}\frac{c_L}{z} - D_{dif,L}\frac{c_L}{z} \qquad (4.11)$$

Where q is the soil water flux ($m^3\ m^{-2}\ d^{-1}$), $D_{dis,L}$ is the coefficient of pesticide dispersion in the liquid phase ($m^{-2}\ d^{-1}$), z is the depth within the soil profile (m) and $D_{dif,L}$ is the coefficient of pesticide diffusion in the liquid phase ($m^2\ d^{-1}$). Further equations describing the calculation of $D_{dis,L}$ and $D_{diff,L}$ can be found in the manual for PEARL. Transport of pesticide within the gas ($J_{p,g}$) phase is calculated by equation 4.12.

$$J_{p,g} = -D_{dif,g}\frac{c_g}{z} \qquad (4.12)$$

Where $D_{dif,g}$ is the coefficient of pesticide diffusion in the gas phase ($m^2\ d^{-1}$). Further information on the calculation of the coefficients for pesticide transport ($D_{dis,L}$; $D_{dif,L}$ and $D_{dif,g}$) can be found within the manual of PEARL.

Pesticide uptake ($M_U$) in PEARL is described by equation 4.13:

$$M_U = R_L PUFC_L \qquad (4.13)$$

here $R_L$ is the water uptake ($m^3\ m^{-3}\ d^{-1}$), PUF is the Plant Uptake Factor (-), and $C_L$ is the concentration of pesticide within the liquid phase ($kg\ m^{-3}$) (Tiktak et al., 2000).

#### 4.3.2.2 PELMO 5.5.3

PELMO (PEsticide Leaching MOdel) 4.01 is another environmental fate model that is used within EU regulatory processes. Originally it was based on PRZM-1 (Pesticide Root Zone Model) which was published by the US-EPA (United States-Environment Protection Agency) but PELMO has been independently developed and updated since 1989. PELMO is a dynamic compartment model whereby the soil profile is divided into small segments throughout, with a tipping-bucket approach being used for the hydrology (Klein, 2012).

The moisture balance and the movement of water within the profile are calculated from the rainfall, runoff, and evapotranspiration at the surface, and the redistribution and storage of water throughout the profile. The tipping bucket approach then works on the basis that once the top layer of soil reaches field capacity, the excess water is passed onto the next layer; this process is repeated throughout the profile until the defined lower boundary. The capillary flow of moisture and compound concentration with plant uptake of water is not considered within this model.

The driver behind the environmental fate of a pesticide is the transport of dissolved pesticide. Within PELMO this is the same as PRZM (Klein, 2012; Suarez, 2005):

$$A\,\Delta z \frac{\partial (C_w \theta)}{\partial t} = J_D - J_V - J_{DW} - J_U - J_{QR} + J_{APP} + J_{FOF} \pm J_{TRN} \quad (4.14)$$

$$A\,\Delta z \frac{\partial (C_s \rho_s)}{\partial t} = -J_{DS} - J_{ER} \quad (4.15)$$

$$A\,\Delta z \frac{\partial (C_g a)}{\partial t} = J_{GD} - J_{DG} \quad (4.16)$$

In these equations: A is Cross-sectional area of soil ($cm^2$); $\Delta z$ is depth of compartment (cm); $C_w$ is dissolved concentration of pesticide ($g\ cm^{-3}$); $C_s$ is sorbed concentration of pesticide ($g\ g^{-1}$); $C_g$ is gaseous concentration of pesticide ($g\ cm^{-3}$); $\theta$ is volumetric water content ($cm^3\ cm^{-3}$); a is volumetric water content ($cm^3\ cm^{-3}$); $\rho_s$ is soil bulk density ($g\ cm^{-3}$); t is time (days); $J_D$ is effect of dispersion and diffusion ($g\ day^{-1}$); $J_V$ is effect of advection ($g\ day^{-1}$); $J_{GD}$ is effect of dispersion and diffusion ($g\ day^{-1}$); $J_{DW}$ is loss due to degradation in the dissolved phase ($g\ day^{-1}$); $J_{DG}$ is loss due to degradation in the vapour phase ($g\ day^{-1}$); $J_U$ is loss due to plant uptake of dissolved phase ($g\ day^{-1}$); $J_{QR}$ is loss due to runoff ($g\ day^{-1}$); $J_{APP}$ is gain due to pesticide deposition on the soil surface ($g\ day^{-1}$); $J_{FOF}$ is gain due to wash-off from plants ($g\ day^{-1}$); $J_{DS}$ is loss due to degradation of sorbed phase ($g\ day^{-1}$); $J_{ER}$ is loss by dissolved removal on eroded sediments ($g\ day^{-1}$); $J_{TRN}$ is gain or loss due to transformation ($g\ day^{-1}$). Further equations for the calculation of the $J_x$ inputs will not be discussed within this chapter but can be found within the manual for PELMO (Klein, 2012)

PELMO takes the same approach as PEARL when it comes to plant uptake ($J_u$ in equation 4.14). This approach is adopted by many environmental fate models and assumes that the uptake of pesticide is related to the concentration within the liquid phase of the soil solution, the input PUF and the amount of soil pore water taken up by the plant during the set time period (Klein, 2012).

#### 4.3.2.3 Model methodologies inter-comparison

##### *4.3.2.3.1 Overview*

As described above, both models have a significantly different approach to environmental fate modelling. Although the above sections provide a more detailed description of the similarities and differences of both models. Below (Table 4.4), has been provided to help draw direct comparisons and the range of options available within each framework.

**Table 4.4 Direct model comparison table for the main features of PEARL and PELMO**

| | Feature | PEARL | PELMO |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **Model Structure** | Model type | One-dimensional, dynamic, multi-layer model | Dynamic, compartmental model |
| | Hydrological methods | Richards equation | Capacity tipping bucket |
| **Hydrological processes** | Evapo-transpiration | Penamn-Monteith | Hamon, Haude or Direct input |
| | Irrigation | Sprinkler or surface. Applied once a week to bring root zone up to field capacity. | Applied once a week to bring root zone up to field capacity |
| | Run-off | Calculated within model | Optional calculation |
| **Pesticide settings** | Plant Uptake Factor (PUF) | Single value applied to each compound. | Single value applied to each compound. |
| | Plant uptake of pesticide by roots | As a function of PUF, concentration in liquid and volume of uptake | As a function of PUF, concentration in liquid and volume of uptake |
| | Sorption | Koc input. Freundlich exponent. | Kom input. Freundlich exponent. |
| | Degradation | First order kinetics. DT50 inputs alongside temperature during the study. | First order kinetics. The option of input as a rate per day, DT50, DT90. The temperature during the study and Q10 temperature coefficient can also be defined. |
| **Scenario settings** | Fallow scenario | Defined within the model as an option. | Unable to give no crop for season so same crop given for one day but harvested the following day. |
| | Application methods | Soil surface, spraying on the crop canopy, application into the topsoil. Dosed at 1 kg/ha, applied at 1 day prior to emergence. | Soil surface, incorporation to defined depth, application to the crop canopy defined by the user, application to the crop canopy calculated by the model. Dosed at 1 kg/ha, applied at 1 day prior to emergence |
| | Crop calendar | User-defined. Emergence and harvest input. Development stages of 0, 0.5 and 1 for wheat. | User-defined, dates given for 4 stages, emergence, maturity, senescence and harvest. |
| | Rooting depth | For winter wheat, depths of 0.1, 0.3 and 0.6 metres are given for their respective growth stages within the crop calendar. | Considered a linear growth from emergence to maturation. For winter wheat, the maximum rooting depth is 1.4 metres. |

### 4.3.3 Meteorological data

Meteorological data is required within environmental fate models to drive the hydrological module. This allows them to simulate realistic environmental conditions by calculating the water balance and hydrological inputs and outputs. Variation in the climatic inputs can have significant impacts on the movement of pesticides which is driven by environmental change. The climate can also have a significant effect on the plant growth, health and stress; therefore it is a very important consideration when modelling plant uptake. Violin plots demonstrate the kernel density of each meteorological variable, whereby the shape of the plot shows the relative spread or clustering of the data points. Therefore, the shorter and wider plots are those that have more points clustered in the same data region; those that are longer and wider have a high spread of data. As no irrigation is being simulated in

Figure 4.6 shows that there are a few locations with a large degree of variation of monthly rainfall each year, for example Porto. Many of the locations have moderate rainfall across the months, as indicated by shorter and wider plots, whereas the bottom three locations have extremes of high rainfall. Porto is a good example of a location that shows extremes of high precipitation (Porto) or dry weather (Sevilla, Thiva).

With regards to *yearly* rainfall data (Figure 4.7), Porto shows high extremes of rainfall with some very long thin plots. The other locations look more like each other, with some variation between years. Thiva and Jokioinen show narrow and thin kernel density plots, indicating a fair amount of variation but low rainfall totals for most years.

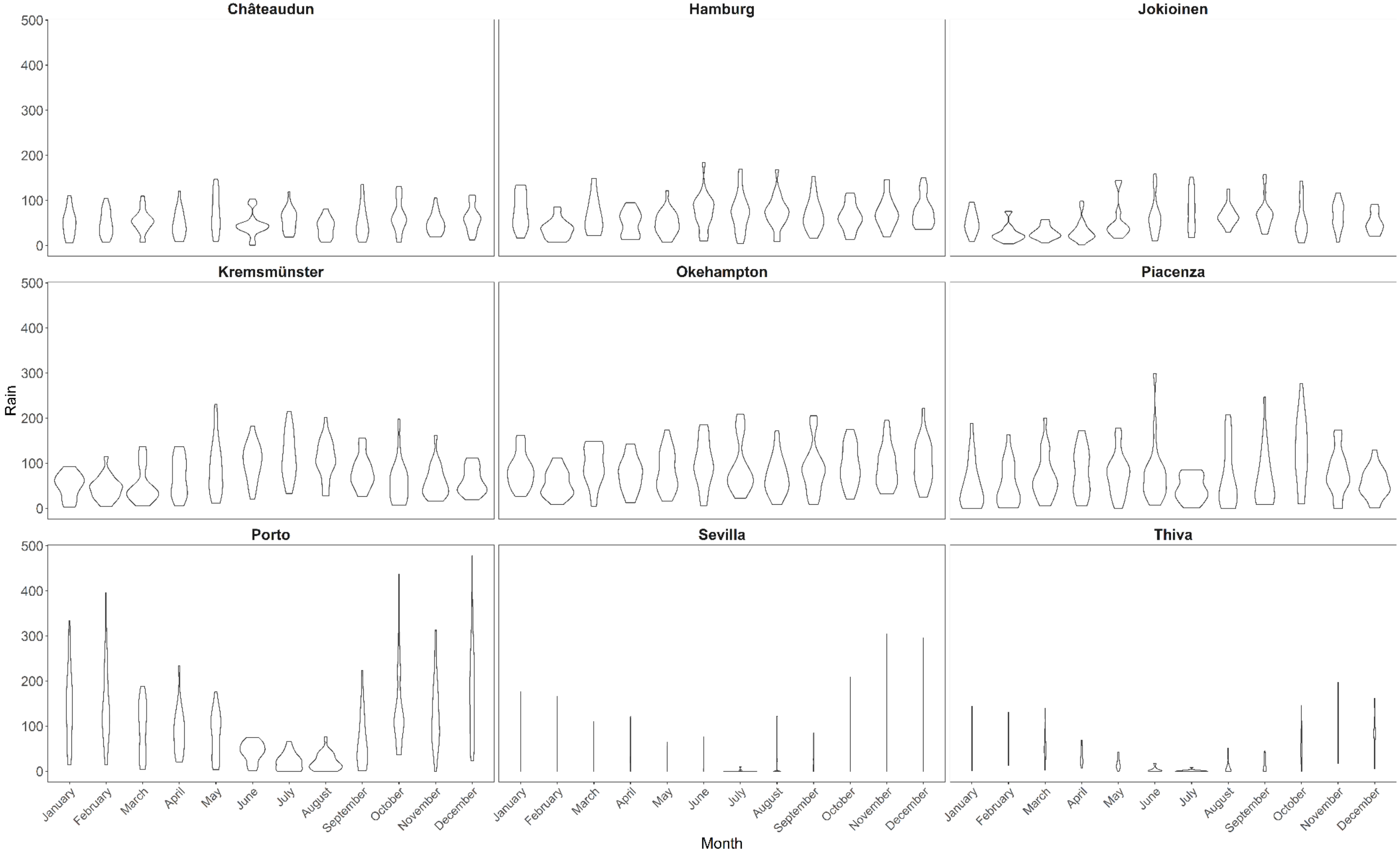

**Figure 4.6. Violin plots of the monthly sums of precipitation**

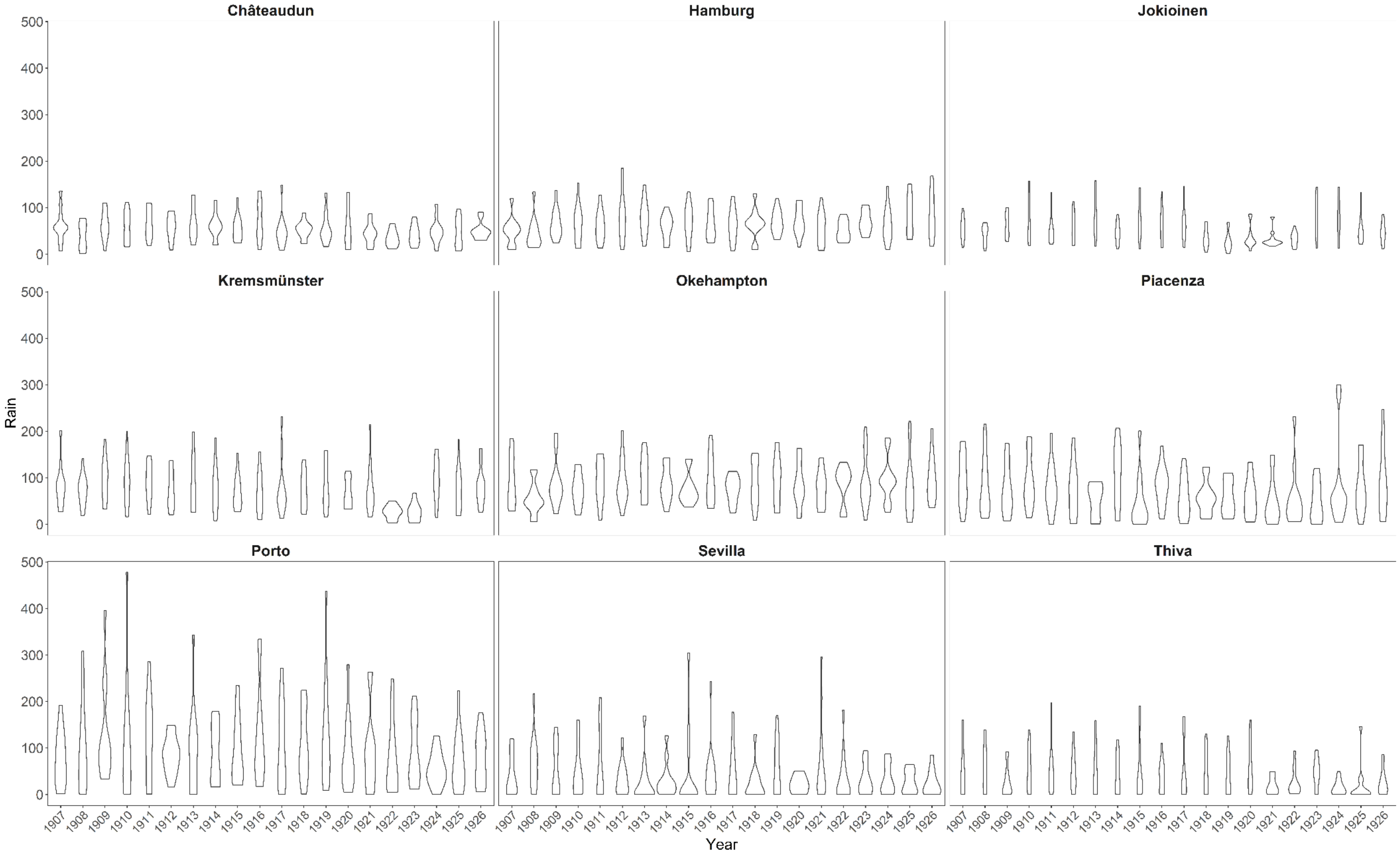

Figure 4.7. Violin plots of the monthly sums of precipitation throughout the simulation period.

# 4.4 Results and Discussion

## 4.4.1 Hydrological boundary fluxes

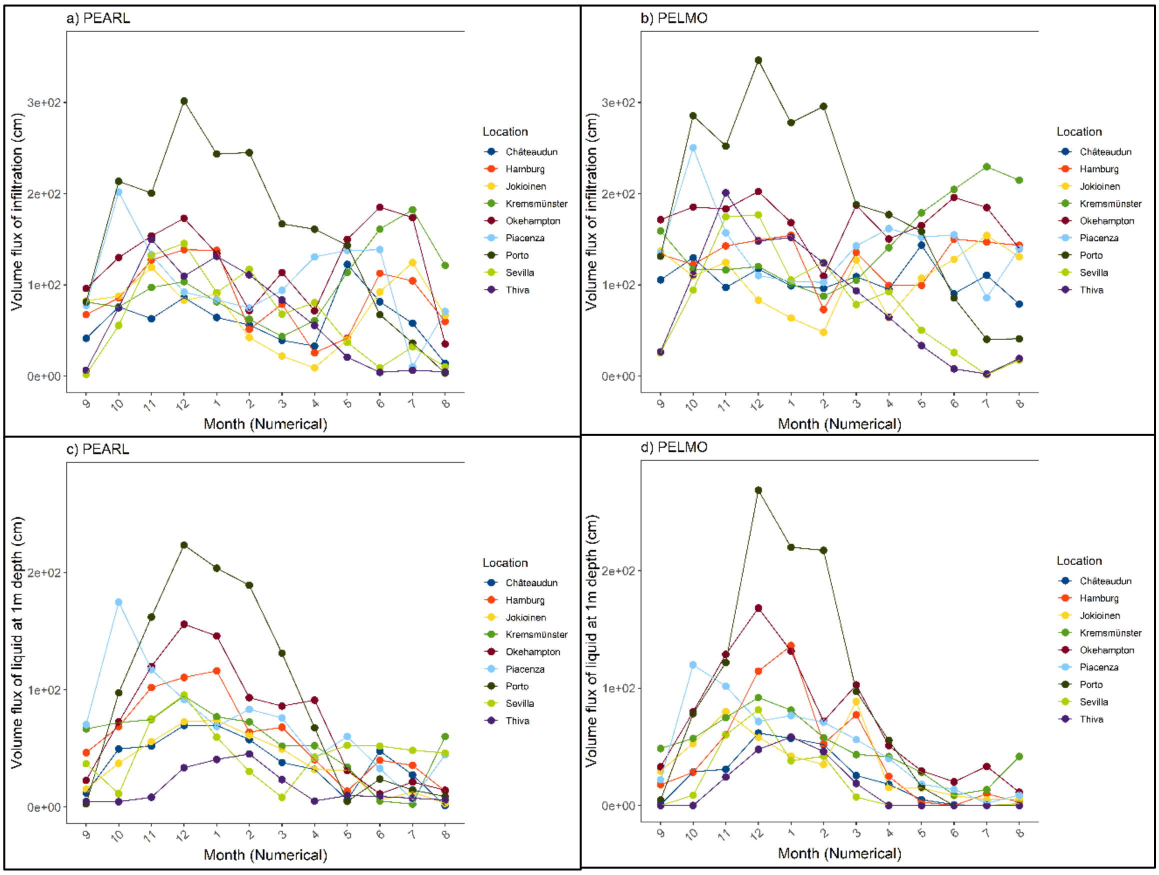

**Figure 4.8. For all 9 FOCUS locations: a) Monthly sum of volume flux of liquid phase infiltration in PEARL, b) Monthly sum of volume flux of liquid phase infiltration in PELMO, c) Monthly sum of volume flux of the liquid phase at 1 metre depth in PEARL, d) Monthly sum of volume flux of the liquid phase at 1 metre depth. The x-axis runs from sowing of the winter wheat in September to its harvest in August.**

The hydrological fluxes at the boundaries (surface infiltration and drainage at the bottom target layer) for both models can aid with the explanation of any potential differences in the leaching at each location (Figure 4.8). Although broadly the shapes in the comparative plots (a) and b), c) and d)) are similar, there are some inter-model and inter-locational differences between the models. For both models, the simulations for Porto initially exhibit the highest water input and output below 1m, with a similar shape for both models. PEARL simulates a large drop in infiltration for Piacenza during July; this drop is much lower for PELMO stopping at just below 100 cm. Also, the cumulative volume flux at 1m for Piacenza in October simulated by PEARL is much higher than that produced by PELMO. This will have implications for the plant water uptake.

## 4.4.2 Areic mass of plant uptake of pesticide

This section presents the plant uptake of the three compounds as simulated by the two models. Despite the topic of plant uptake being much covered within the peer-reviewed literature, there is relatively little discussion on the effect that it has on the leaching out of the profile (Gottesbüren et al., 2000; Köhne et al., 2009). It has therefore been difficult to find examples to present and compare with the model output generated in this Chapter, but some comparative studies will be provided where possible.

### 4.4.2.1 Compound A

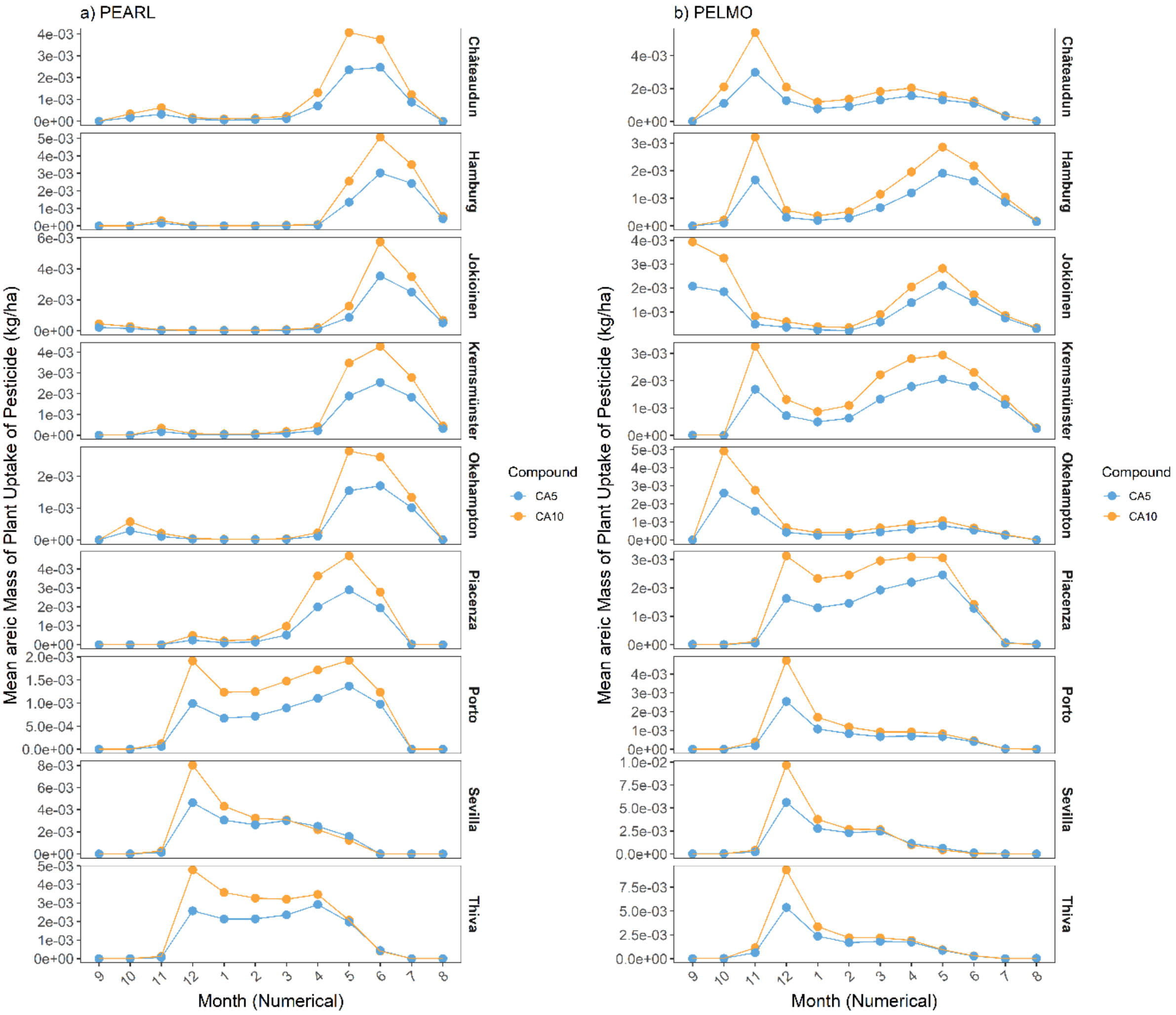

**Figure 4.9. a) The monthly mean mass of plant uptake of Compound A (see Table 4.3) simulated by PEARL, and b) as simulated by PELMO, for the 9 FOCUS locations. The time series starts at the date of the earliest emergence date. The two lines within the subplots represents plant uptake factors of 0.5 and 1.0.**

Plant uptake within the PEARL is considered as a relationship between the concentrations of the pesticide in the soil solution, the amount of soil solution transpired and the PUF of the compound. The amount of compound taken up by a plant is therefore linked to the environmental conditions that the plant is grown in as well as the physical-chemical properties of the compound that is being taken up, and those of the soil medium the plant is growing in. The seasonal evolution of plant uptake simulated by PEARL (Figure 4.9a) for compound A (high mobility (low $K_{OC}$) and medium persistence), shows two distinct features: the first is a slight rise in plant uptake in the month of application followed by a dip that continues until the early spring months when the wheat plants begins to grow at a faster rate and take up much more pesticide, with uptake peaking around May/June and dropping again prior to harvest. This shape is observed at 6 of the locations (Châteaudun, Hamburg, Jokioinen, Kremsmünster, Okehampton and Piacenza). The first rise in uptake around the time of pesticide application is likely to be due to the young plants taking up small amounts of soil solution that is high in concentration of pesticide due to it just having been applied. As the compound concentration in the soil drops over the next few months, the small amount taken up by the plants does not register on the plot. As the temperature increases in the spring-summer months and the plants begin to transpire increasingly large amounts of soil solution, the uptake increases.

The second type of seasonal evolution of plant uptake is seen in the warmer climates (Porto, Sevilla, and Thiva); in these Mediterranean zones the plants can grow much quicker meaning that they transpire more solution sooner after emergence. As the concentration in the soil solution during this period is higher, this results in more uptake of the compound. In the following months the concentration drops slightly and at the start of spring the peak starts to flatten out at the tail, or even increase before falling again shortly before harvest. A previous study compared the uptake of three compounds predicted by a model called SNAPS, using sandy and loamy soils; it showed that the loam soils exhibited lower uptake than sandy soils (Behrendt and Brüggemann, 1993). For compound A, the locations with high sand content (Hamburg and Jokioinen) show slightly higher uptake than a location with high silt content (Châteaudun) but this isn't the case for the other compounds.

PELMO considers plant uptake using the same method as PEARL (see Sections 4.3.2.1 and 4.3.2.2). Results from Compound A (Figure 4.9b) show that the early season uptake shortly following the emergence of the crop and the application of the compounds is much more apparent than for PEARL. Although some of the locations show a higher late-season plant uptake, at none of the locations does it reach a higher peak than the one simulated for the first month, and the plant uptake timeseries are quite different from those simulated by PEARL. Châteaudun, Okehampton, Porto, Sevilla and Thiva all show similar shapes with a sharp increase shortly after application, followed by a sharp decrease and then plateau in the spring and summer months. At the remaining locations (Hamburg, Jokioinen, Kremsmünster and Piacenza) there is a drop of uptake over the winter months before a rise in the spring and summer. The highest peaks of uptake are seen at Sevilla and Thiva in the month following application.

The logarithm of the concentration of the compound within the topsoil is shown below (Figure 4.10), PEARL shows a slightly higher concentration initially and maintains this higher concentration throughout the growing season. Later in the season, PELMO shows a much sharper drop in concentration, this may explain the tendency for PELMO to show lower uptake values than PEARL during this period. PEARL shows much more variability between the profiles, with Porto and Okehampton showing much lower concentration through most of the late growing season but particularly in June, July and August.

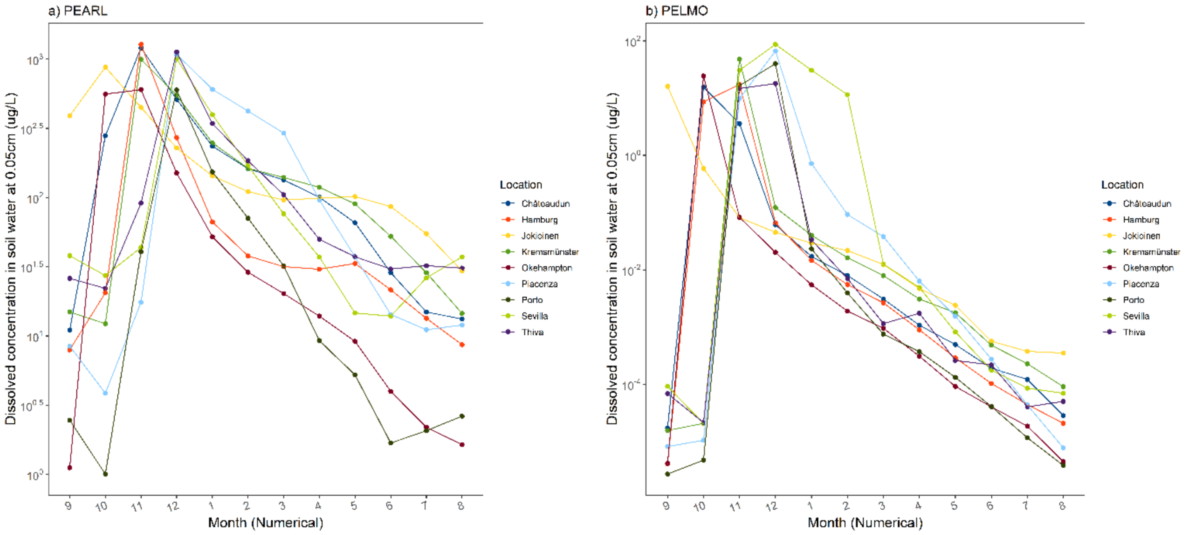

**Figure 4.10. a) The log of the dissolved concentration of compound A in the soil solution in the topsoil for PEARL. b) The log of the dissolved concentration of compound A in the soil solution in the topsoil for PELMO.**

#### 4.4.2.2 Compound B

The overall lower uptake for compound B happens for two main reasons, the higher $K_{OM}$ means that the compound will have a higher affinity to the organic matter within the soil profile and so will be less available to the plant roots, as their will be less of the compound in the soil solution (aqueous phase). The shorter DT50 of compound B by 60 days means that the concentration of the compound will reduce at a much quicker rate, meaning that later on in the season, when the plants are transpiring higher amounts of soil solution, the concentration available to them will be much lower as compound B has been degraded. As expected, the model runs with the higher PUF value show more plant uptake. Interestingly the differences between the model runs with the different PUFs (0.5 and 1.0) seem more pronounced than in Figure 4.11. This is likely to be caused by the higher $K_{OC}$ property, meaning that CB is less mobile and isn't transported down the profile so quickly. This will mean that the compound is more available for uptake later in the season, although the shorter DT50 for CB is likely to interact with this too.

Figure 4.11 (monthly mean mass uptake by the wheat crop) is like that presented in Figure 4.9 for compound A, particularly for PELMO. For PEARL (Figure 4.14.11a) the early uptake shortly following the first application is higher than the second peak in summertime, compared to the results presented in Figure 4.9a. However, note that the y-axis in Figure 4.11 presents uptakes that are approximately a factor 10 smaller than those shown in Figure 4.9. This same effect is observed in the Mediterranean regions (Porto, Sevilla, Thiva), but in this case with the initial uptake being the peak followed by a smooth decline over the following months until harvest.

Modelled uptake of compound B by PELMO (Figure 4.11b) across the months shows lower levels of uptake than those simulated for Compound A, however, the shape of uptake for most locations is very similar to that presented in Figure. 9b. A key difference between the two compounds is that the late season uptake in the summer months has a bigger difference between PUF of 0.5 and PUF of 1. This is apparent at all locations apart from Sevilla and Thiva. Between the models, PELMO appears to estimate consistently higher levels of uptake for all locations, for some locations the predicted uptake is around double that of PEARL at the peak level. This can be seen at Châteaudun, Jokioinen, Kremsmünster, Porto and Thiva.

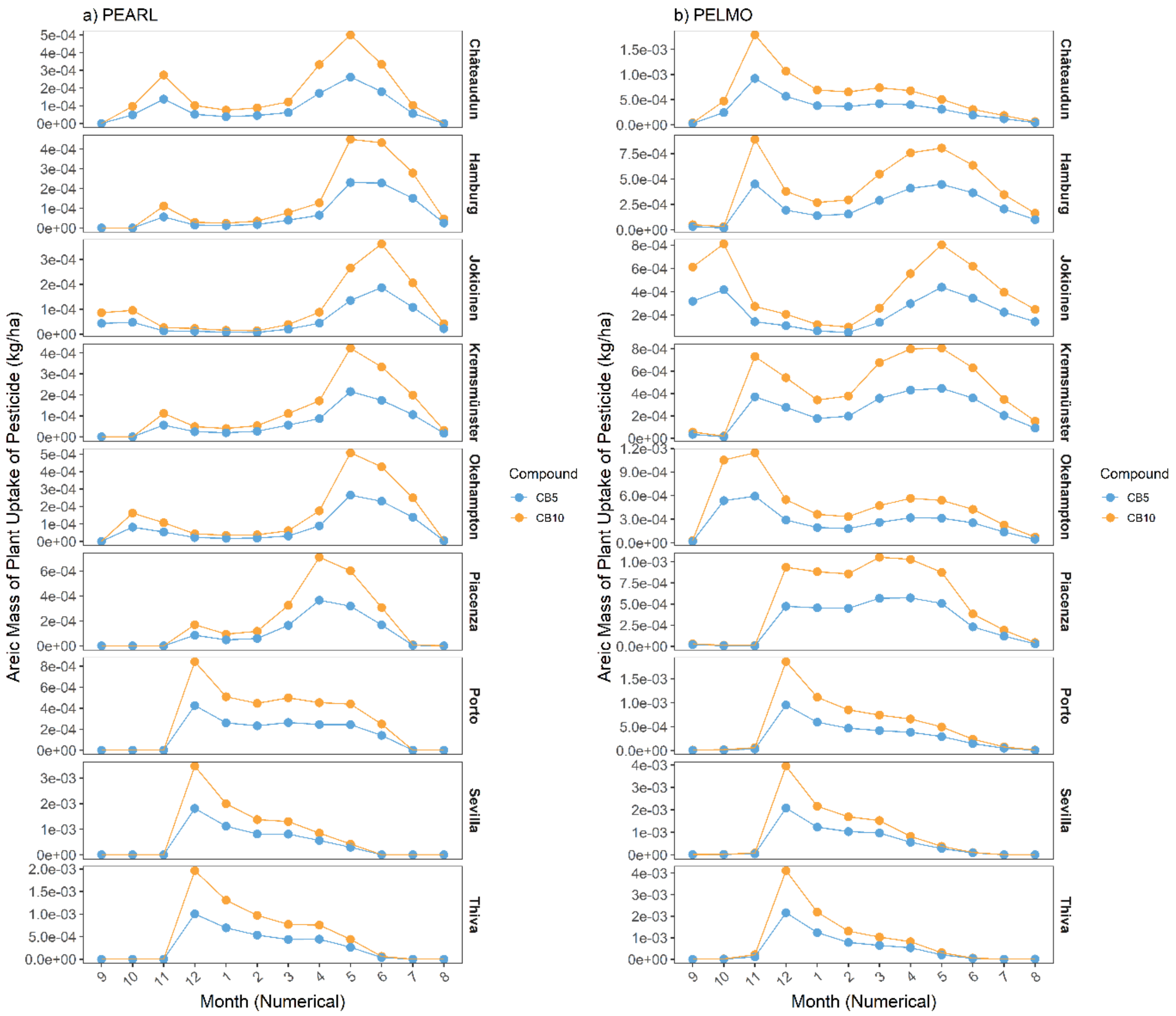

**Figure 4.11. a) The monthly mean mass of plant uptake of Compound B (see Table 4.3) simulated by PEARL, and b) as simulated by PELMO, for the 9 FOCUS locations. The time series starts at the date of the earliest emergence date. The two lines within the subplots represents plant uptake factors of 0.5 and 1.0.**

Figure 4.12 shows the logarithm of the concentration for both models, like Compound A, PEARL shows higher values throughout the growing season and maintains the concentrations late in the growing season. Although the concentrations at Porto and Okehampton remain low later in the growing season, the gap is much less apparent. With PELMO, the pattern of concentrations are very similar for all locations, with the concentration showing a sharp drop shortly after application of the Compound B. The only location that does not show this pattern of a sharp drop is Sevilla, but the concentration is then lost in the spring and summer months. As this is a location with a high mean temperature (Table 4.1), the degradation of the compound is likely to be much quicker here.

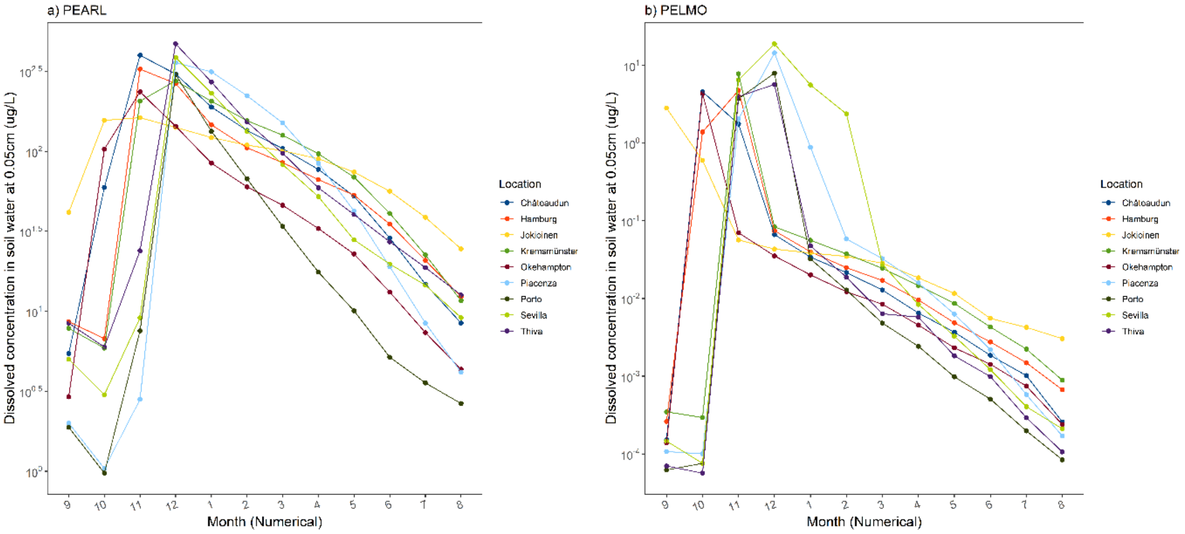

**Figure 4.12. a) The log of the dissolved concentration of compound B in the soil solution in the topsoil for PEARL. b) The log of the dissolved concentration of compound B in the soil solution in the topsoil for PELMO.**

### 4.4.2.3 Compound C

PEARL predicts the uptake of compound C to be higher than compound B but lower than Compound A for most of the temperate regions within this study (Figure 4.13a). This is due to the longer DT50 of this compound, which is 500 days, compared to 40 days for Compound B. Therefore, much more of the compound will be available later in the year as the plant transpiration increases. A high $K_{OC}$ value means that the compound is likely to stick to the organic fraction of the soil composition, this means that while it is present in the soil, it is much harder to access and so uptake will be low in comparison to CA. For the Mediterranean locations, the shape differs slightly from that of Compound A and B as the peak occurs much later in the year.

Uptake of Compound C predicted by PELMO (Figure 4.13Figure 4.1b) has a markedly different shape to what was simulated for Compound A and B (Figures 4.9b and 4.11b). Figure 13b lacks the clear peak in uptake just after the application of the compound, with the later season uptake providing the highest levels across the year. The seasonal evolution of the Mediterranean locations is like those seen in PEARL with an increase of uptake shortly after the application and these high uptake levels are then maintained throughout the season until the harvest of the wheat crop. For the other locations, PELMO simulates low amounts of uptake early in the season, however uptake increases rapidly during winter and remains at a high level throughout the rest of the growing season. What is also more noticeable for Compound C in PELMO is that at some locations, the plant uptake appears to occur all year round, even outside of the peak of the growing season. Jokioinen is the only location where this is possible as the plants emerge 20th September and are harvested on 15th August. For the remaining locations, August is the latest month where there are harvests but the next earliest emergence is in October with Okehampton and Châteaudun being harvest on 17th and 26th respectively. This suggests that uptake is still being considered after harvesting of the plants, this should not be occurring as the after-harvest conditions have been set to residue (Appendix B)

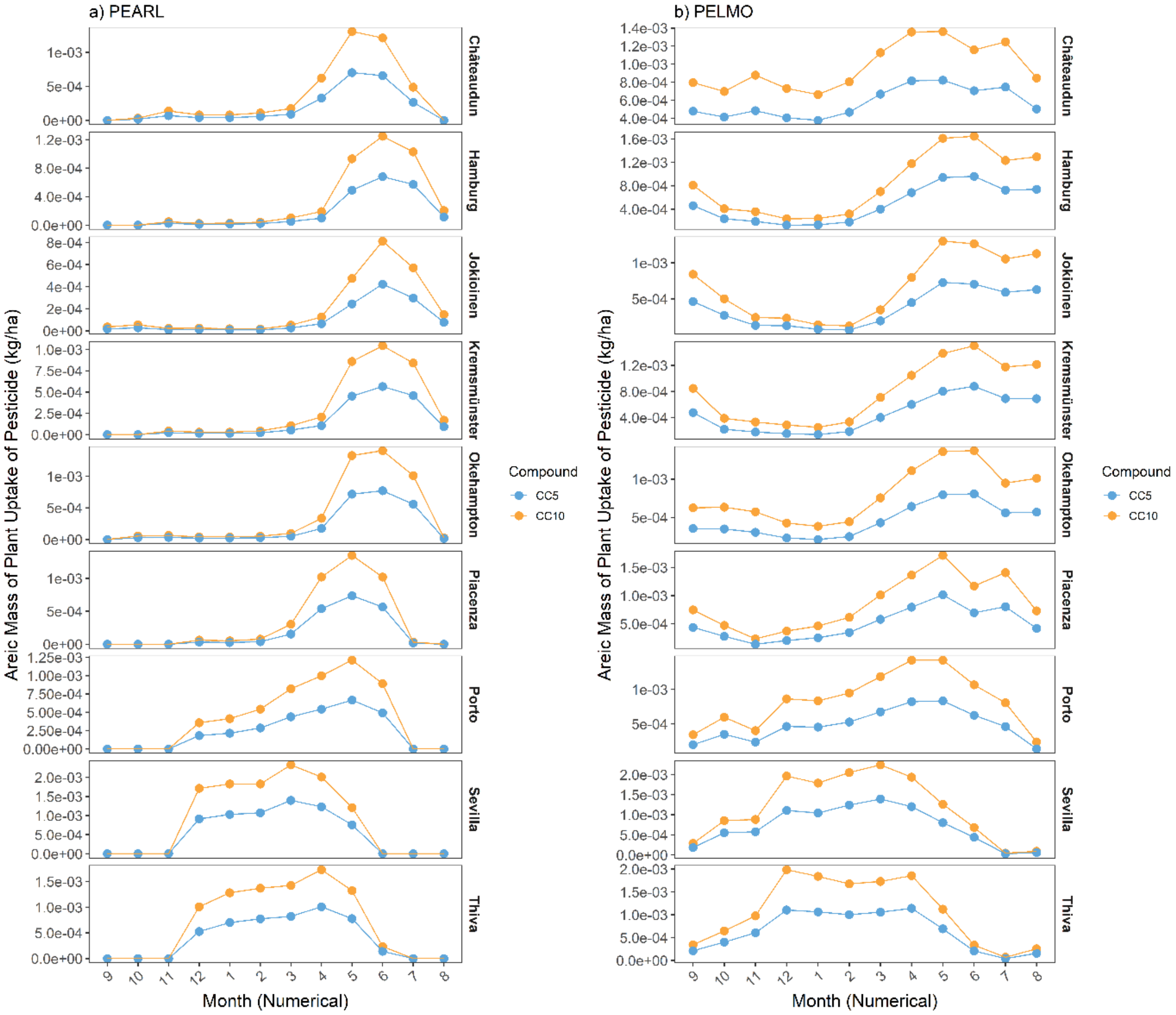

**Figure 4.13. a) The monthly mean mass of plant uptake of Compound C (see Table 4.3) simulated by PEARL, and b) as simulated by PELMO, for the 9 FOCUS locations. The time series starts at the date of the earliest emergence date. The two lines within the subplots represents plant uptake factors of 0.5 and 1.0.**

The log of the concentration in the topsoil is shown in Figure 4.13. The initial concentrations shortly after application are lower for both models than observed in Compound A and B. This is because the $K_{OC}$ value for compound C means that the pesticide is much more attracted to the organic content in the soil. PELMO once again shows a sharp drop in concentration after application but then maintains a much more gradual drop from January until August. PEARL shows a more gradual drop in concentration than for Compound A and B. The gradual drop in the spring and summer months is likely due to a combination of the high $K_{OC}$ and DT50.

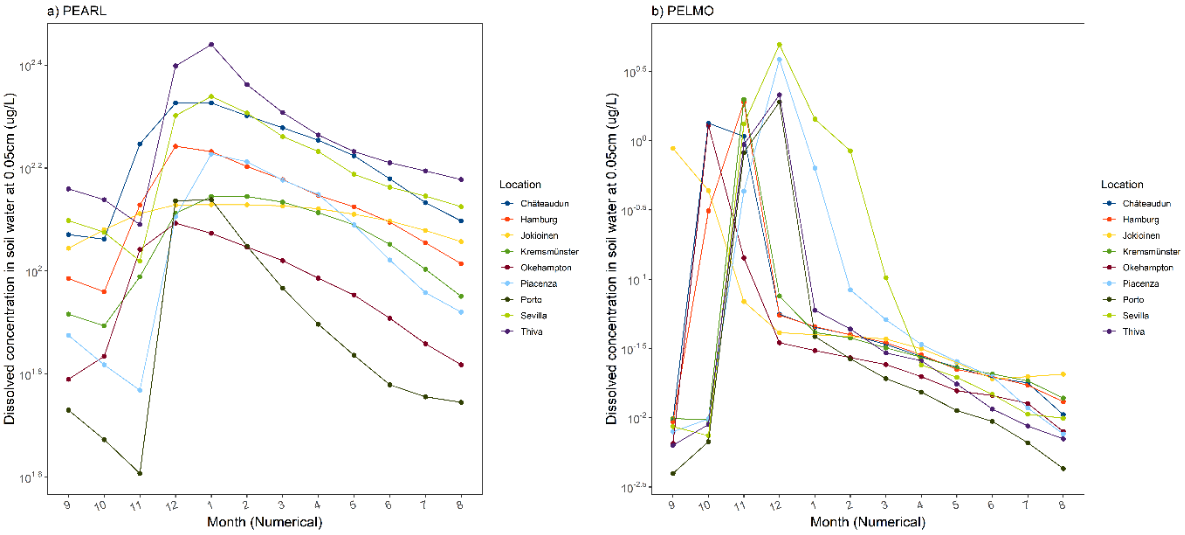

**Figure 4.14. a) The log of the dissolved concentration of compound C in the soil solution in the topsoil for PEARL. b) The log of the dissolved concentration of compound C in the soil solution in the topsoil for PELMO.**

### 4.4.2.4 Plant uptake between compounds

For the more mobile Compounds A and B, PEARL generally simulates a small amount of plant uptake in the months following application, whereas the peak of uptake occurs in the late spring and summer months. The plots of the PELMO simulations show the opposite, with a considerable amount of uptake occurring in a sharp peak shortly after application of the compound, and with only a small amount being taken up later in the season. This is an unexpected result as both models consider plant uptake using the same equation. As the same equation is used by both models, there are two possible explanations for this difference in modelled seasonal plant uptake. Firstly, in PELMO if the concentration in the pore water shortly following application reduces much quicker than in PEARL then the uptake later in the season is likely to be smaller than the initial uptake after application. This would lead to the shape that is evident in Compound A where there is an initial spike of uptake after application which is the peak of uptake for all locations. A second explanation could be that the amount of water transpired by the plant in the early stages of growth is much higher as simulated by PELMO than by PEARL. As both models have differences in their predictions of peak uptake but at different times of year then it is possible that the volume of transpiration is higher in PELMO when the concentration is highest shortly after application, leading to a bigger response in plant uptake. This cannot be corroborated from the model outputs as PELMO does not output a transpiration singularly, instead only the evapotranspiration output can be taken.

Also, in PELMO plant uptake appears to occur all year round, even outside of the emergence and harvest dates; this phenomenon is particularly noticeable for Compound C (Figure 4.13b) but can be seen for Compound B also (Figure 4.11b). This is unexpected as the emergence and harvest dates are fixed so that the model should "know" when the plants roots are present in the soil profile and therefore no growth or abstraction of water would be expected outside of the growing season. A possible explanation for this unexpected behaviour could be sought in the conditions assumed by the model when there is no crop present. Within PELMO, the scenario configuration files can be modified to set the 'conditions after harvest'; there are three options for this: cropping, fallow and residues. If the model has configured to allow for the growth of a cover crop or grass after harvest/before emergence then it could be expected that uptake is still being simulated, however this is not the case in these runs (Appendix B). PELMO does not provide the transpiration by the plants as a separate output, this can only be viewed as evapotranspiration, so this water balance component cannot be compared or discussed.

### 4.4.3 Concentration at target zone

The concentration in the soil solution at 1 m depth ($C_{1m}$) was defined by FOCUS to be the target area. This gives a standard depth for the analysis of leaching that is the same across all locations. $C_{1m}$ is an indicator of the amount of compound that is available for leaching to the groundwater. Hence Figures 4.15-4.17 show $C_{1m}$ values, for three different values of PUF. Using Compound, the compound labelling format is like those of the plant uptake plots (Figures 4.9,4.11 and 4.13), however for the following plots CA0 has been added to show the same compound but with a PUF of 0. In this cluster, the plants will transpire water, but no compound will be taken up within this.

#### 4.4.3.1 Compound A

Compound A was designed to be the most mobile of the compounds. In PEARL (Figure 14a), for the locations Hamburg, Okehampton, Piacenza and Porto the difference between the concentration leaching below 1m in the late winter and early spring months is much reduced (February to May). This would suggest that during this time there is not much plant uptake and that the compound does not spend a lot of time within the upper layers of the soil profile, where most of the plant uptake will occur.

PELMO (Figure 14b) differs from PEARL in that it uses a capacity bucket approach rather than the Richard's equation that is solved within PEARL. The mean concentration of Compound A at the target depth of 1 metre as modelled by PELMO is very similar to PEARL. Throughout the growing season, the difference between CA0, CA5 and CA10 remains reasonably constant, although the difference between the target depth concentrations for the CA0 and CA5 model runs is larger than that found between the CA5 and CA10 runs. For most of the locations, the peak in leaching (concentration) is during the spring months, with a gradual slope throughout the summer months. The location that exhibits the lowest leaching is Sevilla which shares a very similar shape to Thiva; both are in the Mediterranean with low rainfall amounts (see Figure 4.6). The other Mediterranean location (Porto) shows a gradual drop in $C_{1m}$ from October until December, before a rise to a peak in February, followed by a gradual drop for the rest of the season. The difference between CA0, CA5 and CA10 is much reduced at this location.

The 1 m compound concentrations (good indicators of leaching potential) as predicted by both models are very similar, with PEARL showing marginally higher levels for some locations. The effect of variation in PUF, however, appears to be much reduced within PEARL, for Hamburg and Okehampton during the spring and/or summer months.

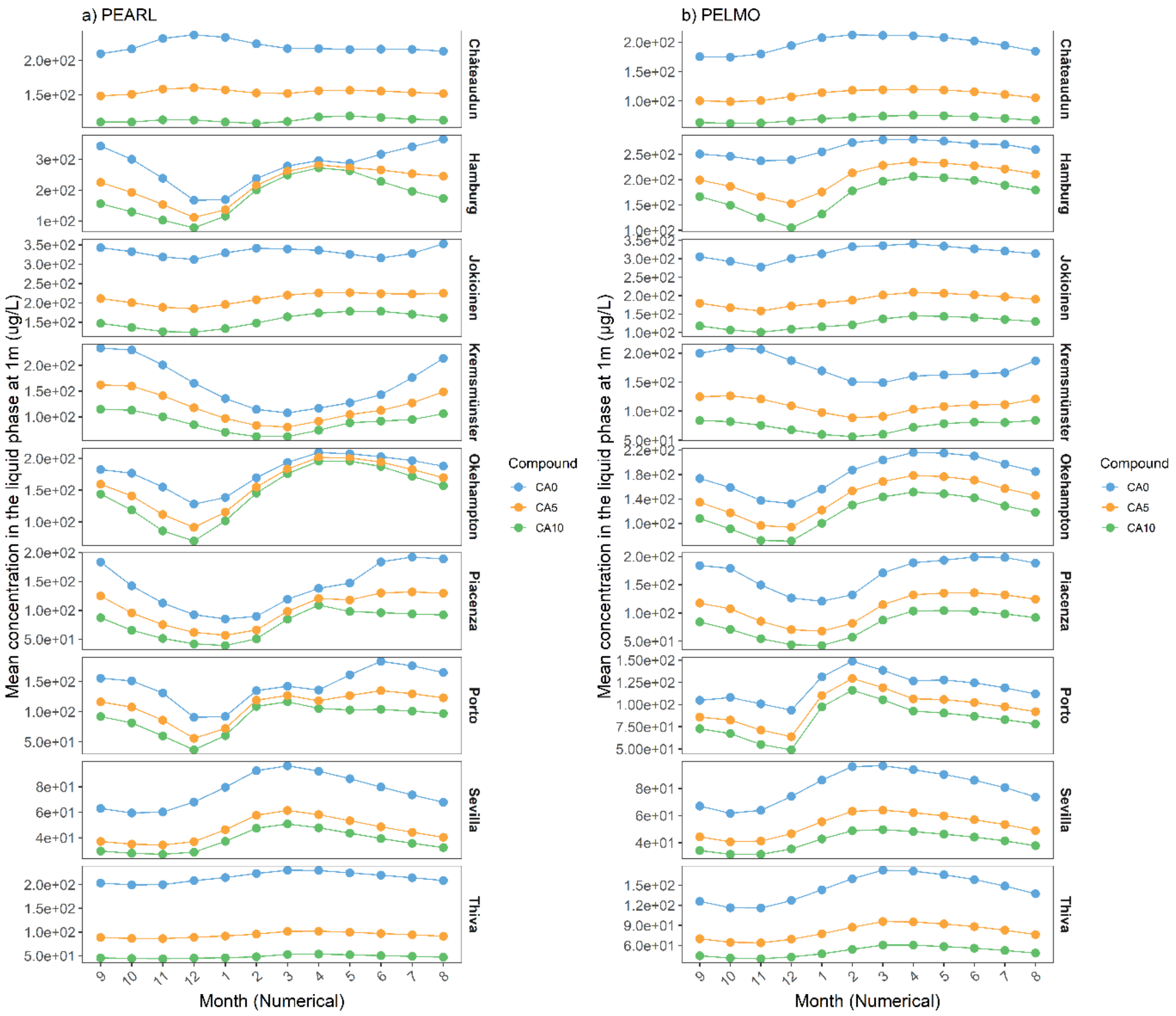

**Figure 4.14. a) The mean concentration of Compound A in the liquid phase at 1 metre depth for PEARL. b) The mean concentration of Compound A in the liquid phase at 1 metre depth for PELMO. Both plots are faceted by each location and the time series is started at the date of the earliest application of pesticide. Each line within a facet represents a compound with different plant uptake factor.**

### 4.4.3.2 Compound B

For compound B, there is a greater spread between the 1 m concentrations for the three PUFs modelled in PEARL (Figure 4.15a), compared to compound A. This is caused by the increased sorption of the compound within the profile (and related reduced mobility), due to the $K_{OM}$ of 100. Compared with Compound A the concentration of the pesticide at 1 m depth is much lower by 1-2 orders of magnitude. Alongside the mobility being reduced, the DT50 was also reduced with a value of 40 being supplied to the model.

In PELMO, the simulation results presented for Compound B show similar seasonal evolutions to those shown for Compound A, but with generally lower levels of leaching for Compound B (Figure 4.15b). For many of the locations a gradual rise in $C_{1m}$ was simulated, until it reaches a peak in the late spring months followed by a decline shortly after with a considerable spread between all PUF runs. Châteaudun, Hamburg, Jokioinen, Kremsmünster and Okehampton all show similar patterns of low concentrations in the autumn and a gradual rise to a peak in March before slowly falling again. Piacenza shows a similar gradual rise in $C_{1m}$ but is followed by a decrease with a slightly sharper slope. The Mediterranean locations (Porto, Sevilla and Thiva) show a much-reduced effect of PUF value in the autumn and early winter months before seeing a sharper rise in concentration in the late winter months to a peak in spring. For these locations, there is a much bigger spread in the effect that the the choice of PUF has later in the year.

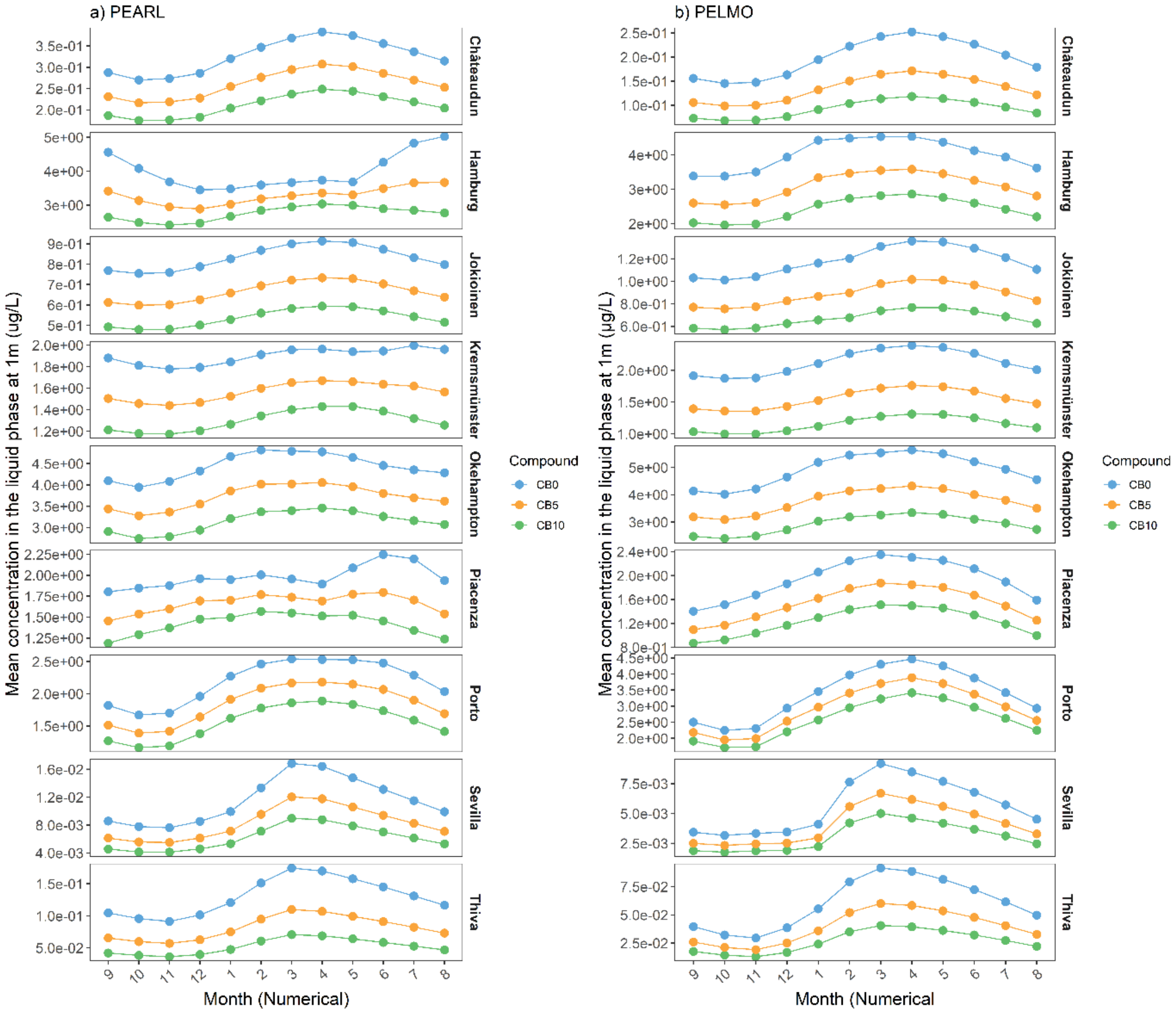

**Figure 4.15. a) The mean Concentration of Compound B in the liquid phase at 1 metre depth for PEARL. b) The mean concentration of Compound B in the liquid phase at 1 metre depth for PELMO. Both plots are faceted by each location and the time series is started at the date of the earliest application of pesticide. Each line within a facet represents a compound with different plant uptake factors.**

Between the models there are slight differences in $C_{1m}$ that can be pointed out. Firstly, although most locations display similar leaching patterns, there are some clear differences. The location where this is most noticeable is Hamburg; in PEARL (Figure 4.15a) the leaching begins to drop in August all the way through to December before a slow and gradual rise until May and then a sharper rise until August. In PELMO (Figure 4.15b), the evolution of $C_{1m}$ for Hamburg follows a similar shape to the other locations, apart from a sharper rise in November to January, but otherwise has a gradual bell-curve shape. It is unclear as to why this location is different in PEARL compared to the other locations with a similar climate, but it is also interesting that the high uptake compound, B with PUF = 1.0, does not show the same shape as CB0. This means that PEARL predicts that there is a much lower effect of PUF on leaching during this period whereas PELMO predicts that the effect of PUF is more important. Piacenza predicts a rise in leaching from May-June, but this effect is not seen in PELMO.

### 4.4.3.3 Compound C

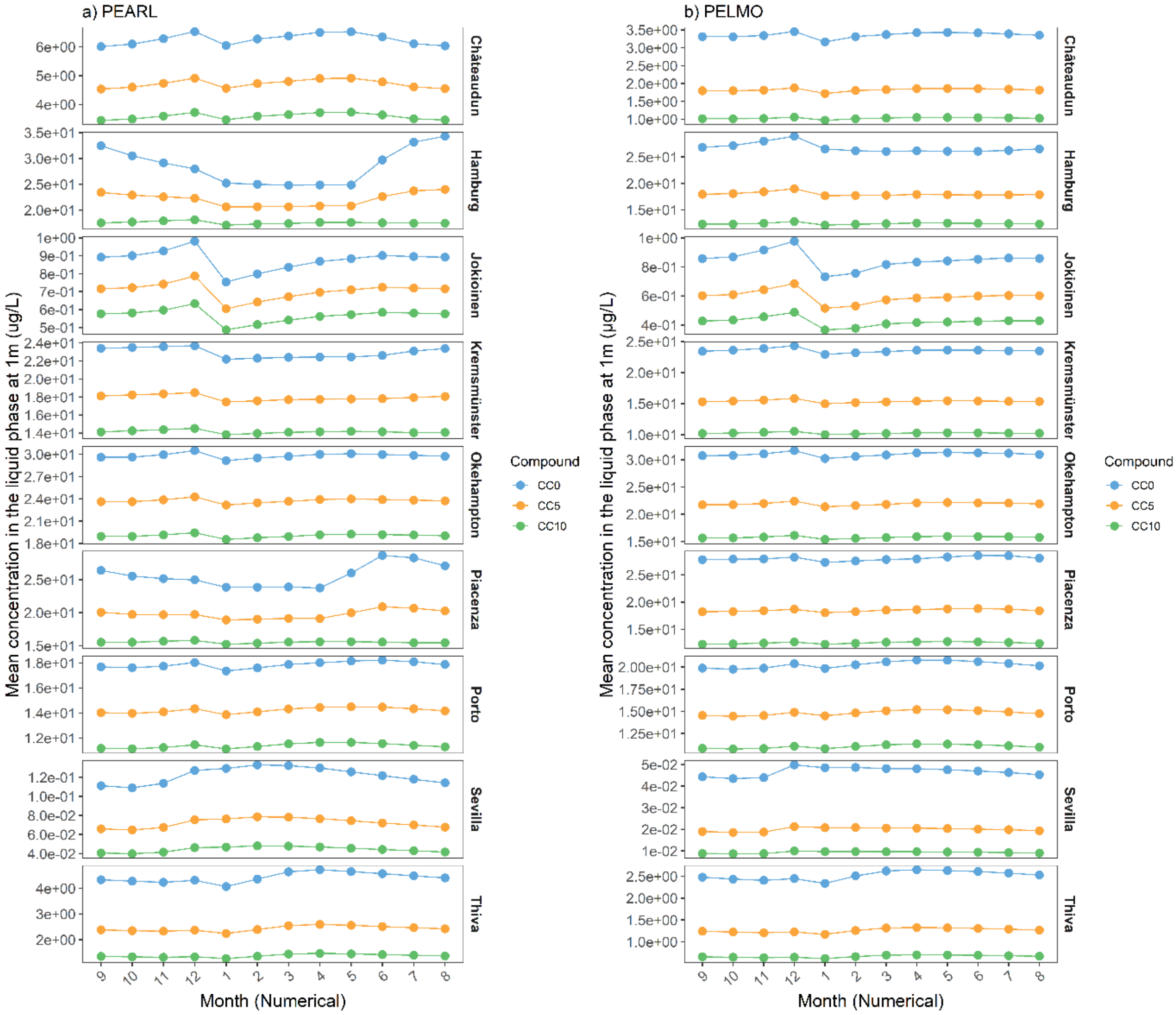

**Figure 4.16. a) The mean Concentration of Compound C in the liquid phase at 1 metre depth for PEARL. b) The mean concentration of Compound C in the liquid phase at 1 metre depth for PELMO. Both plots are faceted by each location and the time series is started at the date of the**

**earliest application of pesticide. Each line within a facet represents a compound with different plant uptake factors.**

For compound C the $K_{OM}$ value has been increased to 500 L/kg and the DT50 to 500 days. The increased persistence means that the concentration of the compound at 1m depth is very consistent throughout the year. In PEARL Figure 4.16a), most leaching patterns are very similar across the locations, all locations leached at a higher level for Compound C than Compound B, this is likely to be due to the persistence (Compound C DT50 = 500, Compound B DT50 = 40).

Compound C shows an almost constant concentration at 1 metre depth throughout the year in PELMO (Figure 4.16b). There are some locations that show a dip in concentration in January but there is a gradual rise in concentration following this throughout the rest of the year. The difference between compound C with varying PUF values remains constant, with the difference between CC0 and CC5 being larger than the difference between CC5 and CC10. This shows that the high persistence and low mobility of the compound, mean that the compound leaches at relatively low levels compared to Compound A but the pattern is more consistent. The leaching behaviour of Compound C is higher than Compound B by 1-2 orders of magnitude.

### 4.4.4 Predicted Environmental Concentration in the groundwater ($PEC_{GW}$)

Based on the advice given by FOCUS, Predicted Environmental Concentration in the groundwater ($PEC_{GW}$) is the value from each model run that is used to determine the leaching potential of each compound. $PEC_{GW}$ is calculated as the 80th percentile of the concentration of pesticide found in the solution at the target depth of 1m. In Tier 1 and 2 assessments, $PEC_{GW}$ is taken and compared with the threshold value of 0.1 μg/L (FOCUS, 2009; Michalski et al., 2004).

As $PEC_{GW}$ is calculated for each individual model run and is calculated using the same method for both models, it allows for a simple comparison between the two models for all locations and compounds. Previous work by the European Food Safety Authority used two locations, two crops and modelled 3 compounds across a range of PUFs (0, 0.5 and 1). Their work found that an increase in PUF reduced the concentration at the target depth by 24-43% (EFSA, 2013). This study found that plant uptake had an effect of 0%-85.7% (Table 1). The percentage change was calculated from the difference between the zero uptake $PEC_{GW}$ (CX0) and the full uptake $PEC_{GW}$ (CX10) and then dividing this number by the zero PUF $PEC_{GW}$. For the PEARL simulations Compound A has the highest average percentage change (54.5%), with Compound B being the lowest (30.4%) while intermediate changes were found for Compound C (44.6%). When using PELMO, Compound C (59.1%) has the highest percentage change, with Compound A (53.7%) being the 2nd highest and Compound B (38.4%) being the lowest. For both models for Compound B at Sevilla, the percent change in $PEC_{GW}$ is 0 which will skew the average result somewhat, although it does not change the overall outcome; values are 34.3% and 43.2% for PEARL and PELMO, respectively, following the removal of the Sevilla result.

Statistical analyses were conducted by t-tests with regards to the percentage change (from PUF = 0 to PUF = 1) for both models for each compound. This found that the only compound where a significant effect of PUF was observed was Compound C, with PELMO showing a higher percentage change compared to PEARL (p-value 0.0238). For compound C, one possible explanation for the higher effect of plant uptake on leaching to the target depth within PELMO is due to the more consistent uptake throughout the year. When comparing the y-axes of the two model outputs (PEARL, Figure 4.13a; PELMO, Figure 4.13b), it is clear that the peak values are slightly higher in PELMO than in PEARL. However, PELMO simulates much more consistent values of $C_{1m}$ for all locations whereas PEARL predicts small rises shortly after application and then a peak of uptake later in in the season shortly before maturity.

Both models simulate that compound B is least affected by the variation in PUF with regards to $PEC_{GW}$. As compound B has the lowest DT50 of the three compounds modelled within this study it is likely that this is the main reason behind this. Although CB has reduced mobility due to the higher $K_{OM}$ compared to CA, the short DT50 means that the concentration within the profile will reduce much quicker and there will be less compound available for uptake by the plants. It is less clear for the remaining compounds as to which an increase in PUF would have the least effect on. Compound A is the most mobile with a much lower $K_{OM}$ and would be more available in the soil pore water but for a shorter period.

**Table 4.4. Predicted Environmental Concentration ($PEC_{GW}$, µg/L) calculated by PEARL and PELMO, for three different PUF values.**

| | | Compound A | | | Percent change | Compound B | | | Percent change | Compound C | | | Percent change |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Location** | **0** | **0.5** | **1** | | **0** | **0.5** | **1** | | **0** | **0.5** | **1** | |
| **PEARL** | Châteaudun | 270.2 | 172.7 | 126.5 | 53.2 | 0.555 | 0.45 | 0.367 | 33.9 | 17.37 | 12.731 | 9.435 | 45.7 |
| | Hamburg | 334.9 | 218 | 154.9 | 53.7 | 5.137 | 4.197 | 3.495 | 32.0 | 45.09 | 33.637 | 25.864 | 42.6 |
| | Jokioinen | 498.5 | 296.1 | 169.6 | 66.0 | 1.126 | 0.921 | 0.759 | 32.6 | 1.89 | 1.553 | 1.289 | 31.8 |
| | Kremsmünster | 195.9 | 140.9 | 102.3 | 47.8 | 2.231 | 1.816 | 1.486 | 33.4 | 33.21 | 25.755 | 20.341 | 38.8 |
| | Okehampton | 186.1 | 161.4 | 142 | 23.7 | 5.636 | 4.758 | 4.039 | 28.3 | 41.22 | 32.613 | 25.904 | 37.1 |
| | Piacenza | 173.8 | 112 | 84.32 | 51.5 | 2.46 | 2.098 | 1.803 | 26.7 | 30.61 | 23.68 | 18.921 | 38.2 |
| | Porto | 143.3 | 111.8 | 99.39 | 30.6 | 3.189 | 2.669 | 2.241 | 29.7 | 25.01 | 19.911 | 16.048 | 35.8 |
| | Sevilla | 89.22 | 21.4 | 12.74 | 85.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.104 | 0.061 | 0.036 | 65.4 |
| | Thiva | 281.4 | 119.7 | 60.6 | 78.5 | 0.162 | 0.104 | 0.069 | 57.4 | 8.216 | 4.726 | 2.771 | 66.3 |
| **PELMO** | Châteaudun | 237.2 | 132.1 | 85.35 | 64.0 | 0.378 | 0.257 | 0.18 | 52.4 | 8.253 | 4.53 | 2.582 | 68.7 |
| | Hamburg | 315.1 | 217.1 | 168.8 | 46.4 | 6.296 | 4.82 | 3.735 | 40.7 | 48.19 | 31.372 | 20.686 | 57.1 |
| | Jokioinen | 410.4 | 230.1 | 138.6 | 66.2 | 1.734 | 1.31 | 1.001 | 42.3 | 1.01 | 0.719 | 0.52 | 48.4 |
| | Kremsmünster | 241.7 | 143.6 | 89.63 | 62.9 | 2.688 | 1.887 | 1.366 | 49.2 | 36.31 | 23.534 | 15.74 | 56.6 |
| | Okehampton | 200.8 | 157.4 | 127.2 | 36.7 | 6.485 | 4.805 | 3.687 | 43.1 | 44.1 | 30.712 | 21.847 | 50.5 |
| | Piacenza | 256.3 | 149.3 | 105.7 | 58.7 | 3.289 | 2.261 | 2.116 | 35.7 | 35.42 | 23.822 | 16.212 | 54.2 |
| | Porto | 160 | 129.8 | 113.5 | 29.1 | 5.135 | 4.308 | 3.702 | 27.9 | 29.27 | 21.714 | 16.372 | 44.1 |
| | Sevilla | 121.1 | 76.08 | 56.88 | 53.0 | 0.001 | 0.001 | 0.001 | 0.0 | 0.014 | 0.006 | 0.003 | 78.6 |
| | Thiva | 212 | 112.5 | 71.28 | 66.4 | 0.111 | 0.074 | 0.051 | 54.1 | 3.416 | 1.71 | 0.899 | 73.7 |

## 4.5 Conclusions

Within previous peer-reviewed model sensitivity analyses Plant Uptake Factor (PUF) has not been mentioned as a highly influential input parameter. However, it is also clear that if there is a compound that has a $PEC_{GW}$ close to the threshold of 0.1 μg/L then a high PUF could potentially reduce the $PEC_{GW}$ below this. Both models show a different seasonal variation in plant uptake. PEARL exhibits the largest uptake later in the growing season, with only a small amount of uptake directly after application, whereas PELMO tends to have the highest uptake earlier on in the season with a secondary peak later in the season. For Compound C, PELMO appears to predict high levels of uptake throughout the year which may be an explanation for the significant difference seen in the $PEC_{GW}$ for this compound. Of the three compounds modelled PELMO predicted a higher effect of PUF for two. This is likely the result of the fact that PELMO predicts a much higher level of uptake in the early growth season soon after the application of the compound. PELMO also appears to consider uptake throughout the year, even out of the supplied growth season for the modelled crop of winter wheat. Comparison of leaching to 1m depth ($C_{1m}$) between the models show very little difference, with statistical tests showing no significant differences in $C_{1m}$.

Current advice from FOCUS is to set PUF = 0 for all compounds unless they are systemic and known for uptake. This is sensible currently as this study shows that plant uptake can have a big effect on the leaching potential of a compound. However, if confidence in laboratory tests improves then PUF parameters would become of serious consideration for pesticide design and regulatory risk assessment. In this case, model selection will come into consideration for high $K_{OC}$ and DT50 compounds as Compound C has shown a much greater effect of plant uptake compared to PEARL.

## 4.6 References

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# 4.7 Appendix A

The settings table for PEARL has been added here to allow for comparison of the parameterisation of both models, the PELMO settings table (Appendix B).

| **PEARL settings table** | | | |
|---|---|---|---|
| .PRL file | | | |
| **Parameter description** | **Value, Source and Comments** | **Model default, FOCUS default, FOCUS file selection, user choice or N/A** | **Rationale** |
| Calling Program | Set to FOCUSPEARL | N/A | |
| Calling Program Version | Set to 4.4.4 | N/A | |
| Init Years | Set to 6 | FOCUS default | Allows for soil moisture to reach equilibrium and allow pesticide to make it's way down the profile |
| TimStart, TimEnd | 01/01/1901 - 31/12/1926 | FOCUS default | 26-year simulation recommended for annual applications |
| AmaSysEnd, Stopcondition(kgha-1) | 0 | Model default | |
| ThetaTol (m3.m-3) | 0.001 | Model default | |
| OptDelTimPrn | Other | User choice | Daily output required |
| DelTimPrn (d) | 1 | User choice | Daily output preferred |
| Repeat Hydrology | No | User choice | Want to see variation over years. |
| OptHyd | OnLine | Model default | |

|  |  |  |  |
|---|---|---|---|
| DelTimSwaMin (d) | 1.00E-07 | User choice |  |
| DelTimeSwqMax (d) | 0.2 | User choice |  |
| PrintCumulatives | No | User choice | Can do cumulative in R if needed |
| GWTol (m) | 1m | User choice |  |
| MaxItSwa | 30 | User choice |  |
| OptHysterisis | No | User choice |  |
| PreHeaWetDryMin (cm) | 0.2 | User choice |  |
| OptScreen | Yes | User choice |  |
| OptPersistency | No | User choice |  |
| OptSys | All | User choice |  |
| OptPaddy | No | User choice | Paddy drainage not required |
| OptMacropore | No | User choice | Macropore drainage not required |
| SoilTypeID | xx_Soil. Prefix of location (xx) followed by _Soil | FOCUS file selection | User input |
| Location | xxyy, Location abbreviation. Xx = two letter abbreviation for country. Yy abbreviation for adminitrative region, varying length. | FOCUS file selection | User input |
| Table Soil Profile | Individual for each location. Consists of 1) ThiHor (depth of soil horizon). 2) NumLay (Number of layers within horizon | FOCUS file selection | 2m soil profile simulated |

| | | | |
|---|---|---|---|
| Table horizon SoilProperties | Individual for each location. Consists of 1) Soil horizon number. 2) FraSand (kg.kg-1), mineral sand fraction 3) FraSilt (kg.kg-1) mineral silt fraction. 4) FraClay (kg.kg-1) mineral clay fraction. 5) CntOm (kg.kg-1) Organic matter content. 6) pH (-) | FOCUS file selection | Properties drainage individual for each location. consists of 1) soil horizon number. 2) frasand (kg.kg-1), mineral sand fraction 3) frasilt (kg.kg-1) mineral silt fraction. 4) fraclay (kg.kg-1) mineral clay fraction. 5) cntom (kg.kg-1) organic matter content. 6) ph (-)t required |
| Table horizon VanGenuchtenPar | Individual for each location. 1) Soil Horizon number. 2) ThetaSet (m3.m-3) Saturated water concent. 3) ThetaRes (m3.m-3) Residual water content. 4) AlphaDry (cm-1) Inverse air entry suction when dry. 5) AlphaWet (cm-1) Inverse of air entry suction when wet. 6) n (-). 7) Ksat (m.d-1), 8) l (-) Lambda. | FOCUS file selection | |
| OptRho | Individual for each location. Table of input for Bulk density. ) Horizon number. 2) Rho (kg.m-3) Bulk density value. | FOCUS file selection | |
| ZPndMax (m) | 0.002 | FOCUS default | Max (m) drainage 0.002 required |
| OptSolEvp | Boesten | FOCUS default | Evaporation drainage boesten required |
| FacEvpSol (-) | 1 | FOCUS default | |
| CofRedEvp (cm1/2) | 0.79 | FOCUS default | |
| PrcMinEvp (m.d-1) | 0.01 | FOCUS default | Evp (m.d-1) drainage 0.01 required |
| Table horizon LenDisLiq (m) | 1) Horizon number. 2) LenDisLiq, 0.05 for all layers | FOCUS default | Liq (m) drainage 1) horizon number. 2) lendisliq, 0.05 for all layerst required |

|  |  |  |  |
|---|---|---|---|
| OptCofDifRel | MillingtonQuirk | Model default | Relative drainage millingtonquirk required |
| ExpDifLiqMilNom (-) | 2 | Model default | Nom (-) drainage 2 required |
| ExpDifLiqMilDen (-) | 0.6667 | Model default | Den (-) drainage 0.6667 required |
| ExpDifGasMilNom (-) | 2 | Model default |  |
| ExpDifGasMilDen (-) | 0.6667 | Model default | Den (-) drainage 0.6667 required |
| OptPnd | Constant | Default | Pond drainage constant required |
| MeteoStation | Location Code | FOCUS input | Station drainage location code required |
| OptEvp | Input. Use ETRef value | FOCUS file selection | ETref value taken from climate files |
| TemLboSta (degC) | ranged from -6.75 to 12.6 | FOCUS input |  |
| FacPrc (-) | 1 | FOCUS default | Prc (-) drainage 1 required |
| DifTem (-) | 0 | FOCUS default | Tem (-) drainage 0 required |
| FacEvp (-) | 1 | FOCUS default | Evp (-) drainage 1 required |
| OptIrr | No | User choice | Irrigation drainage not required |
| OptMetInp | Daily | FOCUS file selection |  |
| OptTraRes | Laminar | FOCUS default | Res drainage laminar required |
| OptRainfallEvents | No | FOCUS default | Events drainage not required |
| OptSnow | No | FOCUS default | Snow drainage not required |
| ZGrwLevSta (m) | Defined by FOCUS per location | FOCUS file choice | No data so set to lower boundary |
| OptLbo | Set to depending on location | FOCUS file choice | Option set by FOCUS depending on location |
| OptDra | No | N/A | Local drainage not considered to keep compound within soil profile and reduce losses |
| OptSurDra | No | N/A |  |
| NumDraLev | 0 | N/A |  |
| MolMas_subst (g/mol) | 400 | User choice | Mid-weight for pesticide |

|  |  |  |  |
|---|---|---|---|
| DT50Ref_xxxx (d) | Ranged between 40-500 depending on compound. CA = 100, CB = 40, CC = 500 | User choice | Varied to observe effects on Plant uptake. |
| TemRefTRa_xxxx (degC) | 20 | User choice | All values set to 20 throughout |
| ExpLiqTra_xxxx | 0.7 | FOCUS default |  |
| OptCntLiqTraRef | Optimum Conditions | User choice |  |
| CntLiqTraRef_xxxx (kg.kg-1) | 1 | User choice |  |
| MolEntTra_xxxx | 65.4 | FOCUS default |  |
| Table horizon FacZTraHor_xxxx | 1) Horizon number. 2) FacZTra, 1 | FOCUS default | not consistent with PELMO which is FOCUS |
| Table horizon FacZSorHor_xxxx | 1) Horizon number. 2) FaczSor, -99 | User choice | Selected to give no variation in sorption over depth |
| MolEntSor_xxxx (kJ.mol-1) | 0 | FOCUS default |  |
| TemRefSor_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| OptCofFre | pH-independent | User choice |  |
| KomEql_xxxx (L.kg-1) | 5.8-290 | User choice | Varied to see effects on plant uptake. |
| KomEqlMax_xxxx (mg.L-1) | 5.8-290 | User choice | Varied to see effects on plant uptake. |
| ConLiqRef_xxxx (mg.L-1) | 1 | User choice |  |
| ExpFre_xxxx | 0.9 | FOCUS default. |  |
| PreVapRef_xxxx (Pa) | 1.00E-12 | User choice | Set to be low to reduce losses from profile to gas fraction. |
| TemRefVap_xxxx (degC) | 20 | User choice | Set to 20 throughout |

|  |  |  |  |
|---|---|---|---|
| SlbWatRef_xxxx (mg.L-1) | 3 | User choice | Set to reasonable solubility but not too high so that losses were drastically increased due to high mobility. Higher solubility means greater concentration in liquid phase, increasing plant uptake and movement down the profile. |
| TemRefSlb_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| MolEntSlb_xxxx (kJ.mol-1) | 27 | FOCUS default |  |
| MolEntVap_xxxx (kJ.mol-1) | 95 | FOCUS default |  |
| CofDesRat_xxxx (d-1) | 0 | User selected | Non equilibrium sorption not considered so set to 0. |
| FacSorNeqEql_xxxx (-) | 0 | User selected |  |
| FacUpt_xxxx (-) | Value of 0,0.1,0.5,1.0 | User choice | Varied to see effects on plant uptake. |
| ThiAirBouLay (m) | 0.01 | FOCUS default |  |
| OptDspCrp_xxxx | Lumped | FOCUS default |  |
| DT50DspCrp_xxxx (d) | 1,000,000 | FOCUS default |  |
| DT50PenCrp_xxxx (d) | 1,000,000 | FOCUS default |  |
| DT50VolCrp_xxxx (d) | 1,000,000 | FOCUS default |  |
| DT50TraCrp_xxxx (d) | 1,000,000 | FOCUS default |  |
| FacWasCrp_xxxx (m-1) | 0.0001 | FOCUS default |  |
| TemRefDif_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| CofDifWatRef_xxxx (m2.d-1) | 4.30E-05 | FOCUS default |  |
| CofDifAirRef_xxxx (m2.d-1) | 0.43 | FOCUS default |  |
| ZTgt (m) | 1 | FOCUS default | Target layer depth at 1m and is standard for all profiles. |
| DelTimEvt (Years) | 1 | User choice | Application repeated each year |

| | | | |
|---|---|---|---|
| table Applications | 01-Emg-01 AppSolSur 1.0 | FOCUS default | FOCUS application schedule, compound applied 1 day prior to emergence of the crop |
| table_TillageDates | No till | User choice | Didn't want to consider tillage for this study |
| Ztil (m) | 0.2 | User choice | N/A as no till selected |
| Table interpolate CntSysEql (mg.kg-1) | 0 | User choice | Set to 0 as no previous applications expected |
| Table interpolate CntSysNeq (mg.kg-1) | 0 | User choice | Set to 0 as no previous applications expected |
| Deposition scheme | No Deposition | User choice | Not considered |
| table FlmDep (kgha-1.d-1) | No Deposition | User choice | Not considered |
| Crop Calendar | Individual for location | FOCUS file selection | |
| Repeat Crops | Yes | User choice | Annual crops to marry annual application |
| OptLenCrp | Fixed | User choice | Crop linear development. |
| table Crops | Individual for location | FOCUS file choice | |
| table IrrigationPeriods | No | User selected option. | Didn't select irrigation so no irrigation periods selected |
| table CrpPar_WCEREALS1<br>0.0 0.0 1.0 0.1 0.0<br>0.5 4.0 1.0 0.3 0.1<br>1.0 0.0 1.0 0.6 0.2 | Table. 1) Development stage (0 = emergence, 1 = Harvest). 2) LAI (m2.m-2). 3) Crop factor for evaporation. 4) Rooting depth (m). 5) Crop height (m). | FOCUS file choice | WCEREALS |
| table RootDensity_WCEREALS1<br>0.00 1.00<br>1.00 1.00 | Root density table 1) Relative rooting depth 2) Relative root density | FOCUS file choice | |
| HLim1_WCEREALS1 (cm) | 0 | FOCUS file choice | |

|  |  |  |  |
|---|---|---|---|
| HLim2_WCEREALS1 (cm) | -1 | FOCUS file choice |  |
| HLim3U_WCEREALS1 (cm) | -500 | FOCUS file choice |  |
| HLim3L_WCEREALS1 (cm) | -900 | FOCUS file choice |  |
| HLim4_WCEREALS1 (cm) | -16,000 | FOCUS file choice |  |
| RstEvpCrp_WCEREALS1 (s.m-1) | 70 | FOCUS file choice |  |
| CofExtDif_WCEREALS1 (-) | 0.39 | FOCUS file choice |  |
| CofExtDir_WCEREALS1 (-) | 1 | FOCUS file choice |  |
| CofIntCrp_WCEREALS1 (cm) | 0.0001 | FOCUS file choice |  |
| TemSumSta_WCEREALS1 (degC) | 0 | FOCUS file choice |  |
| TemSumEmgAnt_WCEREALS (degC) | 0 | FOCUS file choice |  |
| TemSumAntMat_WCEREALS (m) | 0 | FOCUS file choice |  |
| Ztensiometer_WCEREALS1 (m) | 0.2 | FOCUS file choice |  |
| PreHeaIrrSta_WCEREALS1 (cm) | -100 | FOCUS file choice |  |
| IrgThreshold_WCEREALS1 (mm) | 15 | FOCUS file choice |  |

## 4.8 Appendix B

The PELMO settings table to show parameterisation of the model.

.sze file XX_Wcereals

| **Parameter description** | **Value source and comments** | **Model default, FOCUS default, FOCUS file selection, user choice or N/A** | **Rationale** |
|---|---|---|---|
| Program and version | PELMO 4.01 | Most up to date |  |

| Scenario title | XX_WCEREALS or XX_FALLOW | Preferred ID method | |
|---|---|---|---|
| PFAC (0) | 1 | FOCUS default | |
| SFAC | 0.46 | Model default | |
| IPEIND | 0 | FOCUS default | Daily evaporation read from meteorological file |
| ANETD | 20 | FOCUS default | |
| INICROP | 8 | Model default | Cultivated scenario so set to 8 |
| ISCOND | 1 | Default | |
| PFAC (1) | 1.1 | FOCUS file selection | Value set for cultivated scenario |
| PFAC (2) | 0.3 | FOCUS file selection | Value set for cultivated scenario |
| ERFLAG | 0 | FOCUS default | No erosion |
| NDC | 1 | FOCUS default | One crop selected |
| ICNCN | 8 | FOCUS file selection | 8 is crop number for wheat |
| CINTCP | 0 | FOCUS default | No rainfall interception modelled |
| AMXDR | 80 | FOCUS file selection | Rooting depth of WCEREALS in FOCUS |
| COVMAX | 90 | FOCUS file selection | Crop canopy cover 100% - not usual |
| ICNAH | 3 | FOCUS file selection | Defined as residue in FOCUS scenario file |
| CN | Location specific | Model Default | Not usually considered for Tier 1 runs |
| USLEC | N/A | Model Default | Only required if ER flag = 1 |
| WFMAX | 0 | Model Default | |
| RRPPEX | 0.2 | FOCUS default | |
| RRRPEX | 0.2 | FOCUS default | |
| RRVPEX | 0.2 | FOCUS default | |
| RRWPEX | 0.2 | FOCUS default | |
| IRRFLG | 0 | FOCUS default | Non-irrigated crops |
| PEREN | 0 | FOCUS default | Non-irrigated crops |

| | | | |
|---|---|---|---|
| NCPDS | 66 | User input | Longest possible simulation period |
| E_MMDDYY | Location specific | FOCUS file selection | |
| M_MMDDYY | Location specific | FOCUS file selection | |
| H_MMDDYY | Location specific | FOCUS file selection | |
| INCROP | 8 | FOCUS file selection | Number associated with WCEREALS |
| H_MMDDYY | Location specific | FOCUS file selection | |
| T_MMDDYY | Location specific | FOCUS file selection | |
| CORED | Location specific | FOCUS file selection | Depth of soil column |
| DUMMY | 1 | N/A | Dummy number, not applicable currently |
| NCOM2 | Location specific | FOCUS file selection | Total number of simulation compartments in the soil core |
| BDFLAG | 0 | N/A | Not used |
| THFLAG | 0 | FOCUS default | Field capacity and wilting point flag |
| HSWZT | 0 | FOCUS default | |
| NHORIZ | Location specific | FOCUS file selection | Number of soil horizons |
| DELXFLG | 0 | N/A | Not used |
| HORIZN | Location specific | FOCUS file selection | |
| THKNS | Location specific | FOCUS file selection | |
| BDFLAG | Location specific | FOCUS file selection | |
| DISP | Location specific | FOCUS file selection | |
| THETO | Location specific | FOCUS file selection | |
| AD | Location specific | FOCUS file selection | |
| THEFC | Location specific | FOCUS file selection | |
| THEWP | Location specific | FOCUS file selection | |
| OC | Location specific | FOCUS file selection | |
| PH | Location specific | FOCUS file selection | |

| | | | |
|---|---|---|---|
| Biodeg | 0-30cm depth = 1<br>30-60cm depth = 0.5<br>60-100cm depth = 0.3<br>>100cm depth = 0 | FOCUS default | |
| ILP | 0 | FOCUS default | No previous application |
| ITEM1 | WATR | Model default | |
| STEP1 | YEAR | FOCUS default | Set to yearly output |
| LFREQ1 | 0001 | Model default | Output every compartment |
| ITEM2 | PEST | Model default | |
| STEP2 | YEAR | FOCUS default | Set to yearly output |
| LFREQ2 | 0001 | Model default | Output every compartment |
| ITEM3 | CONC | Model default | |
| STEP3 | YEAR | FOCUS default | Set to yearly output |
| LFREQ3 | 0001 | Model default | Output every compartment |
| ROFLAG | 0 | FOCUS default | No runoff |
| DEPRO | 5 | N/A | Not used as run off = 0 |
| DOC | 0 | FOCUS default | Not used |
| DOCFLG | 0 | FOCUS default | Not used |
| DEPMA | 0 | FOCUS default | Not used |
| IC | 0 | FOCUS default | Not used |
| FMAC | 0 | FOCUS default | Not used |
| GEOBREI | Location specific | FOCUS file selection | Latitude of location |

| | | | |
|---|---|---|---|
| .PSM file | | | CompA_10.psm |

| **Parameter description** | **Value source and comments** | **Model default, FOCUS default, FOCUS file selection, user choice or N/A** | **Rationale** |
|---|---|---|---|
| CTITLE | Compound specific | User choice | |
| NHORIZ | 0 | Model default | Not used |
| N_LOC | 1 | FOCUS file selection | 1 location where applied |
| DUMMY | 0 | Model default | Not used |
| REL_ABS_APP | 9 | FOCUS default | Relative application date |
| NAPS | 26 | User choice | 26 applications, 1 per year |
| APD | -1 | FOCUS default | Applied 1 day prior to emergence |
| APM | 0 | FOCUS default | Location specific |
| IAPYR | 01-26 | User choice | 1 per year |
| TAPP | 1 | FOCUS standard | total application rate kg/ha |
| DEPI | 0 | User choice | |
| COVAPP | 0 | Model default | Not used |
| FRPEC | 0 | Model default | Not used |
| APT | 0 | Model default | Not used |
| FAM | 1 | FOCUS Default | Application to soil only - no foliar wash off |
| VAPFLG | 1 | FOCUS Default | 1 = calculated |
| KDFLAF | 1 | FOCUS Default | 1 = calculated |
| HENRYK | 1.33E-10 | FOCUS Default | |
| SOLUB | 3 | User choice | Arbitrary choice for level to not affect mobility too highly |
| MOLMAS | 400 | User choice | Mid-level for pesticide |
| VAPPRE | 1.00E-12 | User choice | Arbitrary value to reduce losses to vapourisation |

|  |  |  |  |
|---|---|---|---|
| DAIR | 0.05 | User choice |  |
| VOLGRE | 0.1 | FOCUS default | Set to 0.1cm |
| T_VOL | 20 | User choice | Set to 20 degrees |
| UPTAKF | Varied between 0,0.1,0.5 and 1.0 | User choice | Varied to see effects of plant uptake on output |
| DKRATE | Varied between DT50 = 40-500 | User choice | Changes to persistence to observe effects to fate |
| TEMP0 | 20 | User choice |  |
| Q10 | 2.58 | User choice | Recommended correction |
| ABSFEU | 0 | User choice |  |
| FELFEU | 100 | User choice |  |
| FREUEXP | 0.7 | User choice |  |
| DEGFLAG | 0 | User choice | 0 = degradation according to degradation factors.  Depth dependant concentration |
| KOC | Between 10-500 | User choice | Changed to observe effects on fate |
| FRNEXKOC | 0.9 | FOCUS default |  |
| PH_KOC | -99 | User choice | Non pH dependent |
| PKA | 20 | User choice | Not used |
| FRNMIN | 0.01 | Default |  |
| ALTERN | 0 | User choice | No increase of sorption over time |
| K_DOC | 0 | Model default | Not used |
| KOC_MOI | 1 | User choice |  |
| KOC2 | -99 | User choice | Non pH dependent |
| PHKOC2 | -99 | User choice | Non pH dependent |
| FNEQ | 0 | User choice |  |
| KDES | 0 | User choice |  |

PELMO.INP

| **Parameter description** | **Value source and comments** | **Model default, FOCUS default, FOCUS file selection, user choice or N/A** | **Rationale** |
|---|---|---|---|
| IYEAR | 26 | FOCUS default | 26-year simulation |
| ISDAY | 1 | User choice | Day of start of simulation |
| ISMON | 1 | User choice | Month of start of simulation |
| IEDAY | 31 | User choice | Day of end of simulation |
| IEMON | 12 | User choice | Month of end of simulation |
| APPLIK | XX_Fallow.sze or XX_Wcereals.sze | FOCUS file selection | |
| CHEM | CompX_XX.psm | User choice | |
| KLIMA | XXXX_metXX.cli | FOCUS file selection | |
| NPLOTS | 20 | User choice | |
| PLNAME1 | PRSN | User choice | |
| MODE1 | TSER | Model default | |
| IARG1 | 0 | User choice | |
| CONST1 | 1 | User choice | |
| PLNAME2 | TETD | User choice | |
| MODE2 | TSER | Model default | |
| IARG2 | 0 | User choice | |
| CONST2 | 1 | User choice | |
| PLNAME3 | INFL | User choice | |
| MODE3 | TSER | Model default | |

| | | | |
|---|---|---|---|
| IARG3 | 100 | User choice | |
| CONST3 | 1 | User choice | |
| PLNAME4 | RUNF | User choice | |
| MODE4 | TSER | Model default | |
| IARG4 | 0 | User choice | |
| CONST4 | 1 | User choice | |
| PLNAME5 | THET | User choice | |
| MODE5 | TSER | Model default | |
| IARG5 | 0 | User choice | |
| CONST5 | 1 | User choice | |
| PLNAME6 | THET | User choice | |
| MODE6 | TSER | Model default | |
| IARG6 | 30 | User choice | |
| CONST6 | 1 | User choice | |
| PLNAME7 | TEMP | User choice | |
| MODE7 | TSER | Model default | |
| IARG7 | 0 | User choice | |
| CONST7 | 1 | User choice | |
| PLNAME8 | TEMP | User choice | |
| MODE8 | TSER | Model default | |
| IARG8 | 30 | User choice | |
| CONST8 | 1 | User choice | |
| PLNAME9 | TPAP | User choice | |
| MODE9 | TSER | Model default | |
| IARG9 | 0 | User choice | |
| CONST9 | 1.00E+05 | User choice | |
| PLNAME10 | TDKF | User choice | |

| | | | |
|---|---|---|---|
| MODE10 | TSER | Model default | |
| IARG10 | 0 | User choice | |
| CONST10 | 1.00E+05 | User choice | |
| PLNAME11 | TUPF | User choice | |
| MODE11 | TSER | Model default | |
| IARG11 | 0 | User choice | |
| CONST11 | 1.00E+05 | User choice | |
| PLNAME12 | TPST | User choice | |
| MODE12 | TSER | Model default | |
| IARG12 | 5 | User choice | |
| CONST12 | 1.00E+06 | User choice | |
| PLNAME13 | PFLX | User choice | |
| MODE13 | TSER | Model default | |
| IARG13 | 100 | User choice | |
| CONST13 | 1.00E+05 | User choice | |
| PLNAME14 | RFLX | User choice | |
| MODE14 | TSER | Model default | |
| IARG14 | 0 | User choice | |
| CONST14 | 1.00E+05 | User choice | |
| PLNAME15 | LEAC | User choice | |
| MODE15 | TSER | Model default | |
| IARG15 | 100 | User choice | |
| CONST15 | 1.00E+09 | User choice | |
| PLNAME16 | INFL | User choice | |
| MODE16 | TSER | Model default | |
| IARG16 | 0 | User choice | |
| CONST16 | 1 | User choice | |

| | | | |
|---|---|---|---|
| PLNAME17 | SPSC | User choice | |
| MODE17 | TSER | Model default | |
| IARG17 | 0 | User choice | |
| CONST17 | 1.00E+06 | User choice | |
| PLNAME18 | SPSC | User choice | |
| MODE18 | TSER | Model default | |
| IARG18 | 50 | User choice | |
| CONST18 | 1.00E+06 | User choice | |
| PLNAME19 | SPSC | User choice | |
| MODE19 | TSER | Model default | |
| IARG19 | 100 | User choice | |
| CONST19 | 1.00E+06 | User choice | |
| PLNAME20 | TVOX | User choice | |
| MODE20 | TSER | Model default | |
| IARG20 | 0 | User choice | |
| CONST20 | 1.00E+05 | User choice | |

# Chapter 5 – The case for updating the FOCUS meteorological files. Measuring the effect of new climatic inputs on the modelled plant uptake of plant protection products

## 5.1 Abstract

Climatic conditions play a very significant role in the study of environmental fate of chemical compounds because of the effect that it can have on the soil transport and degradation processes. It is also something that can have a big impact on the plant uptake, predominantly via transpiration, which in turn could increase or reduce the concentration of the leachate from the bottom of the profile. This chapter consists of two model experiments; this first was designed with the aim of discussing which climatic inputs had the strongest influence on plant uptake. The experiment was designed to keep a constant concentration in the soil profile at one location (Okehampton, UK), with different years used to provide climate variation. The second model experiment used a more recent dataset of climate files (Agri4cast; 1999-2018) to compare with the original FOCUS climate files (1992-2002) when used to run PEARL for 9 locations in Europe, to see if there is a difference between the leaching patterns of the two datasets. Results show that the climatic inputs with the greatest impact on plant uptake were maximum temperature ($T_{MAX}$) and potential evapotranspiration ($ET_{REF}$). Although both $T_{MAX}$ and $ET_{REF}$ showed good linear relationships with plant uptake, plant uptake also became limited by low soil moisture contents as it reduces transpiration. In future studies, $T_{MAX}$ and $ET_{REF}$ should be highlighted when looking at plant uptake. However, generalisation of these findings should be avoided as only one location was used within the first experiment. The FOCUS and Agri4cast datasets showed remarkably similar leaching patterns, however the more recent Agri4cast dataset showed considerably lower levels of leaching. The fact that the more recent climate data, when used to drive PEARL, shows a different level of leaching to the original FOCUS files suggests that the climate files of

FOCUS scenarios should be updated as often as possible to be able to provide the most up to date leaching estimates.

## 5.2 Introduction

Determination of the environmental fate of plant protection products is a highly complex topic and requires consideration of numerous biological, chemical and physical processes (Arias-Estévez et al., 2008). The rates of many of these processes are affected by the conditions encountered by the compound. The meteorological conditions are an example of a set of factors that change regularly and can cause large variation in the transport, degradation or volatilisation of a pesticide (Kookana et al., 2010). Plant uptake is a process whereby a compound is taken up passively during the process of transpiration. Transpiration concerns the uptake of solution from the soil pores; it is intrinsically linked with various meteorological variables such as temperature, relative humidity, incoming shortwave- and longwave radiation, and windspeed. Together, these variables determine potential evapotranspiration (Taiz and Zeiger, 2010). The Earth's climate has been shown to vary naturally throughout history and has been measured through studies such as paleoclimatology. Paleoclimatology then splits geological time into periods across history that represents patterns spotted in the data and can be further subdivided into epochs (Steffen et al., 2011). As a result, it has been proposed that the current geological period that we are living in is to be called the 'Anthropocene' (Barnosky, 2014). The major characteristic of the Anthropocene is that the 'greenhouse effect', that is driven by the abundance of greenhouse gases in the earth's atmosphere, is having an increasing effect on the mean temperature across the globe. The abundance of greenhouse gases in our atmosphere is increased via the burning of fossil fuels which has become a major part of modern industry and energy following the industrial revolution (Steffen et al., 2018). Increase in mean global temperatures does not simply mean a blanket increase in temperatures; it has been predicted that some areas will in fact experience colder temperatures in the future. However, IPCC predictions indicate that there will be an increase in extreme weather events potentially leading to higher frequency of prolonged heatwaves and droughts,  and periods of extreme rainfall (IPCC, 2018).

FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe) were tasked with improving the estimations of environmental concentration of plant protection products through modelling (FOCUS, 2000). They developed nine 'realistic worst-case' scenarios which represented a wide range of climatic zones with corresponding crop calendars. In their final report published in 2009, FOCUS state that their scenarios were setup using a dataset spanning from 1992-2002. This was drawn from the European Commission Joint Research Council Centre (JRC) Monitoring Agricultural ResourceS (MARS) dataset which uses an interpolated 50km x 50km grid (FOCUS, 2009). At the time of writing this thesis, the most recent datapoints used within the FOCUS dataset are now 17 years old; however, the original datasets are still being used for regulatory purposes across Europe. One of the key characteristics of the Anthropocene is not just a changing climate, but the fact that these changes occur at an accelerated pace compared to what has previously been observed (Dalla Valle et al., 2007; IPCC, 2018; Steffen et al., 2018). This means that to get the most accurate predictions of environmental fate, ideally the most recent dataset should be used. Therefore, this chapter contains two separate experiments that have been devised to test two main aims. The first experiment tests which meteorological inputs have the greatest effect on plant uptake. The second experiment uses a new 20-year dataset (1999-2019, as opposed to the original FOCUS 1992-2002) to test the effect of the meteorological files on the uptake of pesticide by the plants and the subsequent effect on leaching down the soil profile. Although the FOCUS dataset only spans over 10 years, the files still cover the required 26 years due to replicate years within the dataset.

## 5.3 Methods

The methodology of the PEARL environmental fate model, and how it treats plant uptake and transpiration, were discussed in detail within Chapter 4 – Quantifying the effect of plant uptake on environmental fate using the FOCUS scenarios. The methodologies of the model will not be repeated here but the approach of this chapter will be detailed.

### 5.3.1 Experiment 1 – Effect of meteorological inputs

The purpose of Experiment 1 was to test the effects of the meteorological inputs on modelled plant uptake. To achieve this, it was important to try and negate the reduction of concentration in the soil profile over time by applying the compound periodically. Plant uptake within PEARL, $M_U$ (kg/m$^3$), is affected by the concentration of the compound in the liquid phase, the plant uptake factor and the amount of solution transpired (Equation 5.1).

$$M_U = R_L PUF C_L \tag{5.1}$$

where $R_L$ is the water uptake ($m^3\ m^{-3}$), PUF is the Plant Uptake Factor (-), and $C_L$ is the concentration of pesticide within the liquid phase (kg $m^{-3}$) (Tiktak et al., 2000). Ideally, $C_L$ needs to remain relatively consistent throughout the growing season, to allow us to reliably investigate the impact of the meteorological inputs on $U_p$. In order to reduce the potential for previous applications affecting the concentration within the profile, it was decided that the application of the compound would not be repeated annually but would be applied periodically through the growing season during one selected year for each model run. Therefore, it was decided that every month there would be an application of the compound at the rate of 1 kg/ha.

It was decided that the FOCUS scenarios would be used for this experiment as they are used most within regulatory assessments. For simplicity, the number of locations selected was reduced to one; Okehampton was selected as this location does not require irrigation to a cereal crop. Spring cereal was selected as the model crop as it is very commonly grown in the U.K. As it is grown in the spring, the growth and plant uptake would be consistent throughout the period that was measured. This was chosen in preference to winter cereals which have the longer sedentary period over the winter where uptake may be more inconsistent.

The PUF that was given to each compound was 0.7 as this gives a high/moderate level of uptake with transpiration but does not give maximum uptake (PUF = 1). The FOCUS dataset replicates years to give the required 66 years of meteorological data. The year for which the compound was applied needed to be selected carefully to ensure that they were unique to give variation in the meteorological variables and see how variation may affect the plant uptake. It should also be noted that the year numbers in the FOCUS dataset do not match those after which they are named. Therefore, the years used within this experiment will represent unique years between the dates of 1992-2002 but not the early 1900s. The years 1907, 1912, 1917 and 1922 were selected for use in this study as they were seen to be unique on further investigation.

The reduction of the model experiment to one location, allowed for the variation in the chemical properties of the compounds that are applied to the profile, i.e., the partition coefficient between water and organic carbon ($K_{OC}$); this was varied from 10 ($K_{OM} = 5.8$) to 1000 ($K_{OM} = 580$). $K_{OC}$ is a chemical property that affects the sorption of the chemical to the soil, therefore it can have a big effect on the concentration of the compound in the liquid phase. This means that an increase in $K_{OC}$ will result in a reduction of the availability of the compound for plant uptake but also an increase in the mobility of the compound down the profile.

### 5.3.2 Experiment 2 – Defining scenarios with new climate data

The purpose of Experiment 2 was to determine whether the addition of climate files with the most recent data would show different leaching outputs to the FOCUS climate files. The FOCUS scenarios were defined to represent 'realistic worst-case' scenarios (see Chapter 4 – Methodology), with the soil profiles and climate files already fitted into the model on installation. However, the original meteorological dataset was drawn from between the years of 1992 – 2002. Given that the rate of climate change is reported to be much faster than previously observed, it is possible that a more recent dataset would provide much different leaching outputs.

Agri4cast is a dataset published by the JRC and is used to present information of recent weather conditions and crop yield forecasts. They have also published a gridded agro-meteorological dataset containing real weather station data that has been interpolated across a 25km x 25km grid across Europe with a date range of 01/01/1975 – 31/12/2018. As this dataset is designed for agro-meteorological purposes, it means that it provides many of the climatic inputs that are used within PEARL for their climate files and the high resolution means that this data can be matched to the locations already used by FOCUS. Each FOCUS location was then matched as closely as possible with an Agri4cast datapoint to provide a new climate file. The runs with the new Agri4cast files were configured for 01/01/1993 – 31/12/2018; this allowed for 6 years of hydrological spin-up (1993-1998) and then 20 years of the most recent climate data. Boxplots of the yearly mean of both the FOCUS and Agri4cast climate files have been presented in Appendix A to show the key differences between the datasets. The model runs were set up to mirror each other with all 9 FOCUS locations being modelled with both the FOCUS climate files and the newly defined Agri4cast files. The three example compounds used within Chapter 4 (Table 5.1) were then applied annually at 1 day prior to emergence. Emergence within the models is supplied as a fixed date based on a crop calendar and this was kept the same between the two datasets, despite the fact this it may be different based on more recent climate data. To model some variation in mobility and persistence and create different behaviours of the compounds within the profile, the only properties that were varied were the partition coefficient between organic carbon and water ($K_{OC}$) and the DT50. Model runs were then setup to mirror each location for both climate datasets, with each compound being modelled with a PUF = 0, 0.5 and 1.0. A fallow scenario was also added to each location of both datasets to act as a control for leaching without cultivation.

**Table 5.1 Example compounds and the physical-chemical properties that were varied to give different mobility and persistence behaviour within the soil profile**

| **Compound** | **$K_{OC}$ (L/kg)** | **DT50 (days)** |
|---|---|---|
| *CA* | 10 | 100 |
| *CB* | 100 | 40 |
| *CC* | 500 | 500 |

To compare the effect of plant uptake on the leaching of the compounds down to 1m depth and the effect that the climate files can have on this, the $PEC_{GW}$ will be used. $PEC_{GW}$ is calculated as the 80th percentile of the mean yearly concentration of the applied compound at 1m depth. This represents a singular value for the leaching within a run and allows for easy comparison between runs.

### 5.3.3 Statistical analysis

Linear mixed effects models were conducted on both datasets. For Experiment 1 looking at the effects of the meteorological inputs on plant uptake, the test will be conducted on the significant effects on the output which shows the areic mass of plant uptake ("AmaUptPro"). For Experiment 2, comparison between the locations modelled with two different climate files, the test will be conducted on the output which shows the concentration of the pesticide in the liquid phase at 1m depth ($C_{1m}$; ConLiq8).

# 5.4 Results and Discussion

## 5.4.1 Experiment 1 – Effects of meteorological inputs on plant uptake

Before presenting and discussing the results within this section it should be stated again that the years reported within the data (1907, 1912, 1917 and 1922) are not data corresponding to those periods in time. Instead, these dates are derived from a dataset between 1992-2002 compiled by FOCUS (FOCUS, 2009).

### 5.4.1.1 Uptake from the soil profile

Figure 5.1 shows that early in the growing season, the uptake each year is very similar among years with very little difference between years. For almost all compounds modelled, the peak of uptake is in July; at this point the simulation for 1922 starts to show much lower uptake than the other modelled years, this trend then continues into August. A similar drop in uptake is also seen in 1907. The interannual difference for the late growing season uptake becomes more pronounced with the higher $K_{OC}$ compounds.

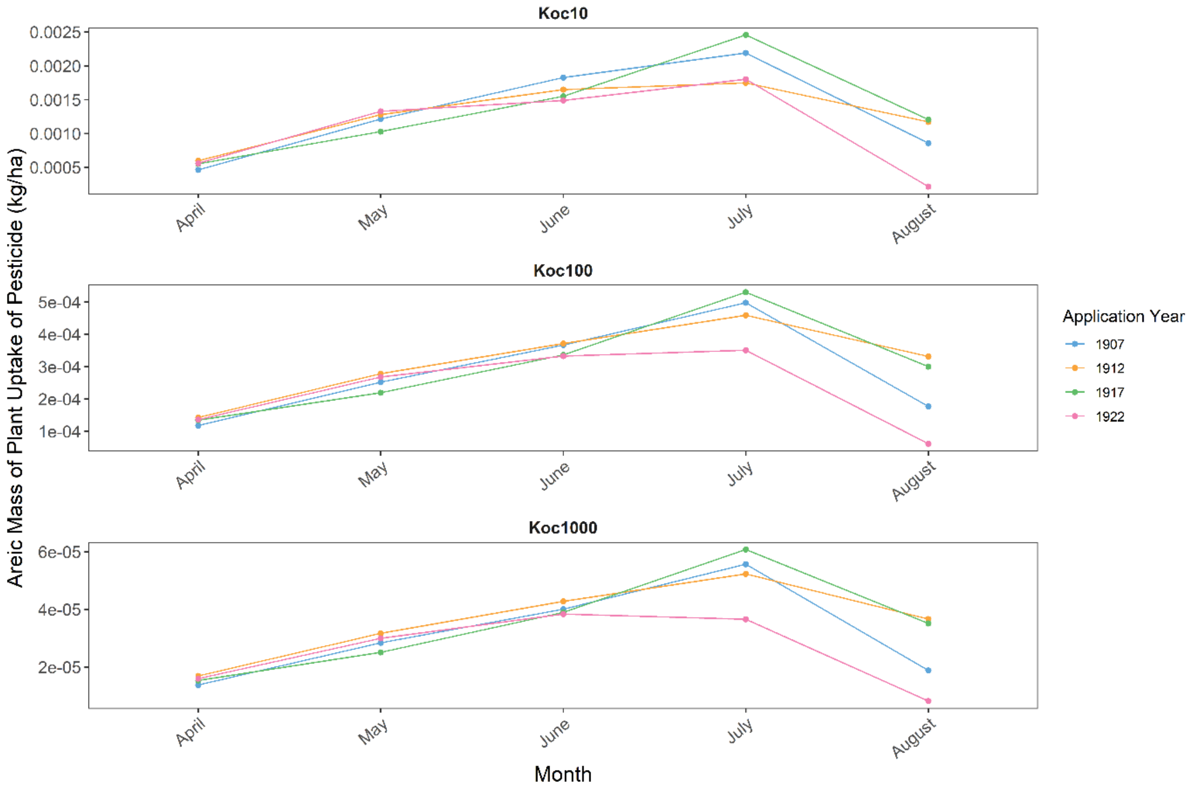

**Figure 5.1. The mean monthly uptake of pesticide with the colour representing the year of application.**

Based on the plant uptake equation used within the models, the plant uptake is a function of the PUF (0.7 in this chapter), the concentration in the solution and the amount transpired by the plant. Figure 5.2 shows the monthly mean concentration in the topsoil for 4 years, with each line representing a different year. Although there are fluctuations, the most constant concentration is found for the compound with a $K_{OC} = 10$, the compounds with a $K_{OC}$ of 100 and 1000 show gradual rises (apart from 1912 for $K_{OC} = 100$) throughout the growing season with $K_{OC} = 1000$ showing the biggest rise.

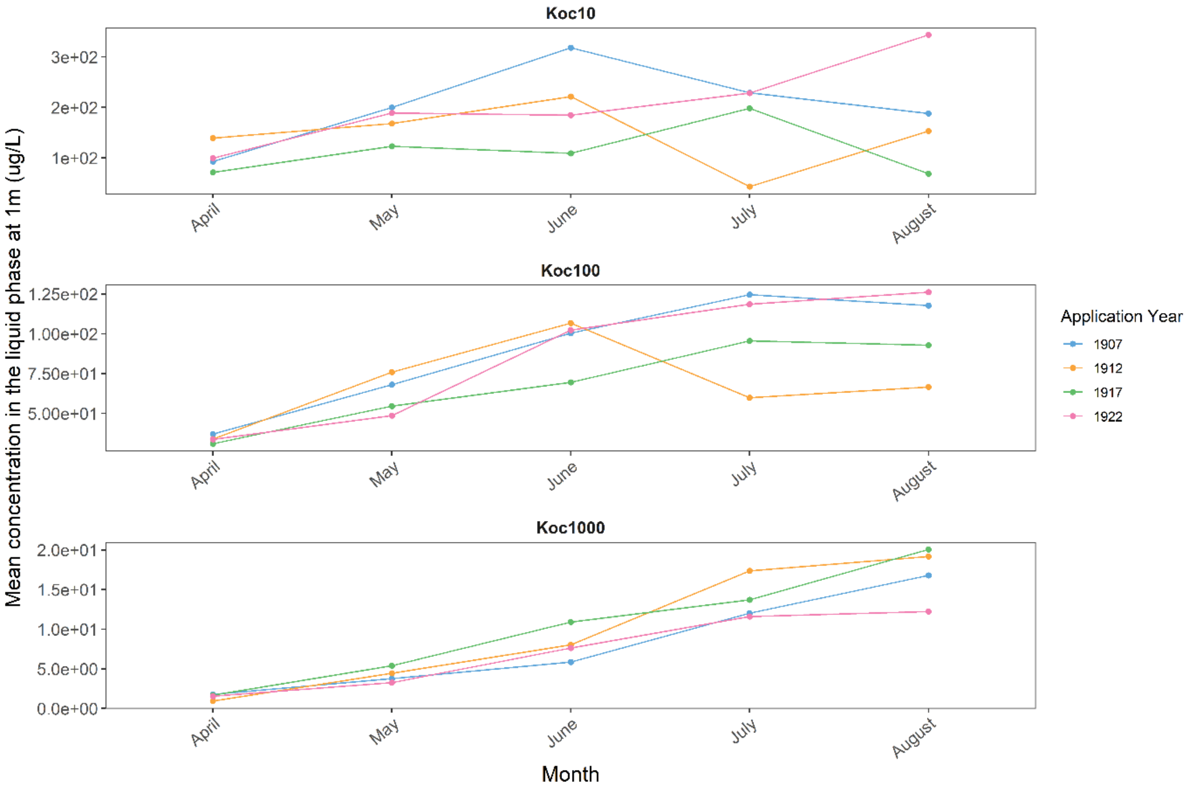

**Figure 5.2. The mean monthly concentration of pesticide in the liquid phase in the topsoil with the colour representing the year of application. Applications of the pesticide at monthly intervals throughout the growing season, at a rate of 1 kg/ha.**

In addition to the concentration, the component that helps explain variations in plant uptake is the transpiration (Equation 5.1). Figure 5.3a shows the transpiration of solution by the plants during the growing season. Within the models, each crop is given a $K_C$ value by the model which estimates the transpiration demand of the crop depending on the growth stage; however, the actual transpiration of the crop is dependent on the climatic conditions and the available soil moisture (FOCUS, 2009). For Winter wheat in PEARL, the $K_C$ value varies from 1.05 (early season) – 1.1 (peak season) – 0.7 (late season). All selected years show a similar shape, with some variation later in the year. 1917 shows the lowest levels of transpiration in May but its values continue to rise in June and July whereas the other years show a peak or plateau in June. The year 1922 shows the lowest levels of transpiration in August. This finding, when considered alongside the concentrations of the compound (Figure 5.3a) in August of 1922, shows that there is a meteorological reason behind the reduction in uptake later in that year. August is the month that shows the greatest variation in uptake between the years, August also shows the greatest variation between the years in transpiration. This modelling experiment has been designed so that the concentration does not limit the amount of compound taken up by the plants. The results reported within this section show that the experiment has worked as designed, by the plant uptake of pesticide not being limited by concentration. This allows the transpiration to have a stronger influence, alongside the Kc value.

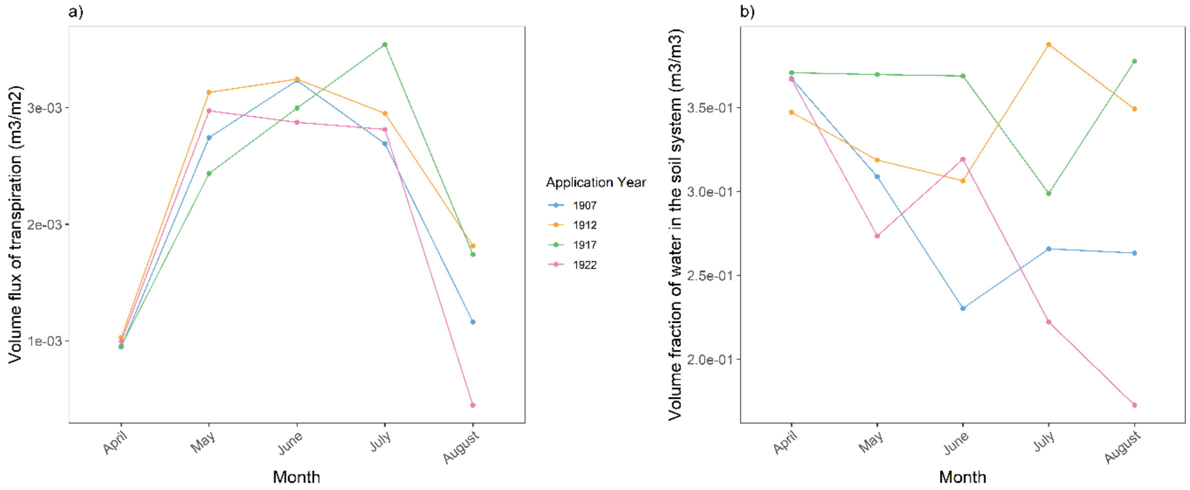

**Figure 5.3. The mean monthly transpiration (a) and soil moisture content (b) with the data coloured by year.**

However, a key point to consider when looking at transpiration is the soil moisture content (Figure 5.3b) as it will affect plant water stress. As transpiration can only be taken from the available soil moisture, it becomes limited when the pool of solution becomes reduced. Figure 5.3b shows that both 1907 and 1922 show their lowest levels of soil moisture in June and August respectively, the lowest overall point is August 1922, this month also coincides with the lowest transpiration and uptake values for all modelled compounds. By referencing that against the concentration data, it shows that although the concentration does not reduce, therefore the transpiration is likely to be limited by the low soil moisture content in that month.

### 5.4.1.2 Relationship between plant uptake and meteorological factors

Within this experiment the transpiration is the only factor within the plant uptake equation which is directly affected by the meteorological inputs. Therefore, to determine the effect that these inputs have on plant uptake, it was important to stop the concentration in the profile becoming a limiting factor in the uptake. The linear mixed model run on this dataset showed that the two inputs from the climate file that have the biggest impact on the $U_p$ were potential evapotranspiration ($\mathrm{ET_{REF}}$) and maximum temperature ($\mathrm{T_{MAX}}$).

Figure 5.4 show plots of $U_p$ against potential evapotranspiration; this demonstrates a clear positive linear relationship for all compounds and across all years. Although the difference between the compounds is separated by a few orders of magnitude for each compound, the pattern of uptake remains very similar across each compound. For 1922, there appears to be a cluster of datapoints that show very low levels of uptake despite relatively high levels of $\mathrm{ET_{REF}}$, these datapoints are likely to be from August as this is the point where the soil moisture drops to the lowest level. This means that despite the meteorological conditions, the uptake is being limited by other factors. As a result of this, further investigation should be conducted into the reason for clusters forming within the data, to show whether further explanation can be given to the variation within the data. Within the models, the potential evapotranspiration is calculated by multiplying the $\mathrm{ET_{REF}}$ by the crop coefficient, therefore it would be expected that $\mathrm{ET_{REF}}$ is closely linked to the plant uptake.

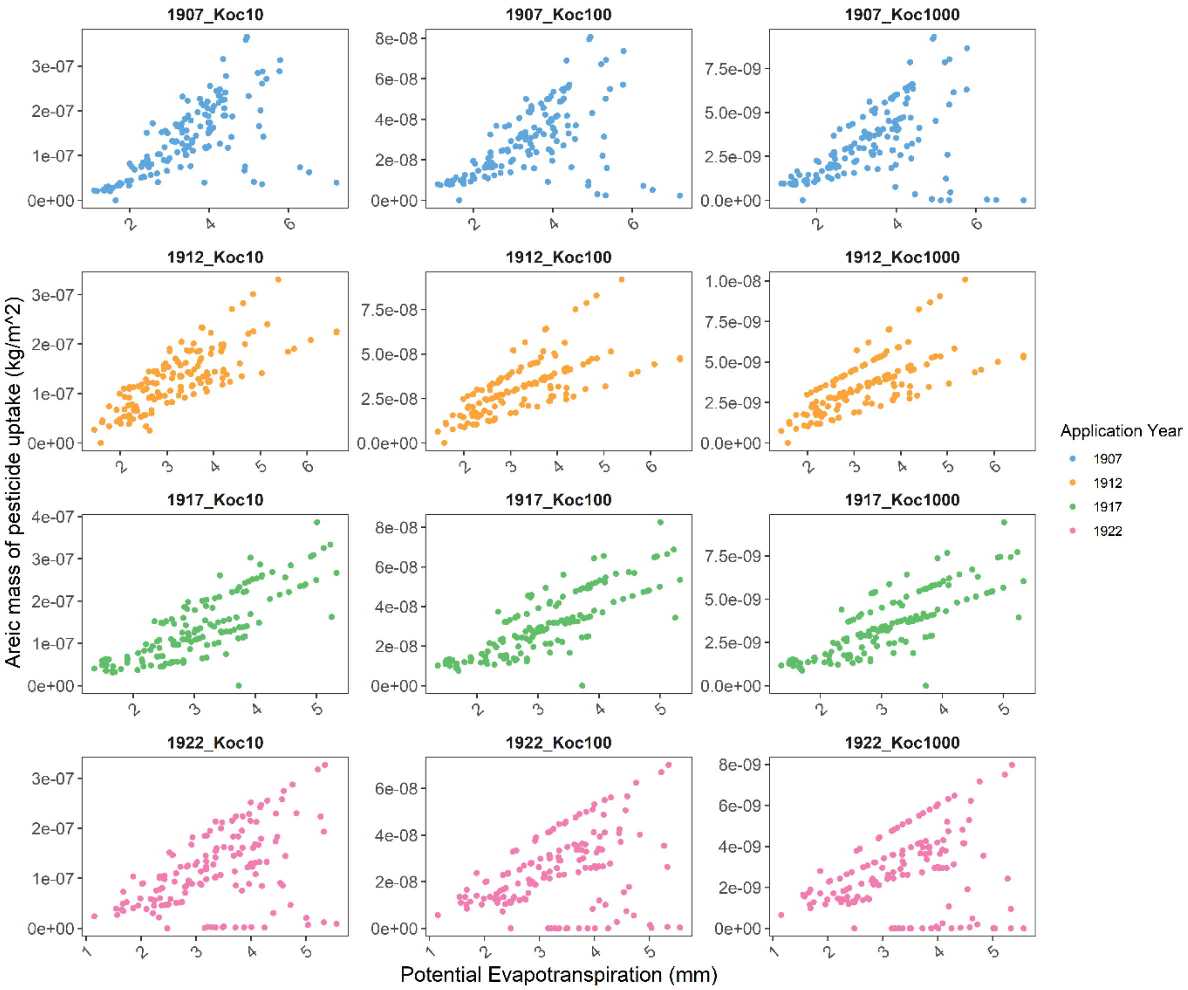

**Figure 5.4. Scatter plots of the areic mass of plant uptake against the potential evapotranspiration.**

Figure 5.5 shows plant uptake against $T_{MAX}$; as in Figure 5.4 there are several points that cluster along the x-axis and appear to show very low levels of uptake despite relatively high $T_{MAX}$. Based on the F-value of the linear mixed model, $ET_{REF}$ was the stronger of the two meteorological inputs, however both had p-values <0.001. Whilst both $T_{MAX}$ and $ET_{REF}$ have been discussed here as they are both separate inputs within the climate files, it should be acknowledged that they are linked due to their calculation. Of course, the maximum temperature is a direct meteorological measurement, but potential evapotranspiration is calculated from a variety of meteorological variables. Therefore, the calculation of the $ET_{REF}$ is dependent on the method used within the dataset that has been imported. With reference to FOCUS, the MARS dataset is used where the Penman-Monteith method is used for calculation of $ET_{REF}$ (FOCUS 2009xx). Penman-Monteith takes the input of radiation, air temperature, relative air humidity and wind speed within the calculation (Van Dam et al, 1997xx).

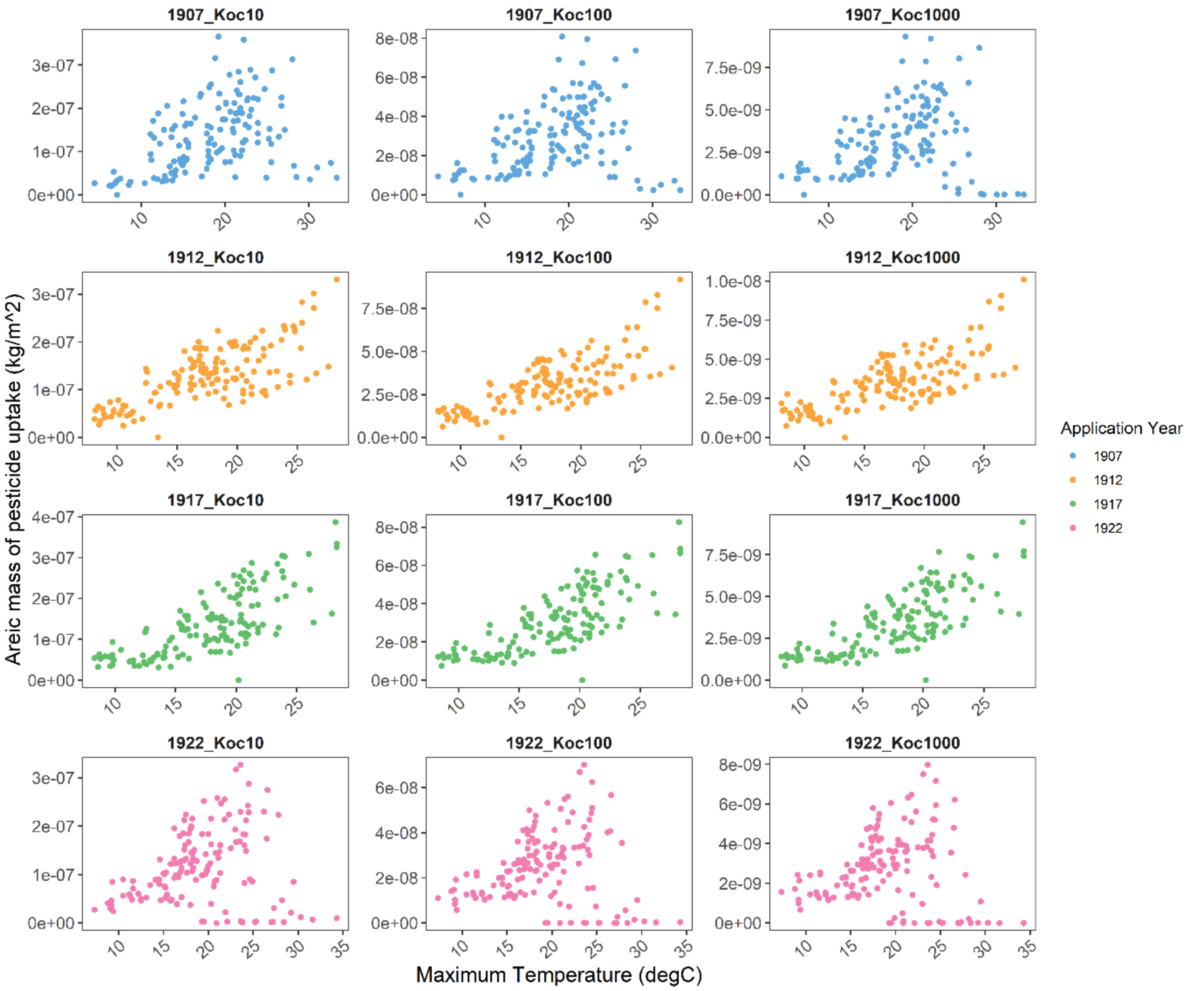

**Figure 5.5. Linear regression of the areic mass of plant uptake against the potential evapotranspiration.**

Although it is possible that all the available locations would react in a similar way to the climate inputs, this is not certain without testing, so care must be taken when trying to apply this universally. Within the models, the transpiration is limited by the crop demand for solution, potential evapotranspiration, and the available water supply. There is however scope for there to be different 'limiting factors' within the climatic conditions that influence the rate of transpiration much more than the $T_{MAX}$ in Okehampton. For example, for Sevilla in the Mediterranean climatic zone, the $T_{MAX}$ and $ET_{REF}$ are likely to be much higher than in Okehampton but there is much less rainfall in this area so this means that while the potential ET may be high, the actual ET is much lower due to the reduced available soil moisture. In practice, transpiration is a biological process and not a mathematical relationship. While it is true in this case that an increase in $T_{MAX}$ and $ET_{REF}$ may lead to an increase in plant uptake, this may reach an asymptote rather than increasing in a linear fashion to extremes. There may be a threshold within the optimum environmental conditions of a plant that mean further increases in temperature beyond this may lead to a decrease in stomatal conductance and hence in plant transpiration and result in reduce plant uptake. Of course, vapour pressure deficit also has an import role to play in the process of transpiration.

### 5.4.2 Experiment 2 – Comparison of the original FOCUS climate files against the Agri4cast dataset for all 9 locations.

The results in this section will discuss the concentration leaching from the profile at 1m depth ($C_{1m}$). The differences between the two datasets have been discussed within the methods section. Whilst it is important to consider the effects that the $T_{MAX}$ and $ET_{REF}$ will have on the leaching output, it is also important to consider that further meteorological inputs may have an effect, such as long periods of no rainfall causing lower levels of water leaching below the 1m depth or higher frequency of rainfall events. Figure 5.6 shows the $C_{1m}$ for Compound A, with the original FOCUS dataset on the left-hand side and the same soil profiles modelled with the new Agri4cast dataset on the right. Within this plot the lines are grouped by the PUF value that has been given to the compound, CA0: PUF = 0; CA5: PUF = 0.5; CA10: PUF = 1. NC_CA0 represents the fallow scenario. Generally, the patterns between the two datasets are remarkably similar as would be expected as the locations are modelling the same location with a slight discrepancy between the years. One of the main differences between the datasets is that locations such as Kremsmünster and Piacenza show relatively high leaching rates when PEARL is run with the FOCUS climate files (see Appendix A for differences between the climate files for each parameter and location). In July, August and September but show comparatively low levels of leaching when using the Agri4cast climate files in the winter months (December, January and February). Within this experiment, the shape of the cultivated scenarios (e.g. CA0, CA5 and CA10), however the fallow scenario can sometimes show a different pattern of leaching. For Compound A the leaching levels in the fallow scenario tend to fall between the range of the cultivated scenarios. The only locations where this is not the case are Okehampton where there is a lower level of leaching and Sevilla where the leaching is higher in the fallow scenario. In the FOCUS climate Sevilla runs, there is a much bigger gap between the CA0 and CA5 data than seen in the runs driven by Agri4cast climate data. This results in the fallow run and the CA0 run showing almost identical values in January, February and March, with the fallow scenario even dropping below the CA0 in February.

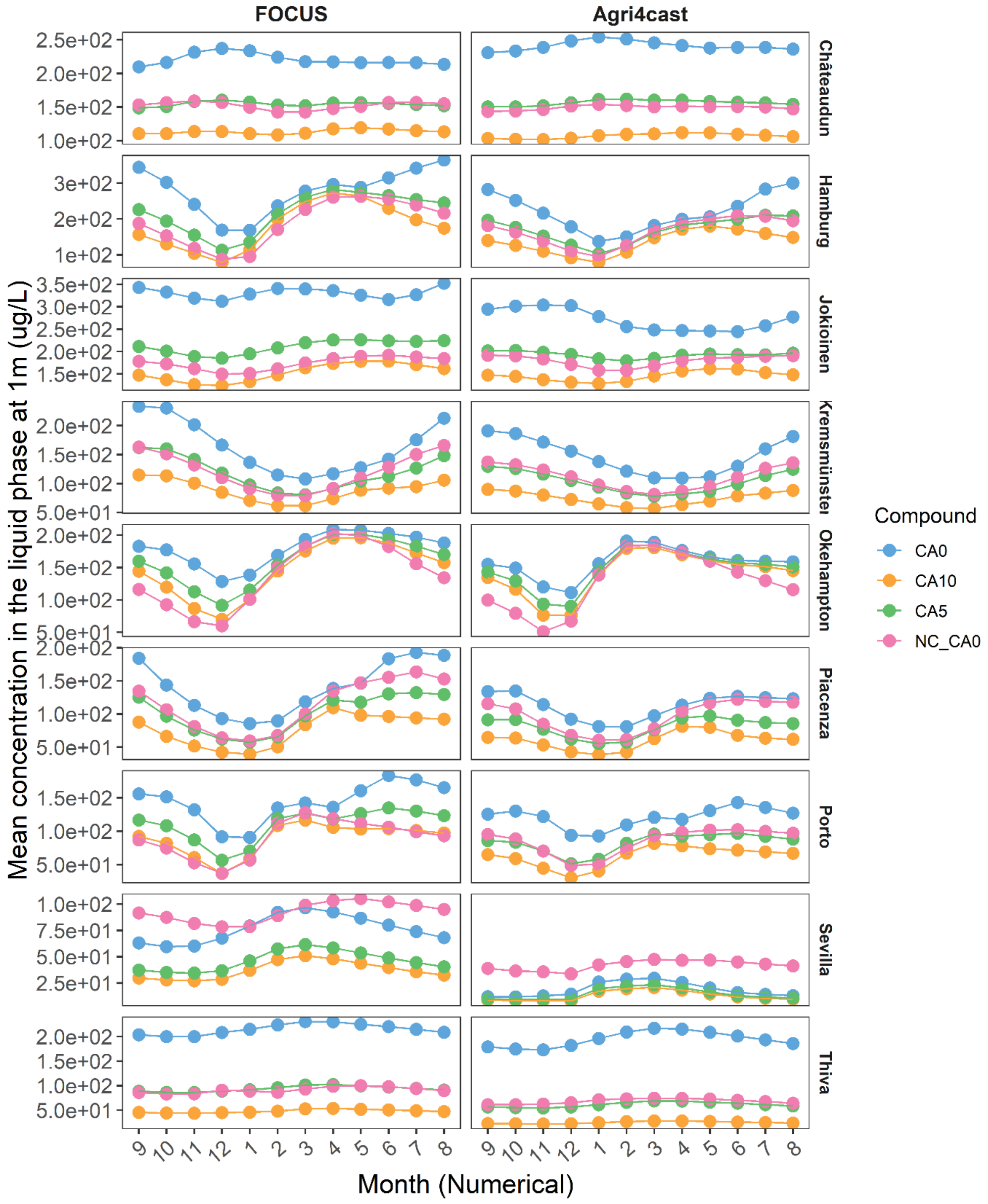

**Figure 5.6. Mean concentration of Compound A at 1m depth ($C_{1m}$) for PEARL model runs driven by both climate files, with FOCUS on the left and Agri4cast on the right. Each plot is split by location.**

Compound B shows much flatter levels of leaching levels of leaching than Compound A and at lower levels by around 2 orders of magnitude (Figure 5.7). For most locations there appears to be a dip in leaching around October, November and December, the leaching levels then pick up and peak in the spring or summer months before dropping again. Hamburg and Piacenza don't follow this pattern, with leaching levels for Hamburg showing a sharper rise in leaching in May – August before peaking and dropping until December and remaining relatively flat during this period. Simulated leaching in Piacenza remains relatively constant throughout the growing season, with a peak in May and June, which is seen much later than for other locations. Both sets of model runs show very similar leaching patterns throughout the year, however the Agri4cast climate files lead to a distinctively lower level of leaching, with Jokioinen and Thiva showing almost flat levels across the x-axis.

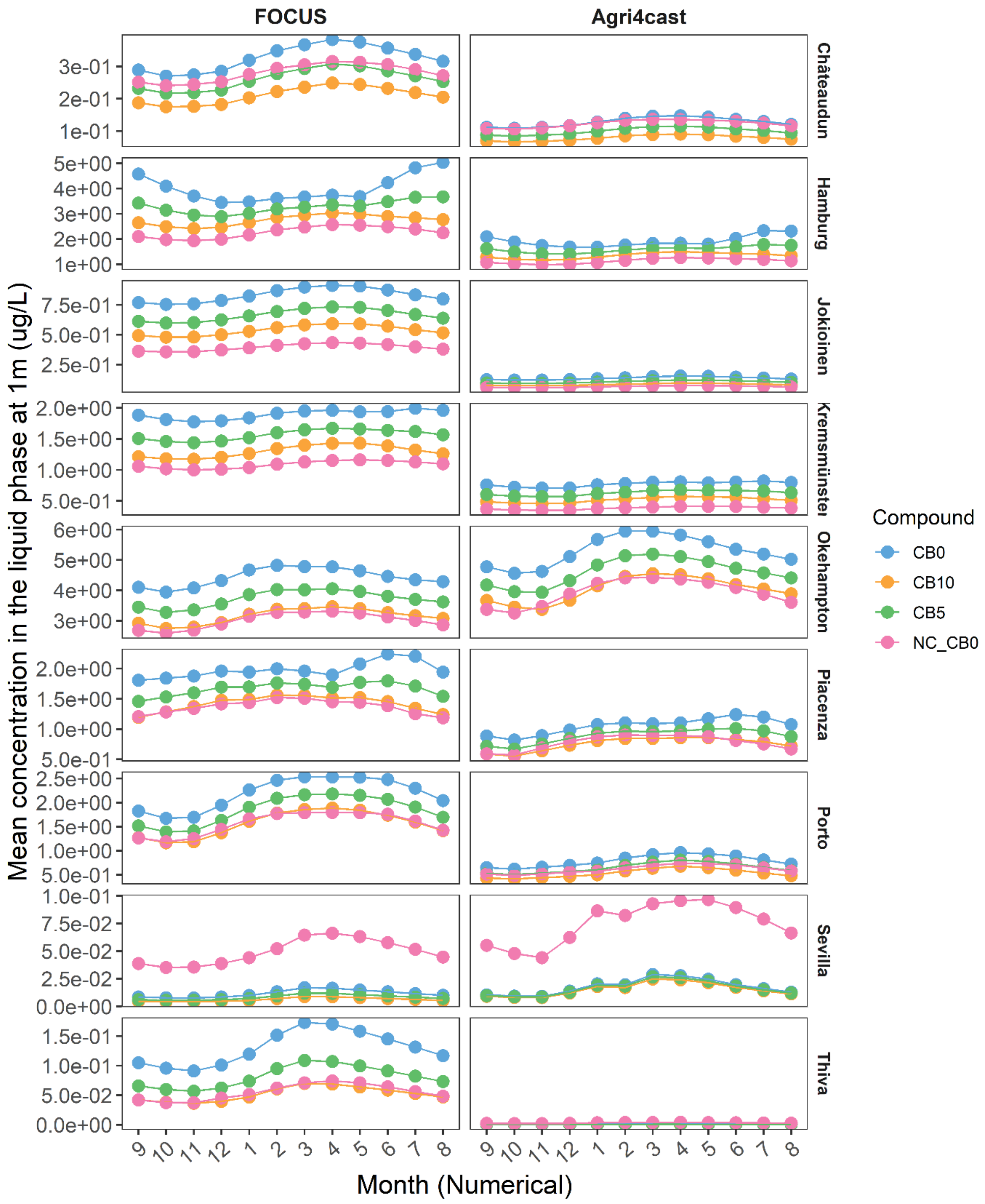

**Figure 5.7. Mean concentration of Compound B at 1m depth ($C_{1m}$) for both climate files, with FOCUS on the left column and Agri4cast on the right. Each plot is split by location.**

The leaching of Compound C (Figure 5.8) is higher than for compound B but lower than what was simulated for Compound A. The pattern of leaching generated by both sets of climate files seems very similar, with the Agri4cast runs predicting lower amounts of leaching than FOCUS. Many of the leaching patterns are similar across the months to those shown for Compound B, however Châteaudun shows a much flatter leaching. The fallow run for Sevilla shows a much high level of leaching when compared to the cultivated scenarios.

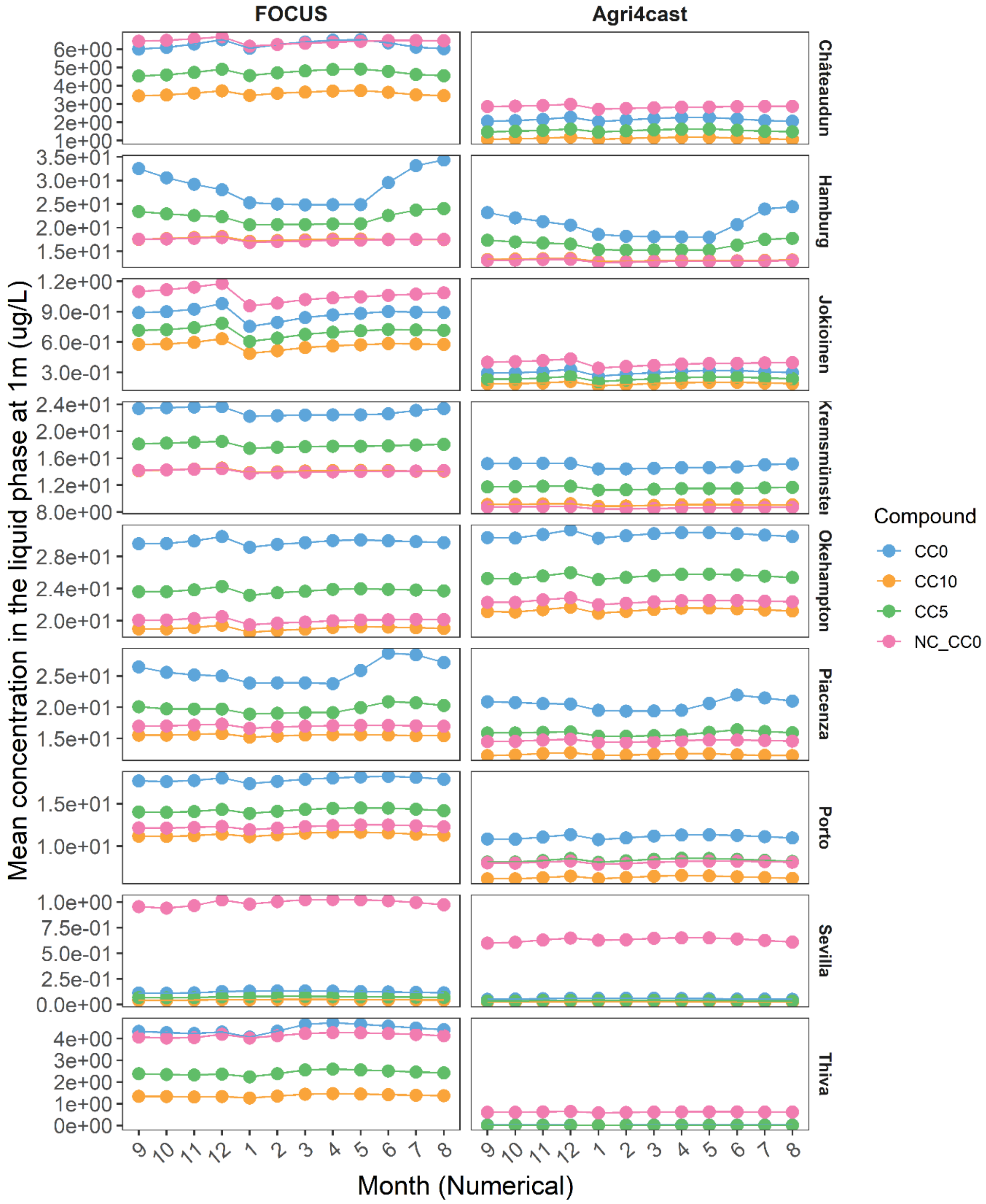

**Figure 5.8. Mean concentration of Compound C at 1m depth ($C_{1m}$) for both climate files, with FOCUS on the left column and Agri4cast on the right. Each plot is split by location.**

A linear mixed model was run on the $C_{1m}$ with the months treated as repeated measures and the locations treated as random effects. The effect of the run, PUF value and the interaction between the two was tested. With the climate files (i.e., Agri4cast and FOCUS) showing a significant difference (p-value = 0.042). The fact that there is a difference between the two datasets adds weight to the fact that the climate files should be updated regularly to allow for the most up to date leaching predictions. Going forward this should lead into investigations on the kind of effect this would have on future leaching predictions. Current climate predictions suggest warmer temperatures and more extreme weather events (IPCC, 2018). This could lead to greater storms which could drive greater levels of leaching. Moreover, higher temperatures could speed up degradation processes and reduce the concentration in the leachate (Bloomfield et al., 2006; Dalla Valle et al., 2007).

### 5.4.3 Predicted Environmental Concentration in groundwater ($PEC_{GW}$)

$PEC_{GW}$ results (Table 5.2) present a singular value for the potential for leaching within each scenario. The results have been presented to allow for easy comparison between the PUF value (0-1) and the two climate files. A t-test on the difference between the percentage change values for each compound shows no significant difference. This means that whilst generally the FOCUS dataset seems to leach at higher amounts than the Agri4cast dataset, there is no significant difference in the effect of plant uptake on leaching using the two different climate files. This suggests that whilst the results in the previous sections show that there is a significant difference between the levels of leaching between the two climate files, there is no difference between the effect of plant uptake within this.

**Table 5.1. Predicted Environmental Concentration in the groundwater ($PEC_{GW}$) values for each run. Percentage change was calculated as the difference between the PUF = 0 run and the PUF = 1 run divided by the PUF = 0 values and multiplied by 100.**

| | | Compound A | | | | Percent change |
|---|---|---|---|---|---|---|
| | **Location** | 0 | 0.5 | 1 | Fallow | |
| **FOCUS met files** | Châteaudun | 270 | 173 | 127 | 172 | 53.2 |
| | Hamburg | 335 | 218 | 155 | 179 | 53.7 |
| | Jokioinen | 499 | 296 | 170 | 189 | 66.0 |
| | Kremsmünster | 196 | 141 | 102 | 135 | 47.8 |
| | Okehampton | 186 | 161 | 142 | 142 | 23.7 |
| | Piacenza | 174 | 112 | 84.3 | 126 | 51.5 |
| | Porto | 143 | 112 | 99.4 | 106 | 30.6 |
| | Sevilla | 89.2 | 21.4 | 12.7 | 114 | 85.7 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Thiva | 281 | 120 | 60.6 | 106 | 78.5 |
| Agri4cast met files | Châteaudun | 294 | 176 | 120 | 173 | 59.1 |
| | Hamburg | 267 | 182 | 140 | 168 | 47.5 |
| | Jokioinen | 368 | 234 | 160 | 202 | 56.5 |
| | Kremsmünster | 199 | 134 | 93.2 | 132 | 53.1 |
| | Okehampton | 170 | 153 | 146 | 129 | 13.8 |
| | Piacenza | 135 | 95 | 68.3 | 108 | 49.4 |
| | Porto | 160 | 107 | 89.6 | 97.4 | 44.3 |
| | Sevilla | 8.25 | 0 | 0 | 37.3 | 100.0 |
| | Thiva | 292 | 87.7 | 32.7 | 84.4 | 88.8 |
| | | **Compound B** | | | | **Percent change** |
| | **Location** | 0 | 0.5 | 1 | Fallow | |
| FOCUS met files | Châteaudun | 0.555 | 0.45 | 0.367 | 0.469 | 33.9 |
| | Hamburg | 5.14 | 4.20 | 3.50 | 3.07 | 32.0 |
| | Jokioinen | 1.13 | 0.921 | 0.759 | 0.603 | 32.6 |
| | Kremsmünster | 2.23 | 1.82 | 1.49 | 1.34 | 33.4 |
| | Okehampton | 5.64 | 4.76 | 4.04 | 4.03 | 28.3 |
| | Piacenza | 2.46 | 2.10 | 1.80 | 2.60 | 26.7 |
| | Porto | 3.19 | 2.67 | 2.24 | 2.43 | 29.7 |
| | Sevilla | 0 | 0 | 0 | 0.037 | 0.0 |
| | Thiva | 0.162 | 0.104 | 0.069 | 0.09 | 57.4 |
| Agri4cast met files | Châteaudun | 0.2 | 0.16 | 0.13 | 0.195 | 35.0 |
| | Hamburg | 2.17 | 1.75 | 1.48 | 1.368 | 31.8 |
| | Jokioinen | 0.15 | 0.12 | 0.096 | 0.080 | 36.0 |
| | Kremsmünster | 1 | 0.827 | 0.703 | 0.466 | 29.7 |
| | Okehampton | 6.38 | 5.45 | 4.734 | 4.841 | 25.8 |
| | Piacenza | 1.14 | 0.968 | 0.829 | 1.228 | 27.1 |
| | Porto | 1.19 | 0.970 | 0.793 | 0.876 | 33.4 |
| | Sevilla | 0 | 0 | 0 | 0.0003 | 0.0 |
| | Thiva | 0.001 | 6E-04 | 3E-04 | 0.003 | 72.9 |
| | | **Compound C** | | | | **Percent change** |
| | **Location** | 0 | 0.5 | 1 | Fallow | |
| FOCUS met files | Châteaudun | 17.4 | 12.7 | 9.44 | 13.4 | 45.7 |
| | Hamburg | 45.1 | 33.6 | 25.9 | 25.8 | 42.6 |
| | Jokioinen | 1.89 | 1.55 | 1.29 | 1.85 | 31.8 |
| | Kremsmünster | 33.2 | 25.8 | 20.3 | 20.7 | 38.8 |
| | Okehampton | 41.2 | 32.6 | 25.9 | 26.9 | 37.1 |
| | Piacenza | 30.6 | 23.7 | 18.9 | 20.9 | 38.2 |
| | Porto | 25.0 | 19.9 | 16.1 | 16.9 | 35.8 |
| | Sevilla | 0.104 | 0.061 | 0.036 | 2.52 | 65.4 |
| | Thiva | 8.22 | 4.726 | 2.77 | 7.54 | 66.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Agri4cast met files | Châteaudun | 5.56 | 4.01 | 2.91 | 6.70 | 47.7 |
| | Hamburg | 33.3 | 26.1 | 20.8 | 21.1 | 37.4 |
| | Jokioinen | 0.71 | 0.57 | 0.459 | 0.89 | 35.4 |
| | Kremsmünster | 24.6 | 19.0 | 15.0 | 13.8 | 38.9 |
| | Okehampton | 40.1 | 33.1 | 27.6 | 28.8 | 31.3 |
| | Piacenza | 26.2 | 19.9 | 14.7 | 17.4 | 44.1 |
| | Porto | 17.5 | 12.9 | 9.36 | 10.3 | 46.4 |
| | Sevilla | 0.091 | 0.06 | 0.04 | 1.21 | 56.0 |
| | Thiva | 0.106 | 0.051 | 0.025 | 1.37 | 76.5 |

# 5.5 Conclusions

Two experiments were conducted in this chapter, with the aim of the first being to determine which climatic input has the strongest effect on plant uptake, the second being to see how PEARL-simulated leaching obtained with updated climate files compared with equivalent runs but using the original FOCUS climate files. It also investigated whether the effect of plant uptake changes within this. Data from the first experiment shows that plant uptake was tested against all climatic inputs with $T_{MAX}$ and $ET_{REF}$ being the strongest drivers, both inputs have a p-value <0.001. Within the FOCUS file, the Penman-Monteith method is used for calculating potential evapotranspiration and therefore $ET_{REF}$ is calculated using many of the other climatic inputs. Despite both $ET_{REF}$ and $T_{MAX}$ showing strong relationships with $U_p$, there were also times when they had less effect on the plant uptake, this was due to low levels of transpiration caused by reduced amounts of soil moisture available to the plant roots which led to plant water stress.

The second experiment showed that there was no significant difference between the leaching of a scenario with PUF = 0 and the scenarios where PUF = 1, suggesting that there wouldn't be a greater effect of plant uptake on leaching despite projected increases in temperature. There was, however, a significant difference between the simulated leaching as produced by PEARL using the FOCUS climate files (1992 – 2002) or the Agri4cast climate files when using the same FOCUS soil data. The runs driven with the more recent Agri4cast climate files (1999 – 2018) showed a reduction in the simulated leaching levels when compared with FOCUS climate driven runs. Although there is a slight temporal overlap between the two datasets, this study provides a strong case for the need to update the climate files as often as possible to allow environmental fate modellers to present the most up to date leaching data. This work can be built on with a look towards future climate predictions, to see how leaching of pesticides is predicted to change in future climates.

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## 5.7 Appendix A

To allow for the comparison of the difference between the original FOCUS climate files and the newly defined Agri4cast climate files, a boxplot of the yearly means have been added within this appendix. A separate plot has been given to each location to show the variability of the changes within this. This is because, there is variation in the comparison of the two datasets, with some clearly increasing, some keeping similar values and others decreasing. Neither climatic input shows a clear pattern across locations. The levels of leaching are likely to be affected by several the climatic inputs, so an interaction of the meteorology is also likely.

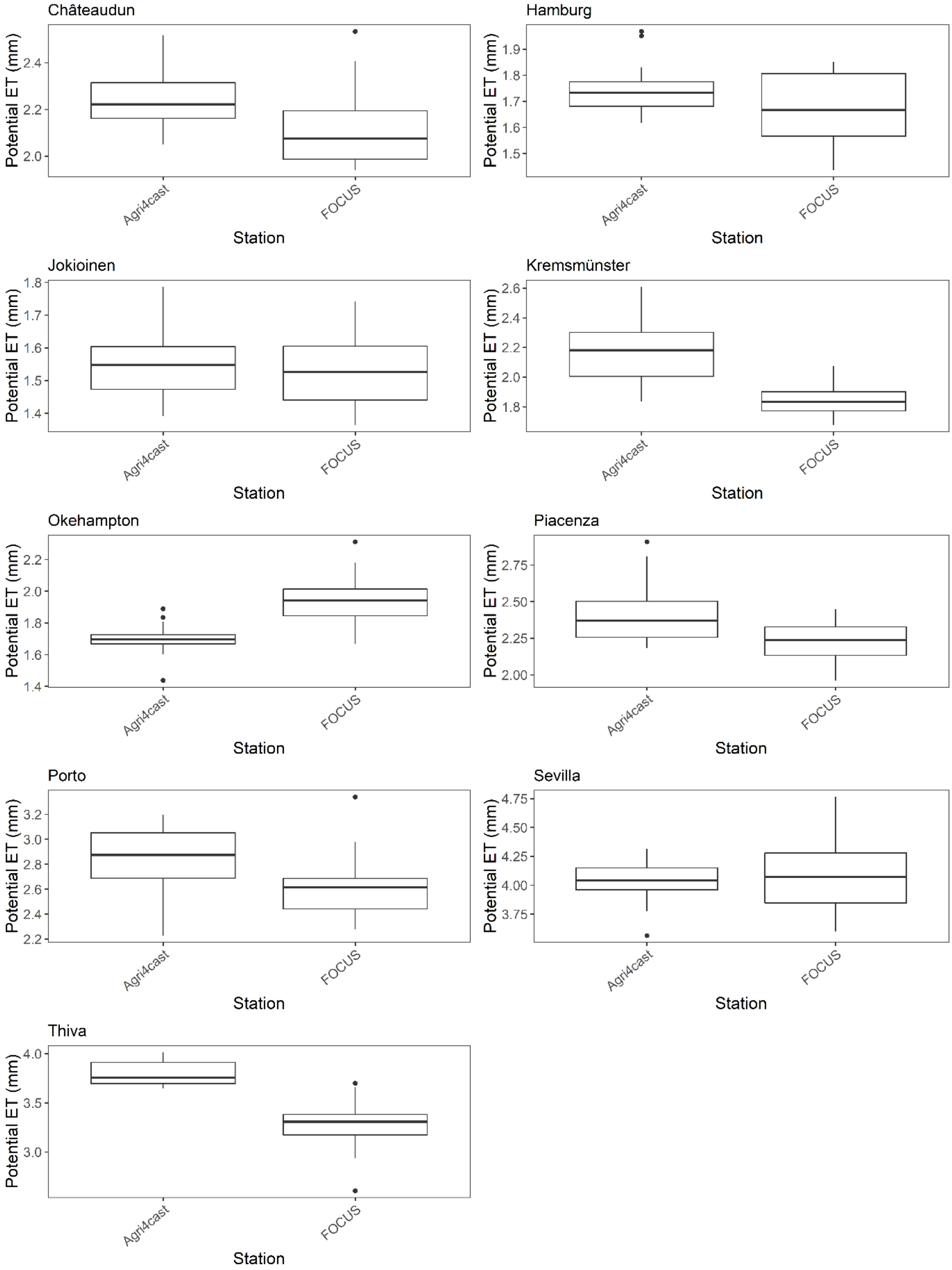

**Figure 5.9. Boxplots of the potential evapotranspiration to allow for comparison between the Agri4cast and FOCUS climate files. Each plot shows a different location.**

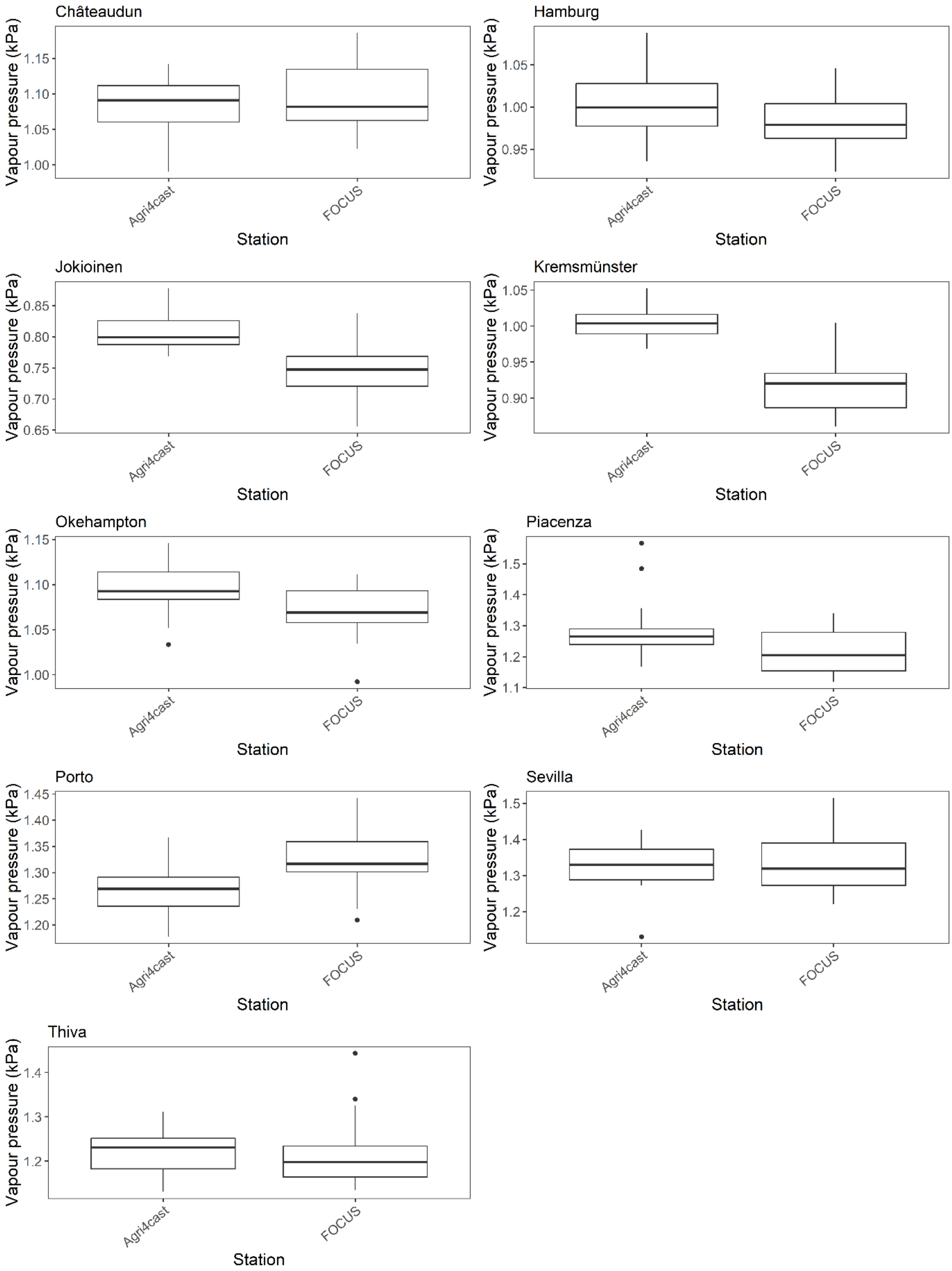

**Figure 5.10. Boxplots of the vapour pressure to allow for comparison between the Agri4cast and FOCUS climate files. Each plot shows a different location.**

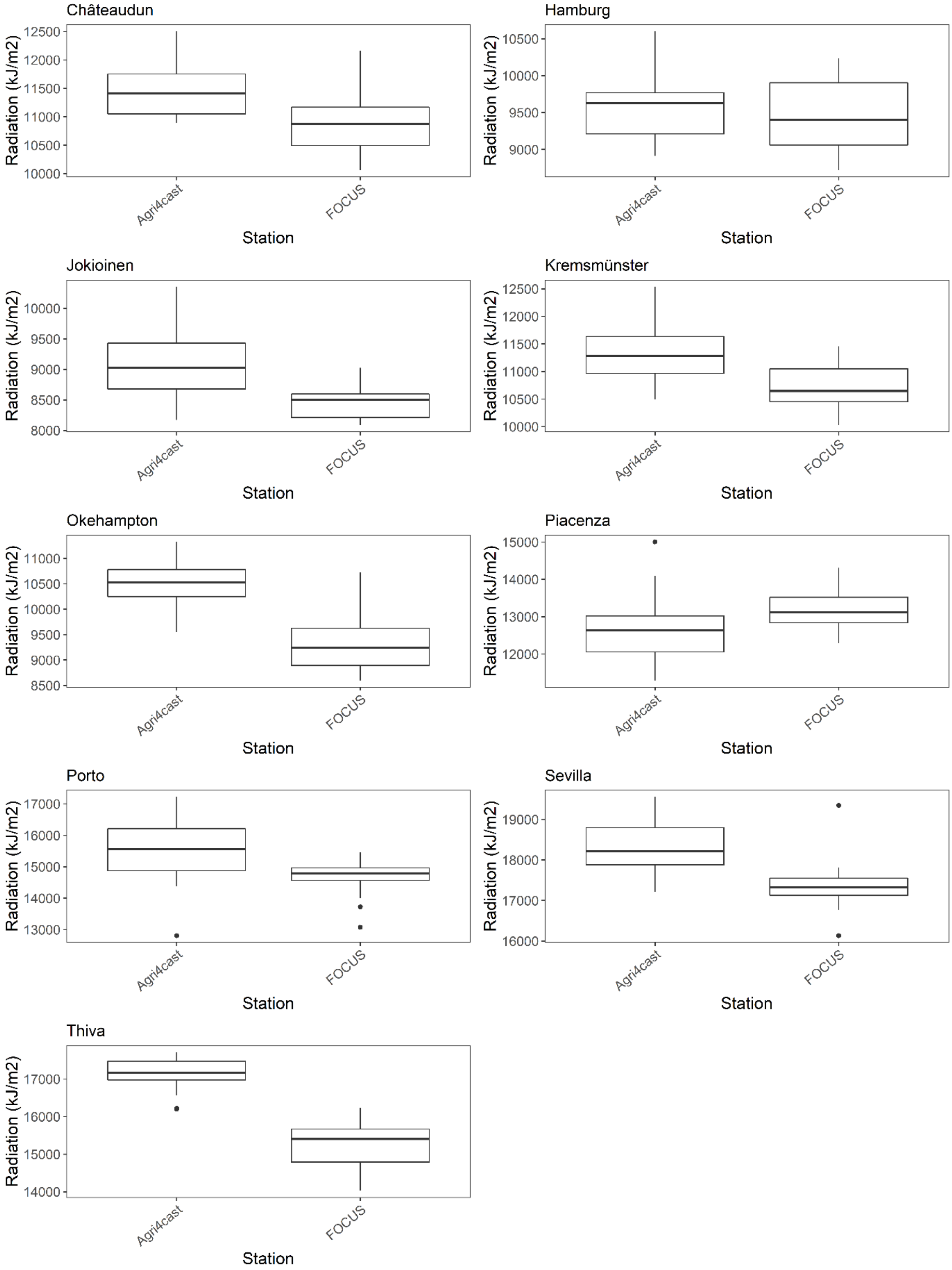

**Figure 5.11. Boxplots of the radiation to allow for comparison between the Agri4cast and FOCUS climate files. Each plot shows a different location.**

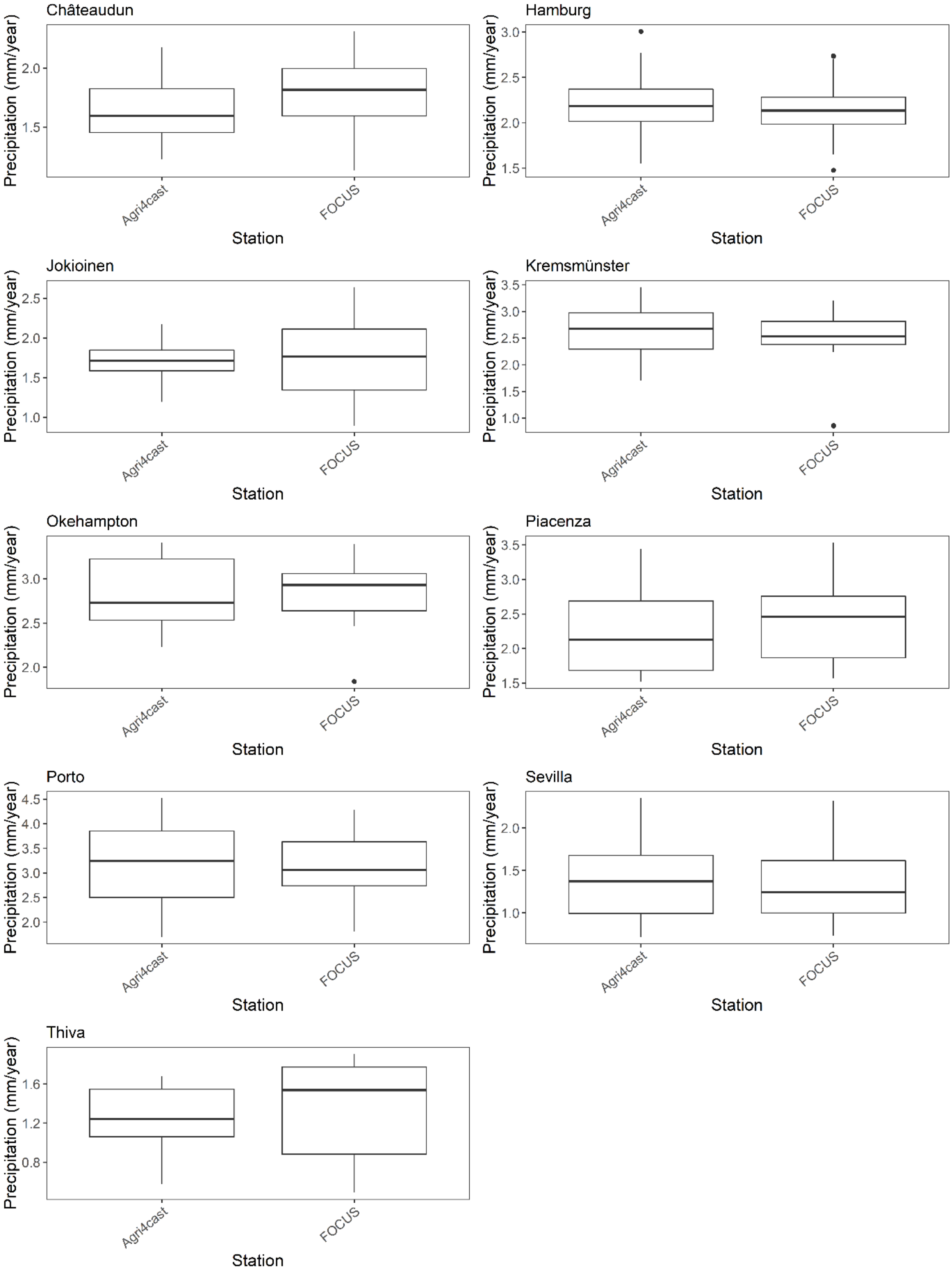

**Figure 5.12. Boxplots of the precipitation to allow for comparison between the Agri4cast and FOCUS climate files. Each plot shows a different location.**

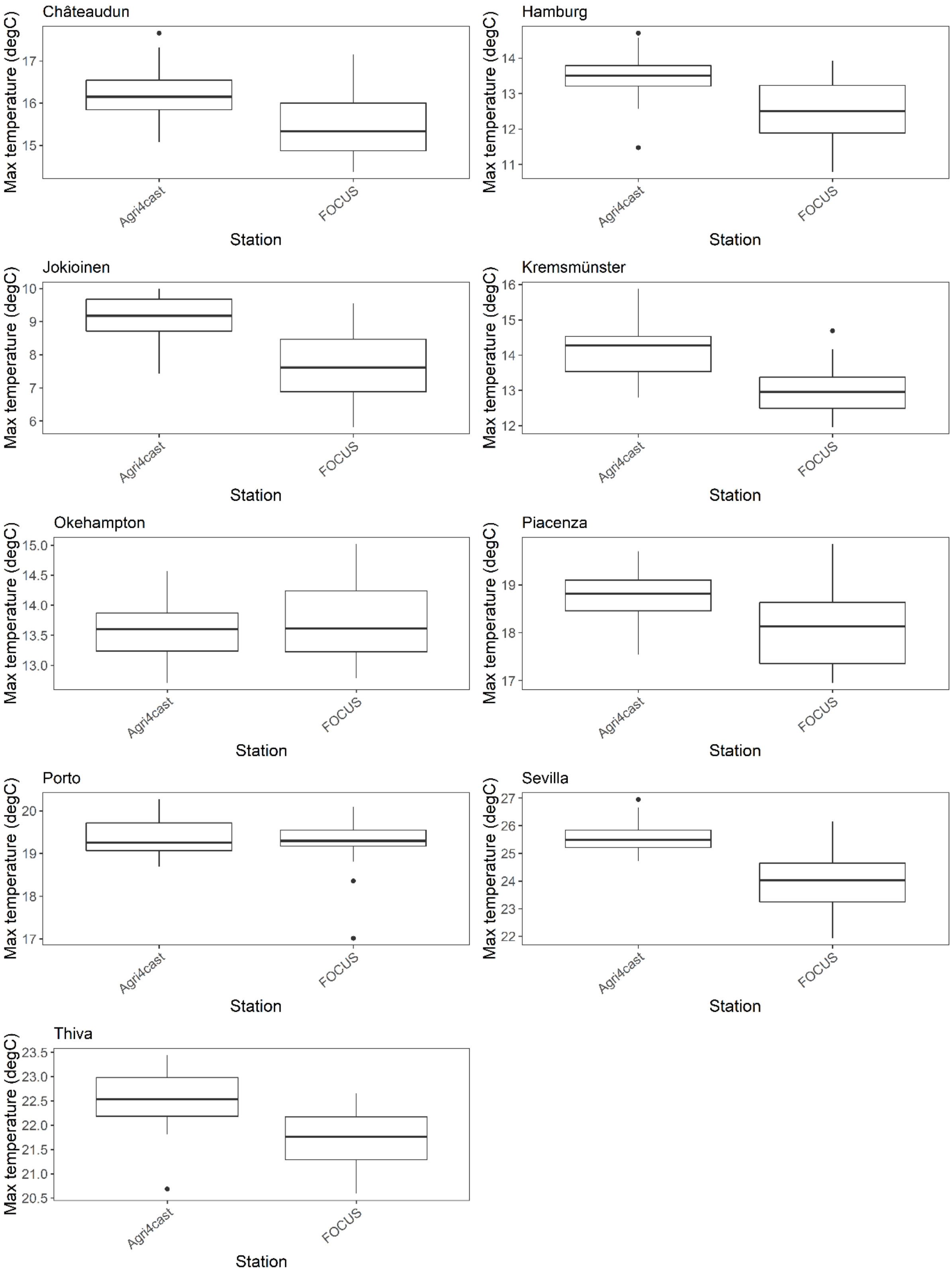

**Figure 5.13. Boxplots of the maximum temperature to allow for comparison between the Agri4cast and FOCUS climate files. Each plot shows a different location.**

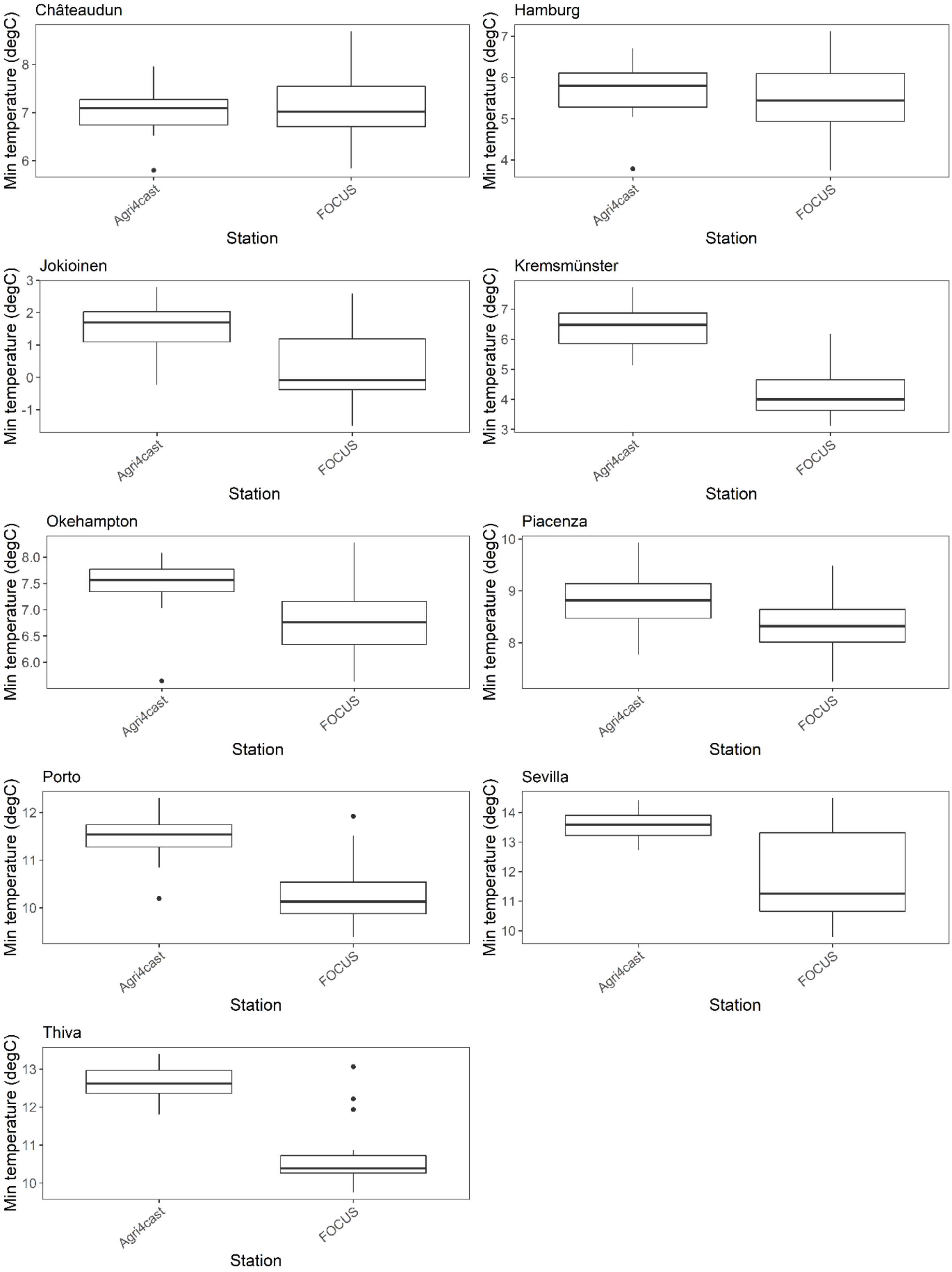

**Figure 5.14. Boxplots of the minimum temperature to allow for comparison between the Agri4cast and FOCUS climate files. Each plot shows a different location.**

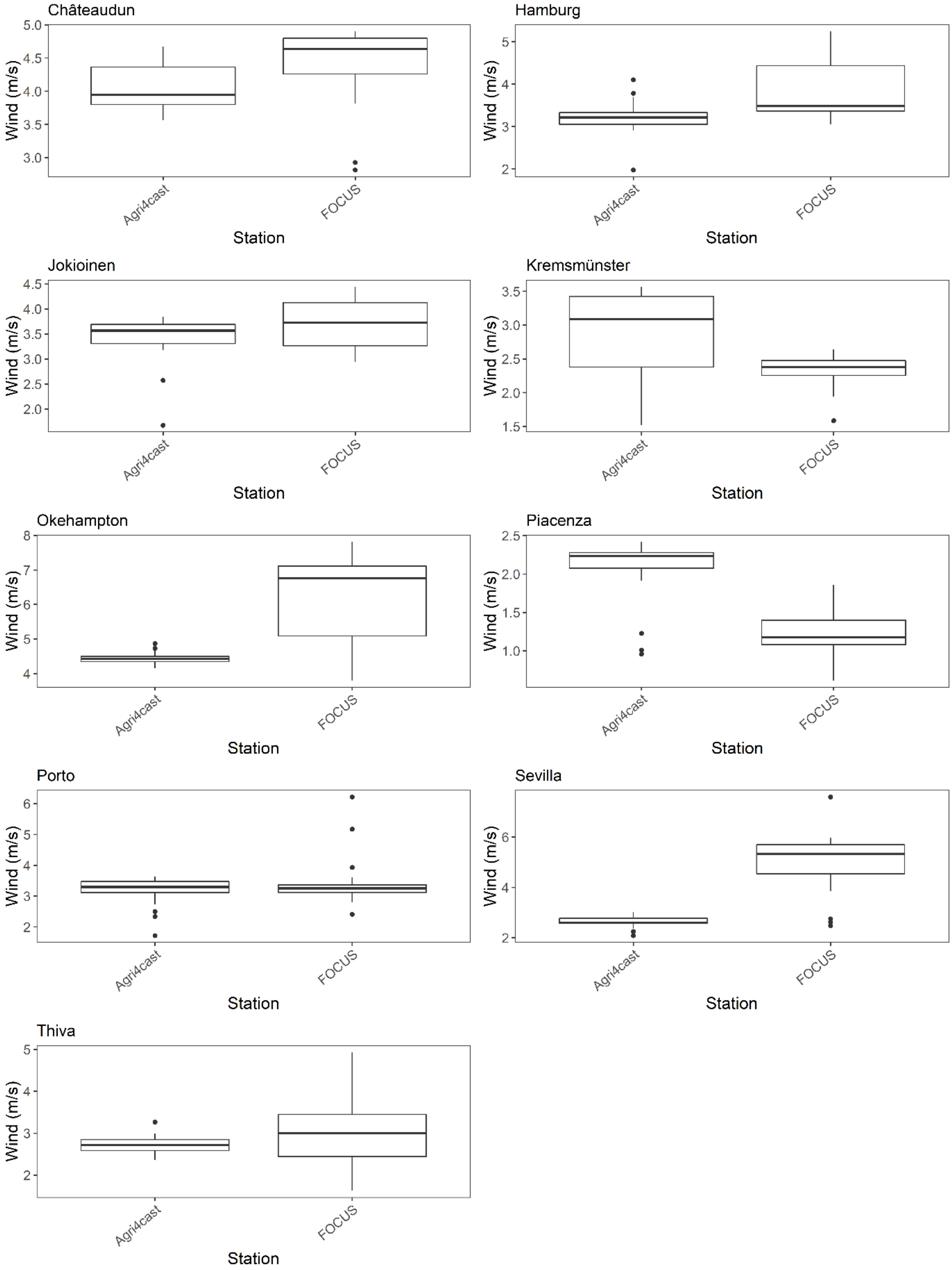

**Figure 5.15. Boxplots of the wind speed to allow for comparison between the Agri4cast and FOCUS climate files. Each plot shows a different location.**

# Chapter 6 – Using ‘typical’ plant uptake scenarios to test the effects of plant uptake on the leaching of pesticides in PEARL

## 6.1 Abstract

Environmental fate is a key issue when it comes to considering the risk posed to groundwater supplies by the application of plant protection products (PPPs) in agriculture. The fate of PPPs following their application is very difficult to measure in the environment and cannot be conduct for compounds that have yet to be declared as safe for release. Therefore, as a part of the regulation process, we rely on environmental fate models to predict the leaching levels of each compound. Currently, assessments are conducted using the FOCUS scenarios, which are described as ‘realistic worst-case’. Plant uptake is a key compound-specific property that has been suggested to significantly affect the levels of leaching into the groundwater. However, when testing the influence of this parameter, the ‘worst-case’ scenarios are being used which could potentially overpredict the effect of plant uptake on compound leaching. This study aims to define an alternative set of scenarios that would represent a ‘typical’ wheat scenario for the most productive regions in Europe with high pesticide sales. These locations were then modelled with three example compounds that were set up to give variation in mobility and persistence of the compounds. Results show that there is some variation in the leaching patterns from these newly defined locations, with high silt locations showing a reduced effect of plant uptake, particularly with the highly mobile compound. Predicted Environmental Concentration in the Groundwater ($PEC_{GW}$) values showed lower levels of leaching when compared to the FOCUS scenarios and a reduced range of percentage change between the high uptake and low uptake scenario. However, there wasn’t a statistically significant difference in the effect of plant uptake within this. Data within this study shows that while plant uptake has been shown to consistently have an effect on the leaching of compounds from the soil profile, there are scenarios where this may not always be the case, and this should be considered when using plant uptake in future assessments.

## 6.2 Introduction

Due to increasing concerns about the potential hazards of plant protection products (PPPs) polluting our natural ecosystems, there has been increasing regulatory interest in pesticide registration and simulation of environmental fate (Wheeler, 2002). The current approach to modelling environmental fate is tiered, with the realism of the environmental systems and processes increasing with the number of tiers. Higher tiered approaches require the highest number of inputs, and hence comprise the most expensive and computationally demanding ways to predict environmental fate (Holdt et al., 2011; Michalski et al., 2004). As with all aspects of modelling, if a less complex approach with fewer inputs to be provided can be used to achieve a result that is not significantly different to a more complex approach, then the simplest approach is recommended (Fryer and Collins, 2003).

Due to the varying regulatory requirements, a few models have been developed to assess pesticide leaching across the world. The main regulatory fate models within the EU are PEARL ver. 4.4.4 (Tiktak et al., 2000), PELMO ver. 5.5.3 (Klein, 2012), MACRO ver. 5.5.4 (Stenemo and Jarvis, 2010) and PRZM ver. 5 (Carsel et al., 1985). PELMO was created and based on PRZM ver. 1 but has been adapted to make it of greater use to regulators in Europe (Klein et al., 1997). As a result, PELMO sees greater use within the EU regulatory framework, although, PRZM is also used sparingly. Largely due to the EU working group, FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe), much research and development has been conducted into these models over the years since their release (FOCUS, 2014). FOCUS was disbanded in 2014, however, their guidance documents cover many of the current issues within environmental fate modelling and are still freely available online (Boesten et al., 2014). Due to many studies into the uncertainty and sensitivity of these models, a lot of scientific interest has been generated into the parameter measuring plant uptake (EFSA, 2013). Variation in nomenclature can mean that this term is sometimes called Transpiration Stream Concentration Factor or Bioconcentration Factor but within this chapter, Plant Uptake Factor shall be used as the general umbrella term for all variations.

Plant uptake is a parameter that has long been discussed within the environmental fate research and regulatory arenas, but knowledge of the behaviour of this parameter within the environmental system is relatively incomplete. This is because it is very difficult to determine the exact concentration of pesticide that a plant root has been exposed to without destructive sampling and disrupting the natural system (Engelhardt et al., 2015). Previous chapters within this thesis have discussed the effect of plant uptake on the outputs from the environmental fate models; however, these outputs were conducted using the FOCUS scenarios. The FOCUS scenarios were set up to represent a 'realistic worst case' for leaching across Europe (FOCUS, 2014). Therefore, it is possible that the effect of plant uptake on the leaching of PPPs within these assessments is different to the effect that would be seen across Europe in the regions where most of the wheat production occurs. This study will use the Eurostat database to determine where are the highest wheat producing regions across Europe. It will combine this information with data on pesticide sales across Europe, and the MARS Agri4cast database to provide climatic data to run the PEARL model. Using PEARL, this study will be conducted over six scenarios and three anonymised compounds. The relative outputs of PEARL will be discussed within the context of variation if plant uptake factor.

## 6.3 Methodology

### 6.3.1 Scenario Selection

The aims of this experiment were to define some new 'typical' scenarios to allow for comparison of the effects of the plant uptake on the concentration of the pesticide leaching to 1m depth ($C_{1m}$). It was decided that a systematic approach would be taken towards the scenario selection to reduce the chance of operator bias. Two European databases were used (Crop production and pesticide sales) to determine the regions with the greatest production and pesticide use.

#### 6.3.1.2 Locations

Although each of the FOCUS models already has the default FOCUS files available upon download. It was decided that the use of 'realistic worst case' scenarios may not represent the typical leaching levels seen across Europe (FOCUS, 2014). Therefore, it is possible that the effect of plant uptake would not be truly represented within a scenario that is designed to represent high leaching. If the . To give a more systematic approach to selection of representative locations, the two Eurostat databases were used to select for the highest wheat producing regions in Europe with the highest pesticide sales: crop statistics (area, production and yield, agr_r_acs) and pesticide sales database (aei_fm_salpest09). The agr_r_acs database is categorised by Nomenclature of Territorial Units for Statistics (NUTS 2) regions. Harvested production was averaged over a 5-year period (2012-2016) for common Wheat and Spelt, with the top 20 producing regions being selected from the database. Once a region was selected from a country that country is then excluded from future selection. The final set of regions were then selected if located within one of the top 10 countries for pesticide sales.

**Table 6.2 Selected regions for the modelling of environmental fate**

| NUTS 2 Region | Crop production (Region rank, without replacement of country) | Pesticide sales in kilograms (Country rank) |
|---|---|---|
| Alfold es Eszak - Hungary | 2,523 (7) | 9,483,759 (10) |
| Bassin Parisien - France | 17,492 (1) | 66,798,227 (2) |
| Bayern - Germany | 4,019 (3) | 45,560,095 (4) |
| Centro - Spain | 3,899 (5) | 72,818,406 (1) |
| East Anglia – United Kingdom | 3,985 (4) | 22,334,905 (6) |
| Macroreguinea Trei – Romania | 2,261 (9) | 11,008,048 (8) |
| Nord-Est - Italy | 1,414 (10) | 60,748,401 (3) |
| Region Polnozny - Poland | 2,372 (8) | 23,996,280 (5) |

**Figure 6.1. Selected locations for analysis mapped onto a map of Europe.**

Figure 6.1 shows that the selected locations for this study cover a wide range in latitude and longitude. Soil data for Germany and Poland were not available using the soil database that was selected for this use and were eliminated at this stage.

#### 6.3.1.1 Selection of crop

This body of work has been designed to be completed alongside experimental laboratory work in designing a test methodology for the measurement of plant uptake from hydroponic solution. During these experiments, Common wheat (*Triticum aestivum L.*) was used due to it being a major crop within European agriculture production. Moreover, it is easy to cultivate in hydroponics. As Eurostat statistics draw no distinction between winter wheat and spring wheat, it was decided that winter wheat would be modelled. This selection increased the chance of high rainfall events shortly after pesticide application (one day prior to emergence) and a longer sedentary period where evapotranspiration losses would be lower, so that leaching was more likely.

**Table 6.1 Growing seasons for selected wheat-growing regions across Europe**

| Location | Emergence date - Harvest |
|---|---|
| Alfold es Eszak (AeE) - Hungary | 15th November – 1st August |
| Bassin Parisien (BP) – France | 1st December – 16th August |
| Centro (C) – Spain | 16th December – 16th July |
| East Anglia (EA) – United Kingdom | 1st November – 16th August |
| Macroreguinea Trei (MT) - Romania | 1st November – 16th August |
| Nord-Est (NE) – Italy | 1st December – 16th July |

### 6.3.1.3 Meteorological data

Agri4cast is a data access portal available via the European Commission Joint Research Centre (EC JRC) to investigate and model the potential effects of anthropogenic climate change on agricultural production. JRC have produced an interpolated gridded agro-meteorological database using data from 4200 Automatic Weather Systems across Europe, to accompany their models and to calibrate their model predictions. Due to this emphasis on agri-environmental applications, many of the weather variables provided by this dataset match to those required by Environmental fate models such as PEARL, PELMO, PRZM and MACRO. The high spatial resolution of the dataset meant that this database was ideal for use in this study. The daily meteorological variables downloaded from this database are: maximum air temperature (°C), minimum Air temperature (°C), mean daily wind speed at 10m (m/s), mean daily vapour pressure deficit (hPa), sum of precipitation (mm), potential evaporation from a bare soil surface, global radiation (kJ/m$^2$/day). The chosen dates ranged from 01/01/1990 to 31/12/2015.

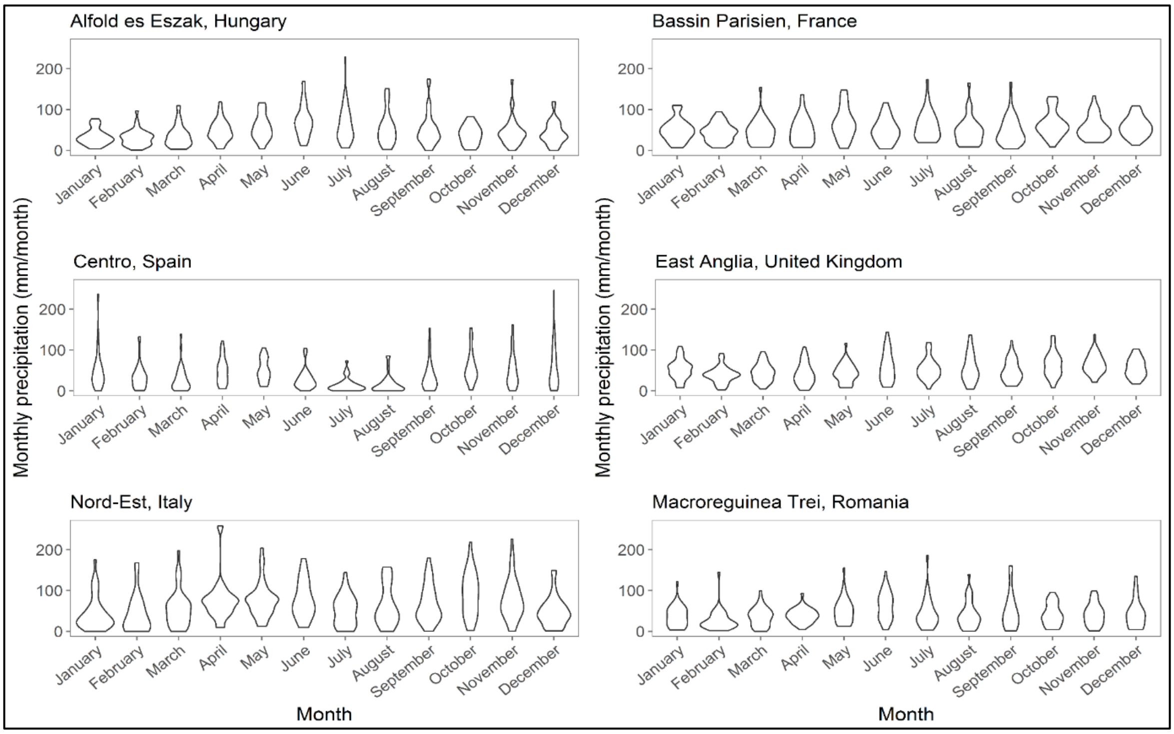

**Figure 6.2. Violin plots of precipitation across the months for each location**

In Figure 6.2, Violin plots depict the kernel density (i.e. the probability density function) of monthly precipitation; the shape of the plot demonstrates the variation and the distribution across the scale. Therefore, the shorter and fatter plots are those that have a smaller range of monthly precipitation values and a higher probability of lower values of precipitation. The longer thinner (e.g. Centro in December and January) violins show high variation in monthly precipitation and higher probability of extreme rainfall events. Plots with a more rhombus shape are those that regularly have a moderate amount of rainfall but show little variation and extremes. East Anglia, U.K. overall appears to be the driest location (Figure 6.2) with very little variation across the months. On the other hand, Centro, Spain shows a lot of variation across the months, with some very dry months in July and August but some particularly wet Januarys and Decembers in some years. Alfold es Eszak in Hungary appears to be the opposite of this, with little variation in rainfall in January and February but particularly wet months in June and July.

### 6.3.1.4 Soil data

Information on soil properties data was obtained using the European Soil Database v2.0. This was established by the European Commission and the European Environment Agency (EEA) to give easier access to soil data across Europe. The key reasons for the selection of this database are the wide geographical range available within the same dataset and the availability of data from several land uses. This meant that that when selecting a location within the soil database, preference was given to the land use and only agricultural soil profiles were selected.

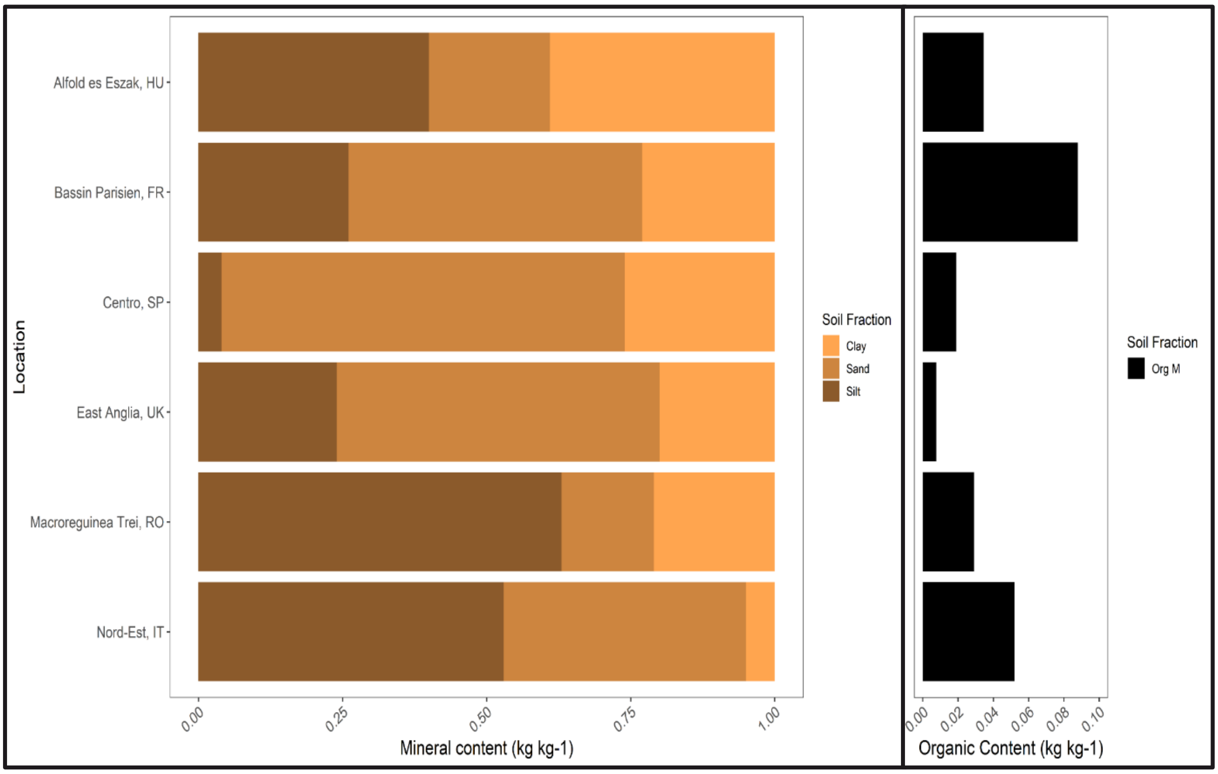

**Figure 6.3. Mineral soil fractions for all modelled locations.**

Figure 6.3a shows the mineral soil component within the topsoil of each location. This should provide substantial variation in the outputs of environmental fate model runs. Figure 6.3b shows the organic matter content (kg kg$^{-1}$) of each location within this study. Bassin Parisien has the highest organic matter content, with East Anglia showing the lowest content.

### 6.3.1.5 Van Genuchten Hydraulic Parameters

To be able to model the hydrological behaviour of a soil profile, the water retention curve and hydraulic conductivity curve are required to provide (i) the relationship between the soil water content and the soil water potential, and (ii) between either of these variables and hydraulic conductivity. The van Genuchten parameters provide the values that determine the shape of the curves, provided by Van Genuchten (1980). Ordinarily the van Genuchten parameters are derived through field and lab assessments, however, this can be a time and labour-intensive study. Also, with modelling studies, it is also not always possible to visit the exact site where the original sample was taken and take further samples for assessment of soil hydraulic properties. This led to the development of Pedo-Transfer Functions (PTFs) to provide an alternative method of obtaining the soil hydraulic curves from the basic soil texture values (e.g., Wösten et al., 1999), see Table 2 for the PTFs used in this study. Although known to not be as accurate as a direct assessment, they do provide a readily available parameter value and can be useful for initial estimates for a large number of sites, catchments or regions (Liao et al., 2014).

**Table 6.3. Pedo-transfer functions used (and related literature reference) to calculate the van Genuchten parameters for all locations within the study. Silt, Sand, Clay and OrgC represent the mineral and organic content of each soil and these were substituted for the value of each component when running the equation.**

| Van Genuchten Parameter | Equation | Reference |
|---|---|---|
| Dry Bulk Density (ρ) | $\rho = 0.99915 - 0.08415 * \log(Silt) + 0.07712 * \log(Clay) + 0.09371 * \log(Sand)$ | (Martín et al., 2017) |
| Theta Sat ($\theta_{sat}$) | $\theta_{sat} = 0.81 - (0.283 * \rho) + (0.001 * \mathrm{Clay})$ | (Vereecken et al., 1989) |
| Theta Res ($\theta_{res}$) | $\theta_{res} = 0.015 + 0.005 * Clay + 0.014 * OrgC$ | (Vereecken et al., 1989) |
| Alpha (α) | $\alpha = \exp(-14.96 + (0.03135 * Clay) + (0.0351 * Silt) + (0.646 * OrgM) + (15.29 * \rho) - (0.192 * TopSoil) - (4.671 * \rho^2) - (0.000781 * Clay^2) - (0.00687 * OrgM^2) + (0.0449/OrgM) + (0.0663 * log(Silt)) + 0.1482 * log(OrgM)) - (0.04546 * \rho * Silt) - (0.4852 * \rho * OrgM) + (0.00673 * TopSoil * Clay$ | (Wösten et al., 1999) |
| Lambda (λ) | $\lambda = 0.0202 + (0.0006193 * Clay^2) - (0.001136 * OrgM^2) - (0.2316 * \log(OrgM)) - (0.03544 * OrgM * Clay) + (0.00283 * \rho * Silt) + (0.0488 * \rho * OrgM)$ | (Wösten et al., 1999) |

| | | |
|---|---|---|
| Ksat | $K_{sat} = 7.755 + (0.0352 * Silt) + (0.93 * TopSoil) - (0.967 * \rho^2) - (0.000484 * Clay^2) - (0.000322 * Silt^2) + (0.001/Silt) - (0.0748/OrgM) - (0.643 * \log(Silt)) - (0.01398 * \rho * Clay) - (0.1673 * \rho * OrgM) + (0.02986 * TopSoil * Clay) - (0.03305 * TopSoil * Silt)$ | (Wösten et al., 1999) |
| n | $n = 1.0 + \exp(-25.23 - (0.02195 * Clay) + (0.0074 * Silt) - (0.1940 * OrgM) + (45.5 * \rho) - (7.24 * \rho^2) + 0.0003658 * Clay^2) + (0.002885 * OrgM^2) - (12.81/\rho) - (0.1524/Silt) - (0.01958/OrgM) - (0.2876 * \log(Silt)) - (0.0709 * \log(OrgM)) - (44.6 * \log(\rho)) - (0.02264 * \rho * Clay) + (0.0896 * \rho * OrgM) + (0.007818 * TopSoil * Clay))$ | (Wösten et al., 1999) |

### 6.3.1.6 Example compounds

To contrast the mobility and persistence of a compound at each location, three example compounds were input into the model with only two physical-chemical properties being altered. Table 6.4 is reproduced from Chapter 4 to aid the reader with referring back to these properties if needed. The range of $K_{OM}$ and DT50 were selected to represent a wide range of properties that would be observed in true PPPs. These parameters were $K_{OM}$ (the partition coefficient between organic matter and water) and DT50 (the time taken to for the compound to degrade to half the original concentration).

**Table 6.4. Physical-chemical properties of the modelled compounds that were varied to alter the mobility and the persistence.**

| Compound | $K_{OC}$ (L kg$^{-1}$) | DT50 (days) |
|---|---|---|
| CA | 10 | 100 |
| CB | 100 | 40 |
| CC | 500 | 500 |

Appendix A shows a full break down of the parameterisation of the scenarios and the inputs supplied to the model with a justification for the selection provided. As the example compounds were not based on real PPPs, some of these parameters were selected to be the same as those observed with the FOCUS example compounds as this would provide a parameter value that would fit with those selected for compounds proposed for registration.

### 6.3.2 PEARL 4.4.4

As the methodology for PEARL was discussed at length in Chapter 4 (Heading#) it will not be reiterated here, however the plant uptake equation has been reproduced as this is specifically relevant to this chapter and allows for the reader to find this information easily (Equation 6.1)

$$U_p = R_{u,L} * PUF * C_L \tag{6.1}$$

Where $R_{u,L}$ is is the volumic volume rate of water uptake (-), PUF is the Plant Uptake Factor, $C_L$ is the concentration of pesticide within the liquid phase (Tiktak et al., 2000).

### 6.3.3 Hydrological balance

Many the hydrological input events throughout the year, come from irrigation rather than rainfall (Figure 6.4). Within PEARL, the irrigation applied is weekly and is continued until the soil profile is recharged back up to field capacity. Therefore, soils that drain more quickly will receive higher irrigation inputs than other locations.

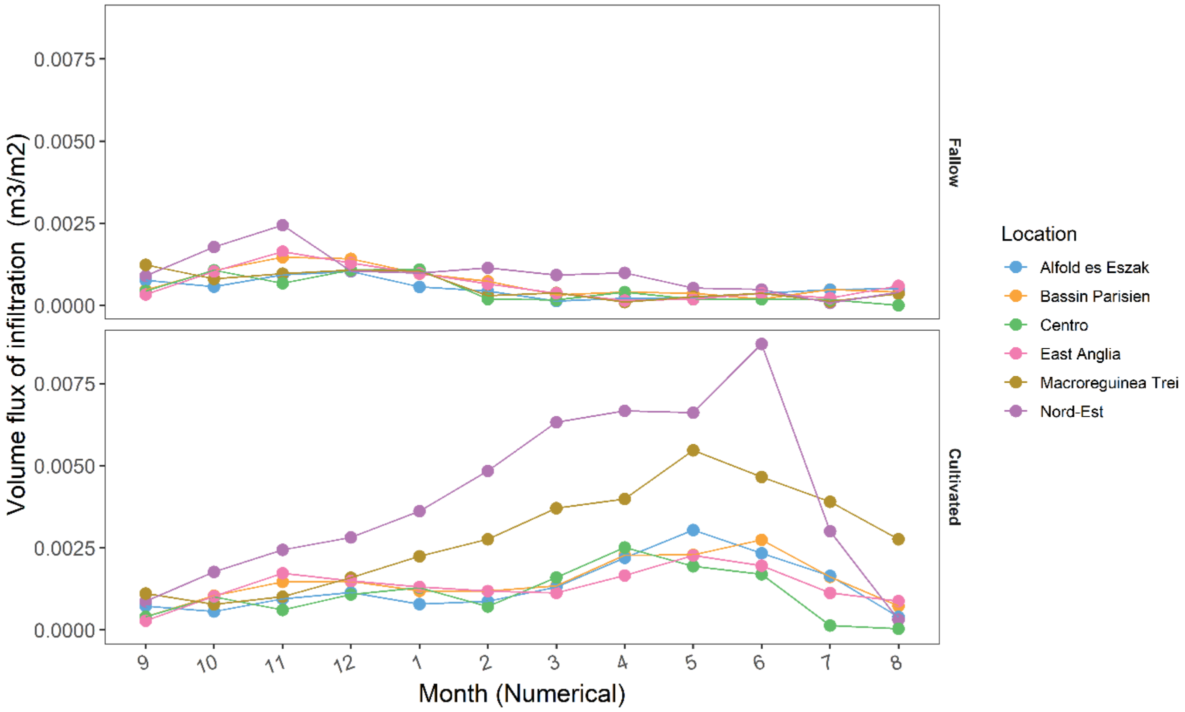

**Figure 6.4 Average infiltration of water entering the upper boundary each month across all locations. The top facet is the fallow scenario without irrigation and the lower facet contains the cultivated scenarios with both rainfall and irrigation as inputs. For reference, 0.005 $m^3$ $m^{-2}$ is equivalent to 5 mm.**

As the soils are recharged up to field capacity each week, there are only minor changes over time at most locations with regards to the soil moisture content (Figure 6.5). However, there are some locations which show a marked difference between the fallow and cultivated locations, such as Macroreguinea Trei and Nord-Est. These locations are those with large portions of silt and sand in their mineral content, meaning that their water retention capabilities are reduced compared to some of the other locations with a higher clay content. These locations with coarser-textured soils show a much higher soil moisture content in the spring and summer months in the cultivated runs when compared with the fallow scenario (Figure 6.5). This is due to their high requirement of irrigation which is also obvious from their higher rates of infiltration (Figure 6.4).

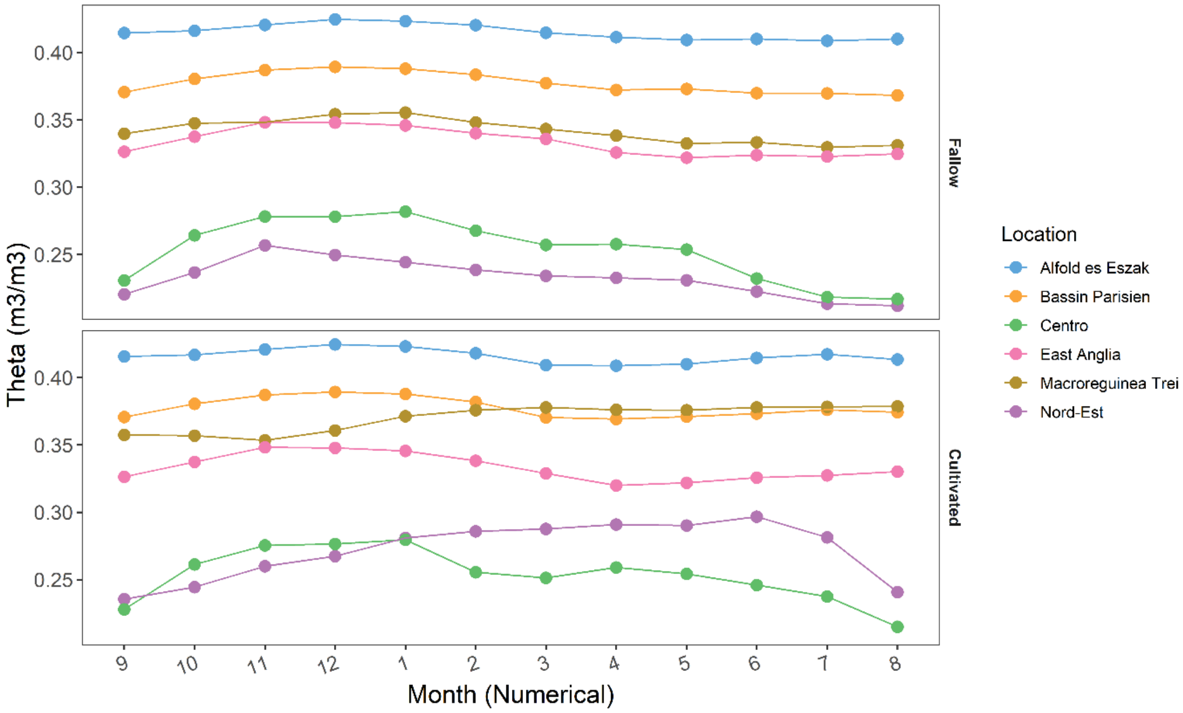

**Figure6.5. The soil moisture content of all locations presented as a mean value across all months.**

## 6.3.4 Statistical analysis

A linear mixed model analysis was conducted on this dataset containing the mean concentration of pesticide leaching below 1m ($C_{1m}$) value across the years (average across 20 years dataset) to show the effect of the compound, PUF and Month on $C_{1m}$. Each location will be treated as a random effect and the data will not be considered as a time series between years but across the years.

# 6.4 Results and Discussion

## 6.4.1 Concentration of pesticide at 1m depth

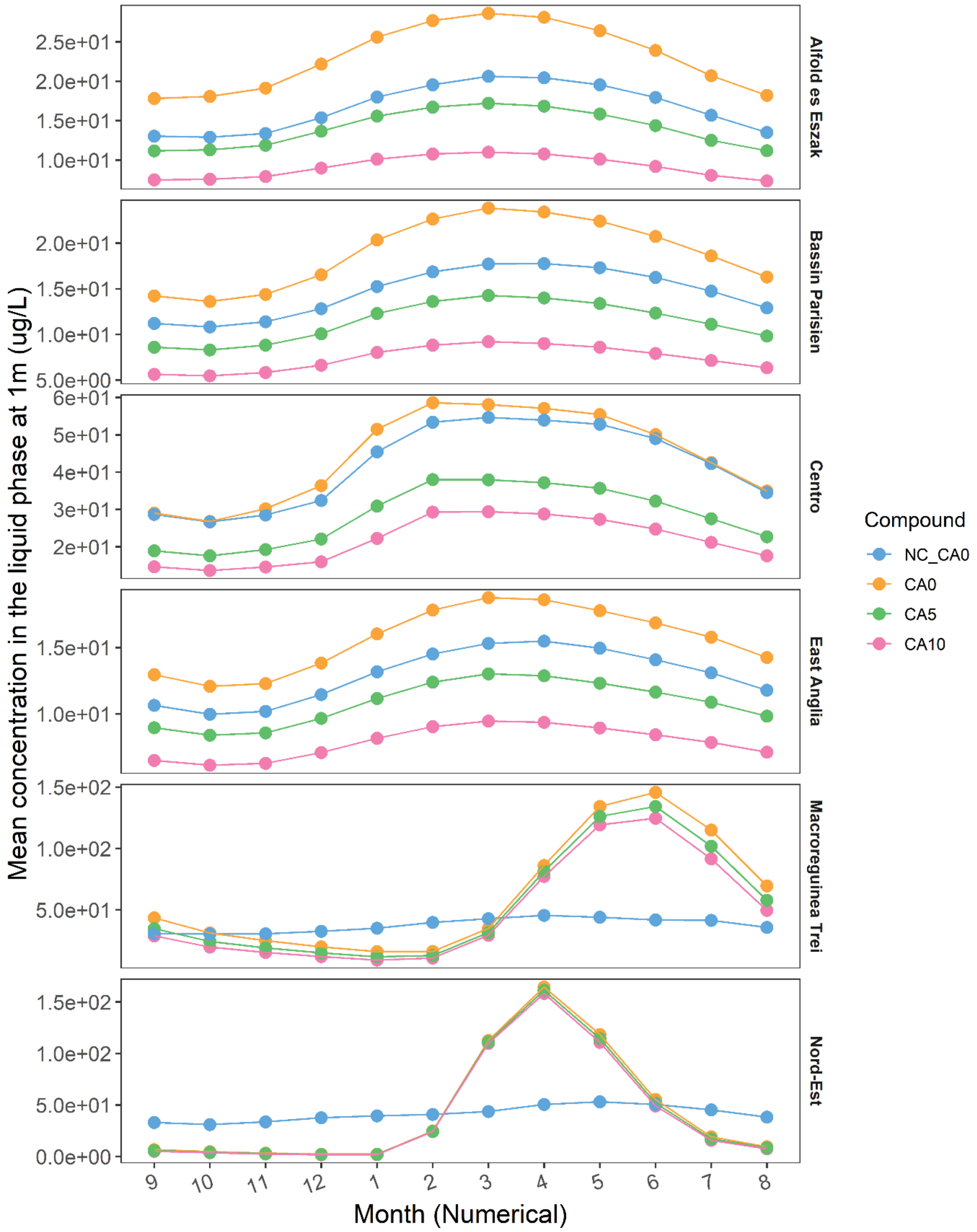

Figure 6.6. Concentration in the liquid phase at 1m depth for Compound A. With each location represented within a facet and each plant uptake scenario being represented by different colours. Each plant uptake scenario is given a different colour and labelled with an acronym in

**the legend: NC_CA0 = No crop, Compound A with PUF = 0; CA0 = Winter Wheat, Compound A with PUF = 0; CA1 = Winter Wheat, Compound A with PUF = 0.1; CA5 = Winter Wheat, Compound A with PUF = 0.5; CA10 = Winter Wheat, Compound A with PUF = 1.0**

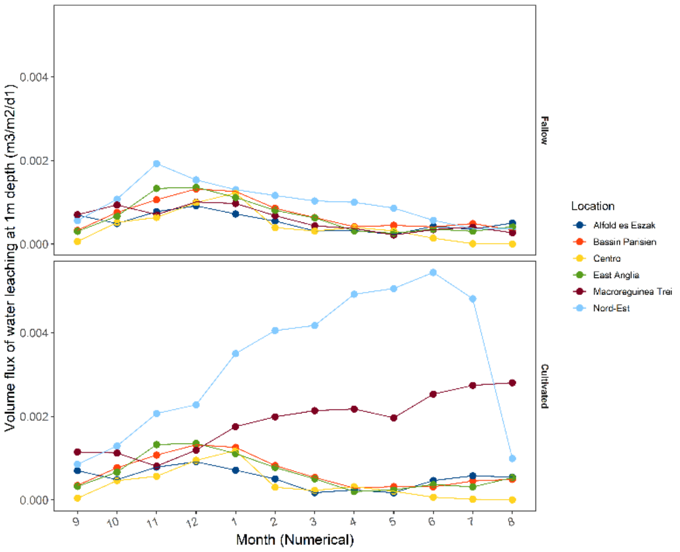

**Figure 6.7. Volume flux of water leaching out of the system over time. The plot is facetted by scenario and coloured by location. The plot is facetted by scenario with the 'Fallow' scenario on the top and the 'Cultivated' scenario at the bottom. Data points are coloured by location.**

For compound A (Figure 6.6), most of the locations have very similar shapes, that is, an increase in leaching in the few months following pesticide application, with the peak in leaching being reached in around March and April, followed by a steady decrease in the months following. Macroreguinea Trei (MT), Romania, and Nord-Est (NE), Italy, appear to show a slightly different shape to the other locations. NE has very low leaching levels over the autumn and winter months but sees a sharp rise in February and March, reaching a peak in April, before slowly declining through May, June and July and reaching low levels in August and remaining at approximately the same level of leaching rate for the remainder of the year. MT exhibits a similar shape except that is demonstrates a slow decline in leaching rate until January followed by a sharp rise through March, April, and May, before reaching a peak in June and a decline back through to January. MT and NE have medium-to coarse textured (Sandy Clay Loam and Sandy Loam, respectively). Within these locations, the difference between the high plant uptake scenario and low plant uptake scenario appears to be smaller than seen in the other 4 locations, with the difference between the lines being almost indistinguishable in Figure 6.7. For Macroreguinea Trei and Nord-Est, the fallow scenario also exhibits much lower leaching levels than that other locations. Alfold es Eszak (AeE), Bassin Parisien (BP) and East Anglia (EA) show a reasonable spread of leaching across the year, with the expected pattern of CA0 leaching the highest mass of compound (no plant uptake) and CA10 showing the lowest concentration. For the top 4 locations (AeE, BP, C and EA) the fallow scenario appears somewhere between the 0 PUF and 1 PUF scenarios, whereas for Macroreguinea Trei and Nord-Est the Fallow scenario (NC_CA0) exhibits a much different shape and a more constant course compared to the peak seen in the cultivated scenarios.

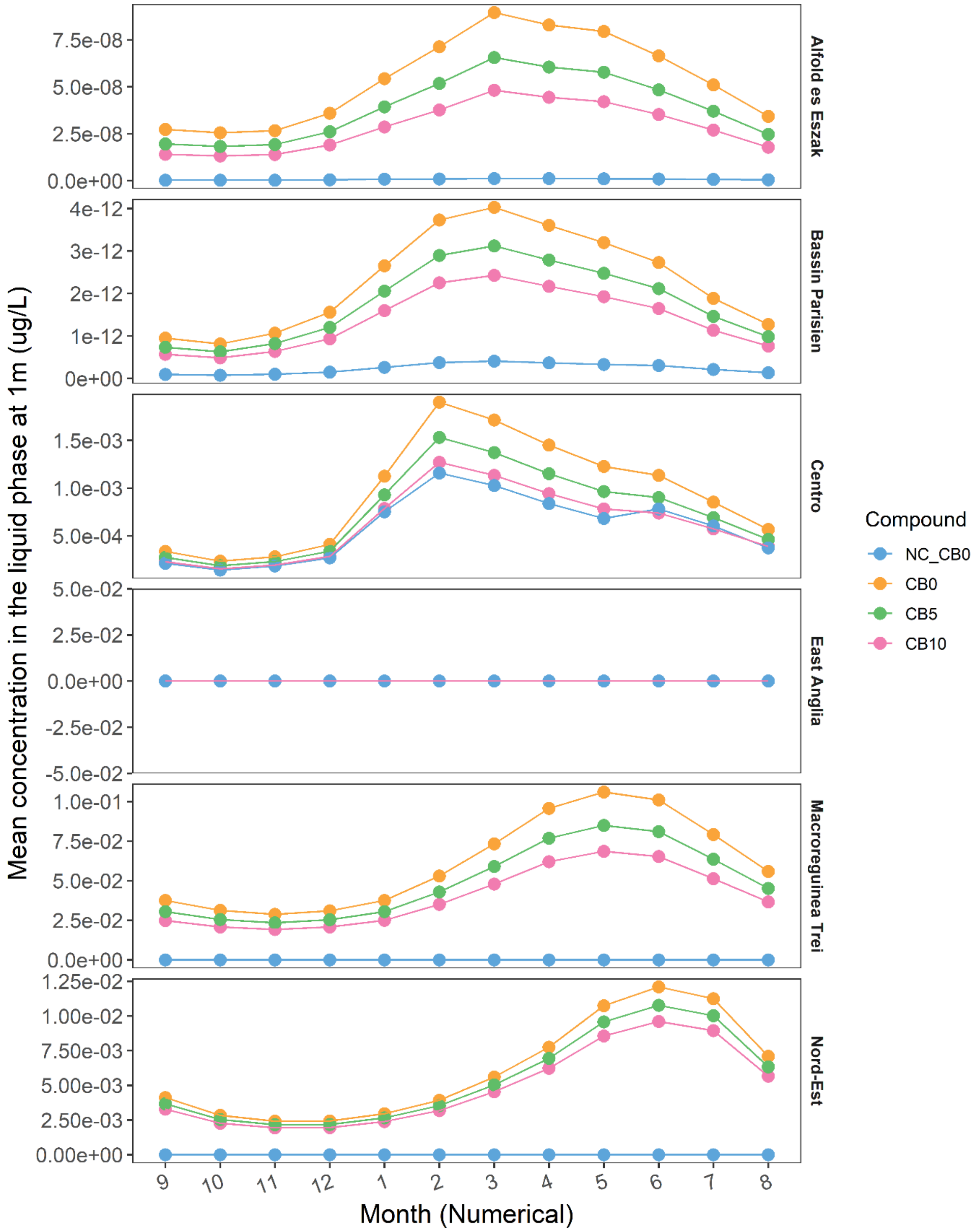

**Figure 6.8. Concentration in the liquid phase at 1m depth for Compound B. With each location represented within a facet and each plant uptake scenario being represented by different colours.**

Compound B has a $K_{OM}$ of 100 which is higher than Compound A (10) but a shorter DT50 of 40, compared to 100. AeE and BP demonstrate a similar pattern to the one observed for compound A (see Figure 6.8), with the effect of plant uptake becoming slightly more marked. Whilst Centro shows a peak in concentration at a similar time as AeE and BP, the peak is much sharper with a rise in January and February and a more gradual drop for the rest of the growing season. MT and NE show a much later peak, although a similar trend to the other locations. The fallow scenario for these two locations has a markedly different shape of leaching, possibly owing to less irrigation, meaning that the highest input of water occurs in the winter months. It should be noted that although some of the patterns within the Compound B results are quite similar to those presented for Compound A, the concentration from Compound B is at a much lower order of magnitude. For compound B (Figure 6.8), no leaching was predicted to occur in East Anglia, United Kingdom and therefore no pattern can be described.

Compound C is expected to be even less mobile, with a $K_{OM}$ value of 500. However, the DT50 for this compound is 500 which is much higher than those for Compound A and B with half-lives of 100 and 40, respectively and hence this compound is more persistent. The seasonal evolutions of Concentration in the liquid phase at 1m depth (Figure 6.9) for Macroreguinea Trei and Nord-Est once again show similar shapes to those presented in Figures 6.7 and 6.8; i.e., a much flatter shape compared to the other locations with the difference between the fallow and cultivated scenarios being more marked. It should also be noted that leaching from Compound C is higher than Compound B for all locations and scenarios but not higher than what was found for Compound A. There are not any major differences between the leaching patterns for Compound C compared to Compound B. Centro shows a much more pronounced effect of plant uptake in the months of December and January.

As mentioned, the aim of this chapter was to compare the response of PEARL when the fate of three different compounds with varying chemical and physical properties was modelled. These compounds were designed to simulate varying levels of sorption and persistence within the soil profile. The aim of this study was not to suggest new scenarios to supplant those used by and proposed by FOCUS but to test a 'more typical' scenario against the 'realistic worst case' (Boesten et al., 2009). Within the FOCUS scenarios (Chapter 4) there was a good spread of leaching and the $C_{1m}$ was relatively consistent with much smaller peaks the typical scenarios in this chapter. The concentrations reported in this chapter show sharper peaks with generally lower levels of concentration at 1 m depth for all compounds. The leaching of Macroreguinea Trei and Nord-Est, particularly for Compound A, show a much different to those in the FOCUS scenarios. For these locations there was a much-reduced effect of plant uptake for all compounds when compared with the other locations. This is likely due to their high mineral content of silt and sand, meaning that water leaches through the system much faster, which results in a much higher input of irrigation. Note that the work conducted with the FOCUS scenarios did not include irrigation and this is perhaps a reason why this effect was not seen in that study.

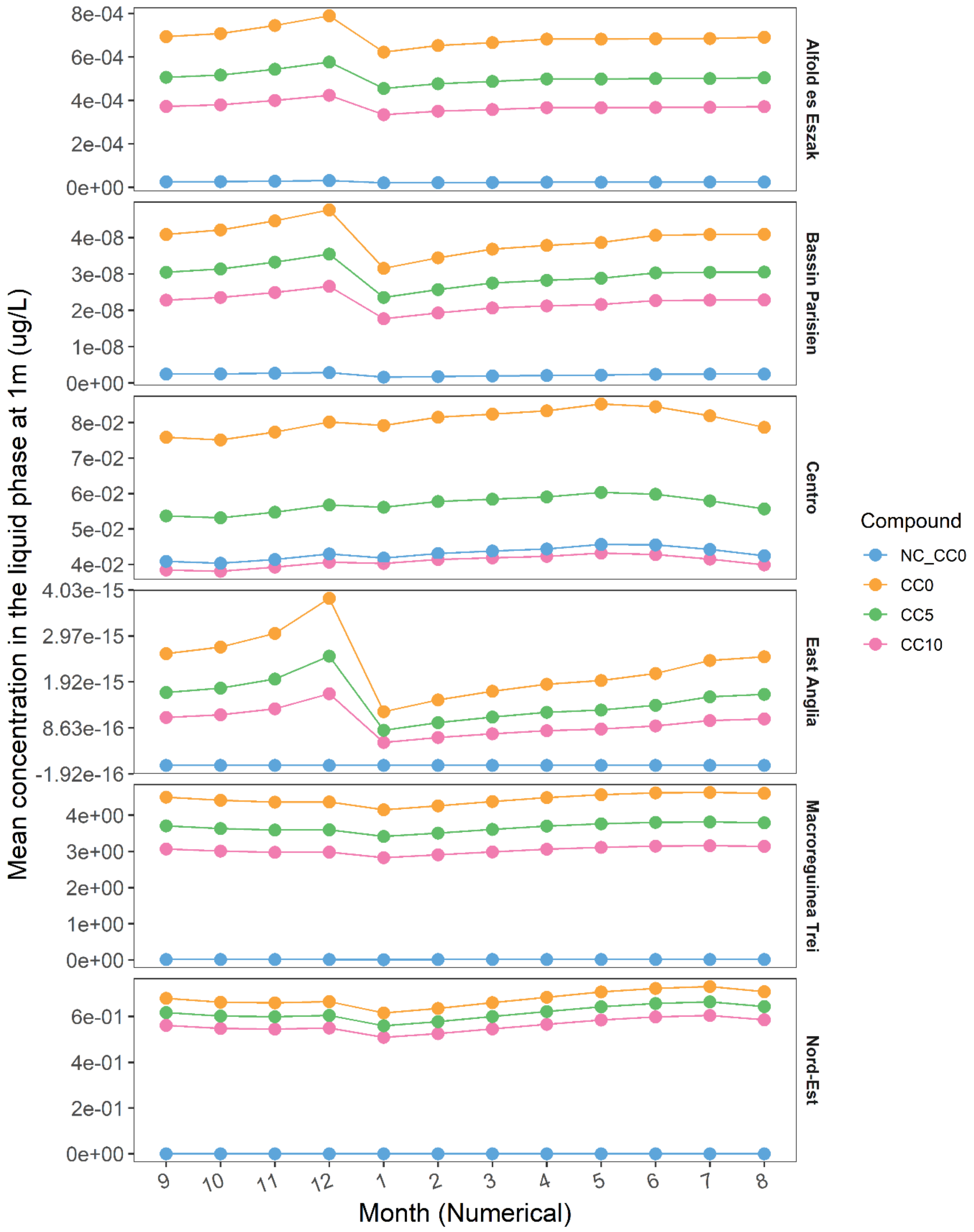

**Figure 6.9. Concentration in the liquid phase at 1m depth for Compound C. With each location represented within a facet and each plant uptake scenario being represented by different colours.**

### 6.4.2 Plant Uptake of Compounds from Soil

Plant uptake is calculated as the relationship between the concentration of the pesticide in the soil solution, the transpiration of solution by the plant roots and the PUF values (Equation 6.1). Therefore, although the concentration in the profile may be high, the plant growth stage or the meteorological conditions means that transpiration is low and high uptake is not observed. The PUF was varied between 0-1 within this chapter

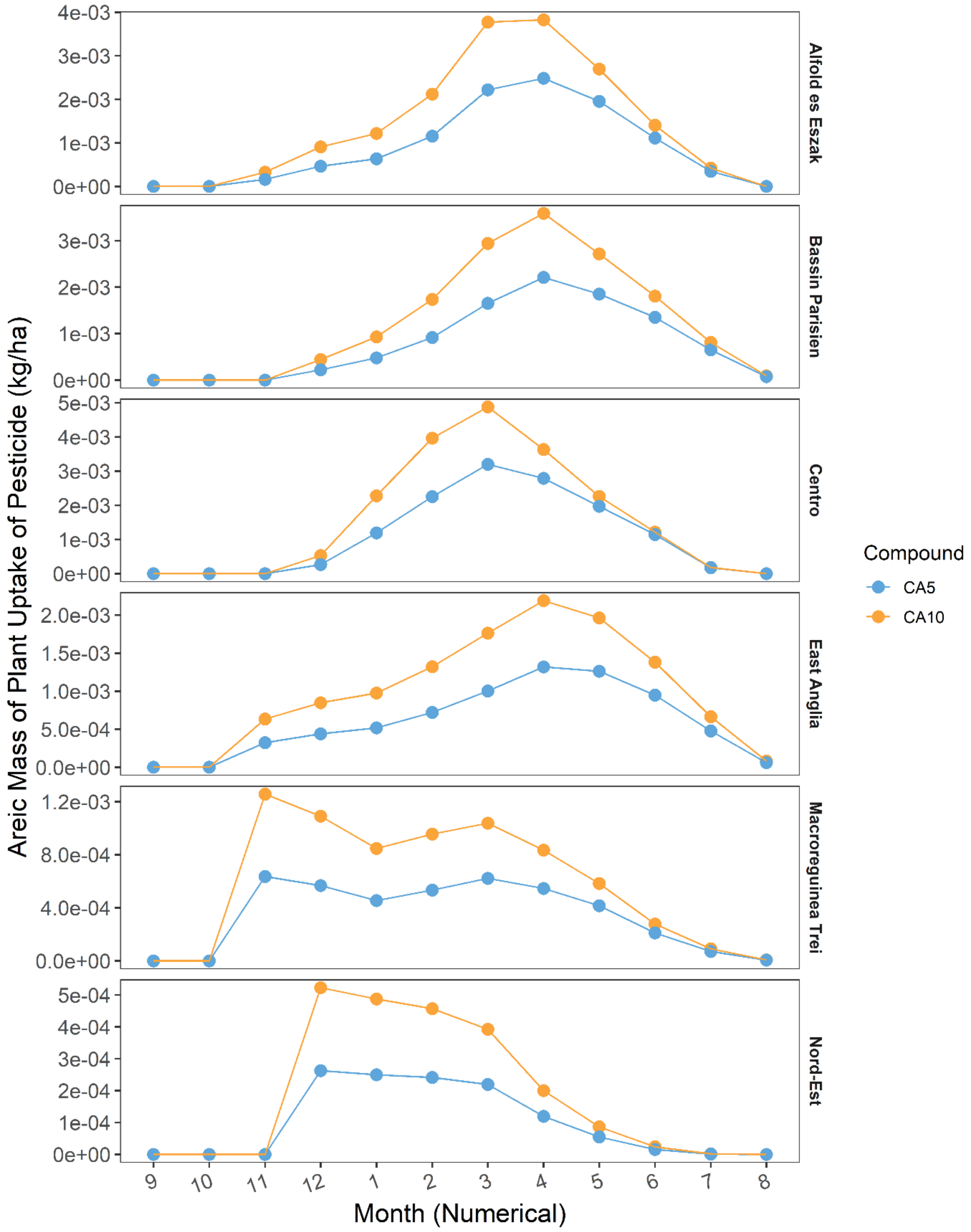

**Figure 6.10. Plant uptake of Compound A. Plot facetted by location and coloured by PUF, with CA5 denote a PUF of 0.5 and CA10 a PUF of 0.5.**

Compound A has the lowest $K_{OM}$ of the 3 compounds analysed within this study. Therefore, this compound should be the most available within the soil profile for plant uptake due to having a higher concentration within the soil profile. Plant uptake is described by Equation 6.1 and is therefore directly proportional to the Plant Uptake Factor, Concentration in Liquid and Volume of water transpired by the plant. Macroreguinea Trei shows high uptake (Figure 6.10) during the early months after the beginning of the growing season (1st November); this is due to high concentrations following application and a high transpiration rate. Nord-Est has a very similar pattern of uptake with large amounts being abstracted by the roots during the early growing season, although the early transpiration rate is much lower than N-E and MT. Although the transpiration rate increases to values that rank the third highest of all locations in May (Figure 6.12), MT and N-E show the lowest concentration by this time (Figure 6.11). Like the results concerning the simulated amounts of leaching out of the profile, Nord-Est and Macroreguinea Trei show very similar levels of plant uptake and the concentration of the dissolved pesticide within the soil pore water is consistently lower than for the other locations. Alfold es Eszak and Bassin Parisien show very similar shapes of plant uptake, with Bassin Parisien showing the highest levels of uptake for compound A. Bassin Parisien does not show very high levels of transpiration, it consistently has a higher concentration of the compound within the soil pore water. Therefore, the highest levels of compound concentration within the soil pore water of the upper layers are simulated to occur during the period shortly after application and this is when the compound will be most accessible to the plant roots (Figure 6.11).

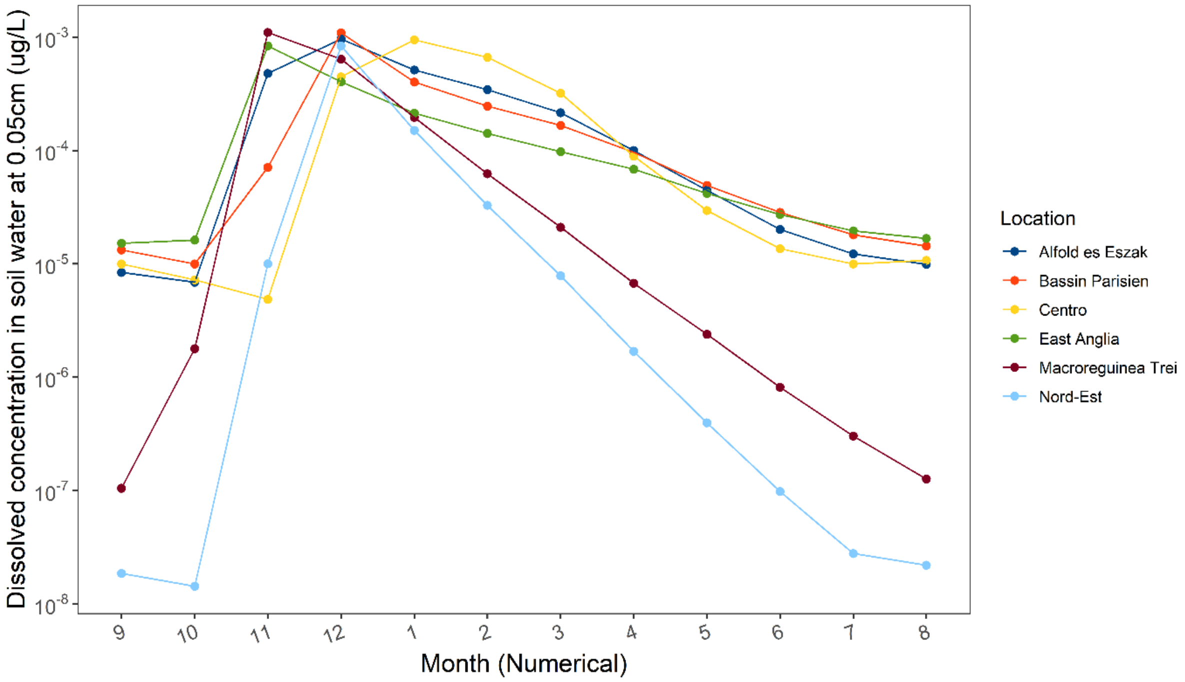

**Figure 6.11. Concentration for Compound A in the soil water at 0 cm on a logarithmic scale. Points and lines are coloured by location**

As shown by Figure 6.12, the highest levels of transpiration shortly after the application period are by East Anglia and this explains the slightly positive skew to the shape of the curve. Although the concentration of the compound within the profile remains relatively high, the plant roots do not take up high levels of pesticide due to the much lower transpiration rate than the other locations, meaning that the relative uptake remains low.

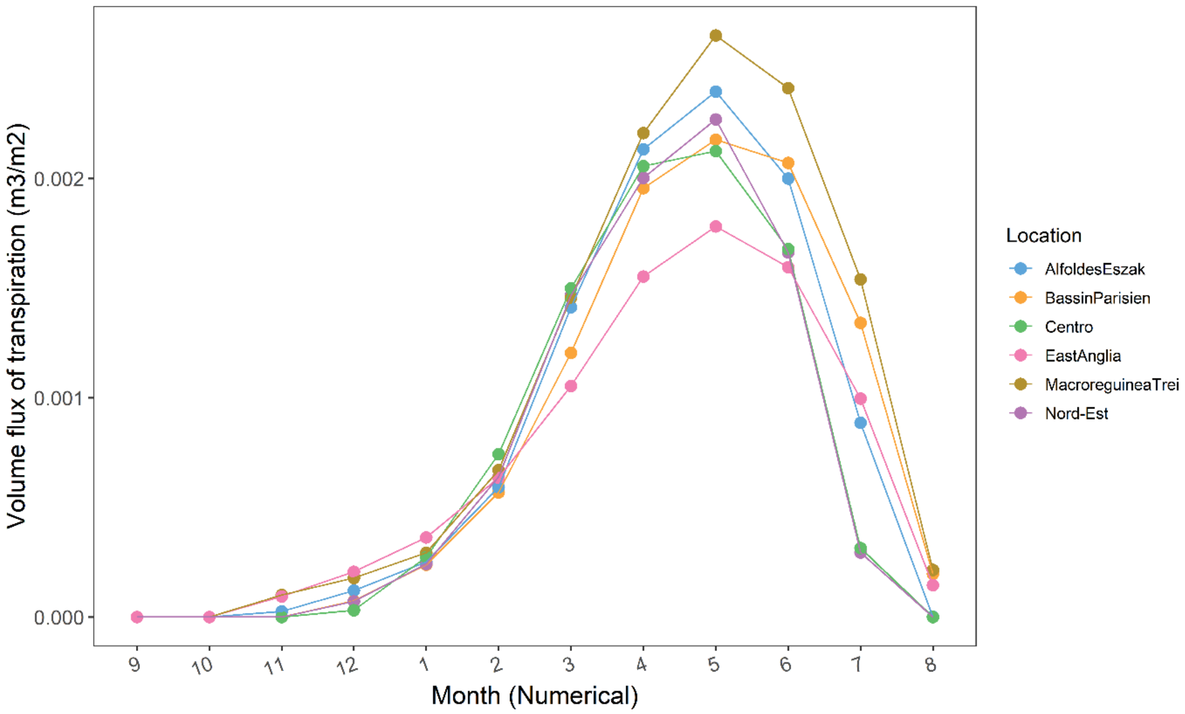

**Figure 6.12. Transpiration across the growing season.**

As expected, the amount of uptake for Compound B (Figure 6.13) is reduced compared to Compound A, as a result of its higher $K_{OM}$. Alfold es Eszak, Bassin Parisien and Centro show remarkably similar levels of uptake, albeit with slightly different patterns depending on the concentration within the profile and the rate of transpiration. East Anglia shows a slightly more exaggerated skew than for Compound A, with similar levels of uptake in the early growing season and until peak levels are reached during the later spring and early summer.

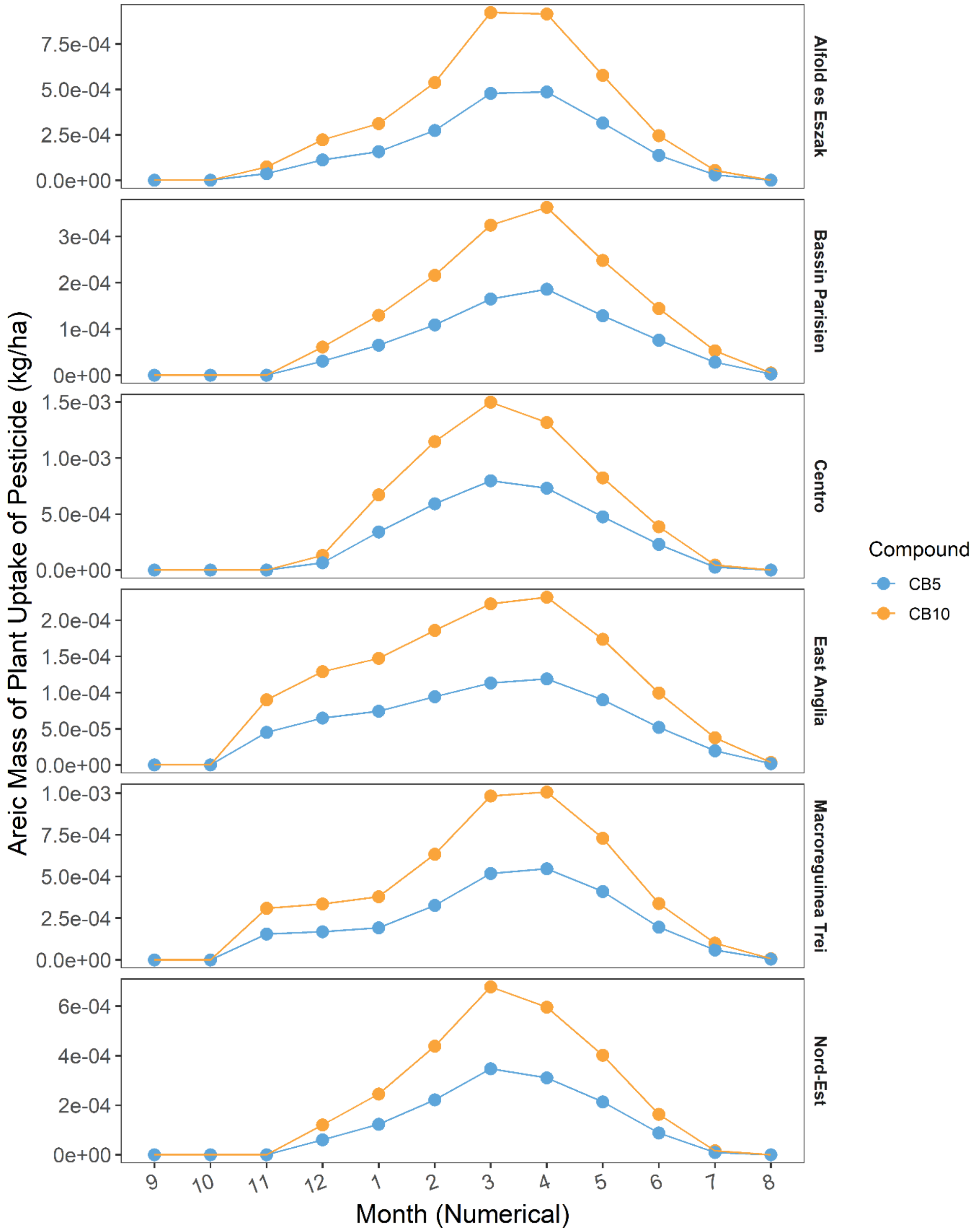

**Figure 6.13. Plant uptake of Compound B. Plot facetted by location and coloured by uptake.**

Although Compound B has a higher $K_{OM}$ than Compound A, the DT50 is much shorter, therefore although it is much more mobile it does not persist as long in the profile. Consequently, this compound does not show as high a persistence as would be expected of a compound with a $K_{OM}$ of 100 (Figure 6.14). For all locations, the occurrence of highest concentration within the soil pore water is immediately after the application of the compound; simulations for MT and NE show very similar concentrations, and AeE and C display similar behaviour also. East Anglia maintains a more constant concentration throughout the year more so than the other locations, exhibiting a shallower negative gradient than AeE and C. This is probably due to the low transpiration rate and temperatures at EA, leading to lower losses from the profile. Concentration of compound B at BP also shows a shallow negative gradient, however, this location also shows one of the highest levels of plant uptake.

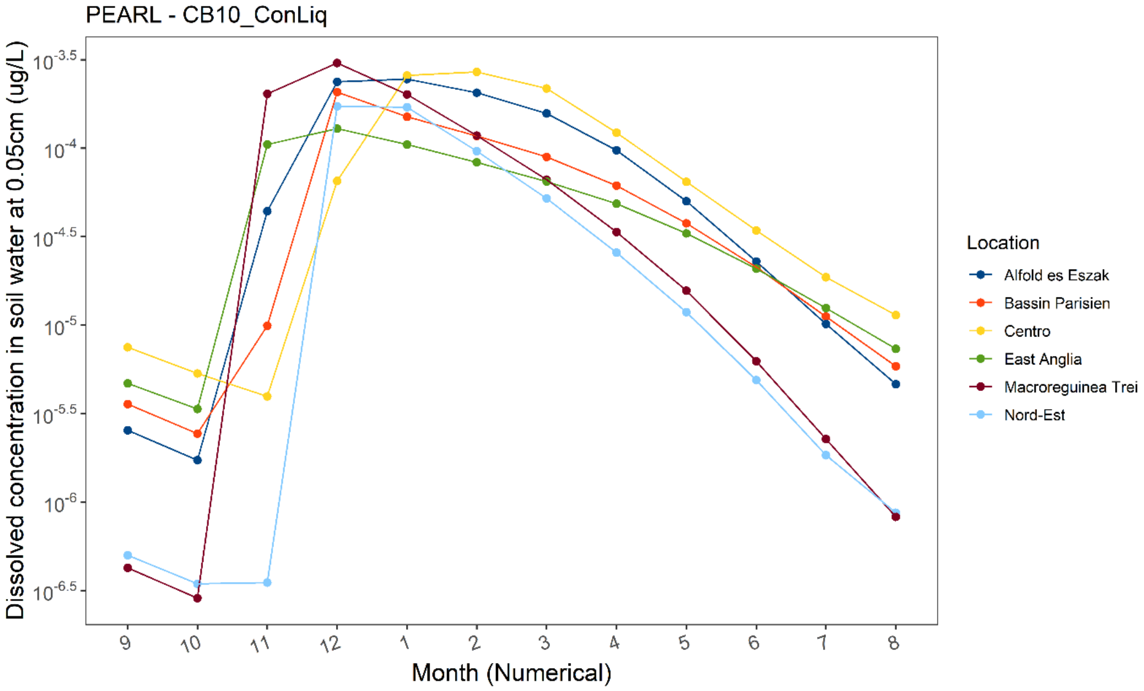

**Figure 6.14. Concentration for Compound B in the soil water at 0 cm on a logarithmic scale. Points and lines are coloured by location**

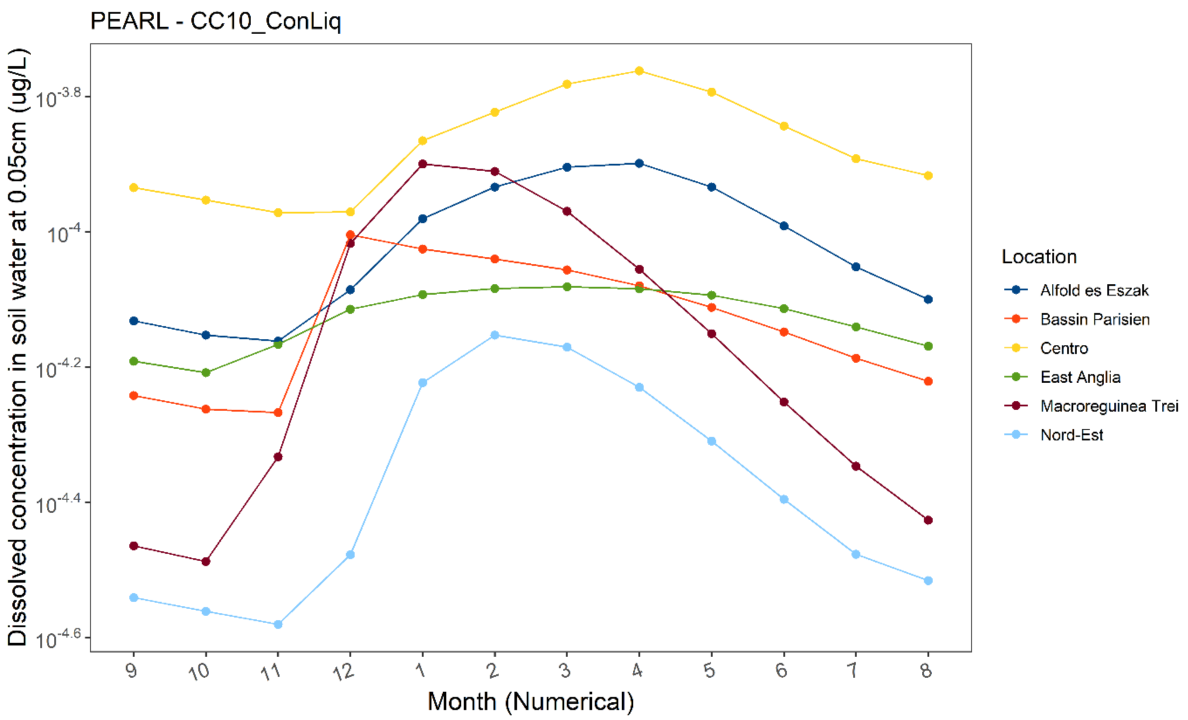

**Figure 6.15. Concentration for Compound C in the soil water at 0 cm on a logarithmic scale. Points and lines are coloured by location**

Contrary to the other compounds, for most locations Compound C does not show the peak concentration in the soil pore water immediately after application to the soil (Figure 6.15), with locations such as Centro, East Anglia and Alfold es Eszak peaking in the months following. The difference between the sandier locations (MT and NE) and the others becomes more pronounced for Compound C (Figure 6.16). For this compound the DT50 is the longest of all three modelled compounds and therefore, although the higher $K_{OM}$ means that the compound is not as available. The increased persistence within the profile means that the plant uptake is higher than for Compound B but smaller than Compound A.

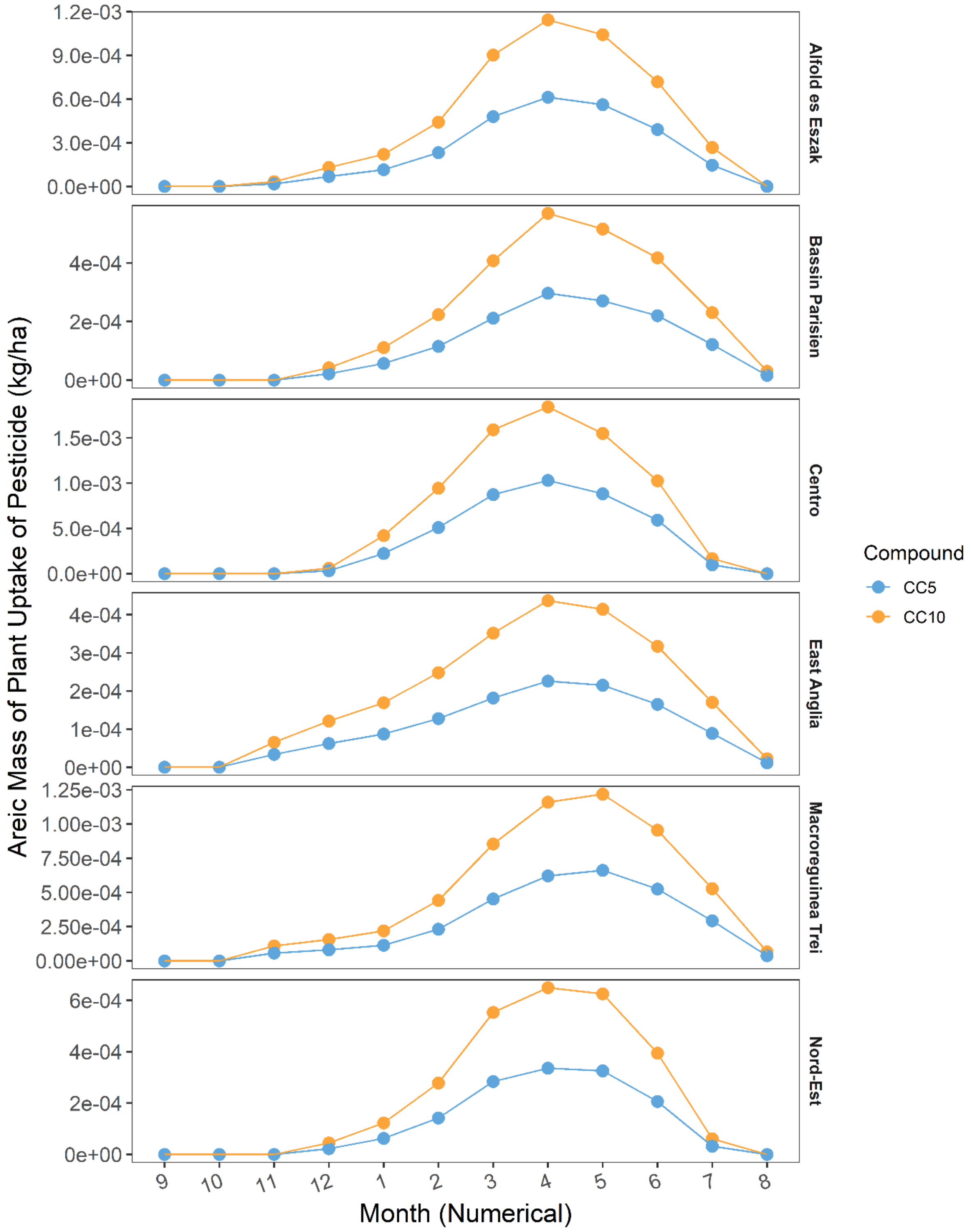

**Figure 6.16. Plant uptake of Compound C. Plot facetted by location and coloured by compound.**

There have been several studies into the sensitivity of environmental fate models and the effect of this on the simulated leaching of compounds from the soil profile. Within these studies the plant uptake factor does not feature heavily, however it has come to prominence more recently since manipulation of PUF has been being suggested as a method to reduce pesticide mobility within the profile. PEARL simulations predict a bell-shaped uptake pattern across the growing season, with low levels of uptake at the beginning of the season and higher levels in the spring and then reduced levels in the summer when the plants reach maturation prior to harvest.

### 6.4.3 Predicted Environmental Concentration in the groundwater (PEC$_{GW}$)

Current model regulatory advice is that PUF is set to 0 for all compounds, unless they are known to be systematic, in which case a value of 0.5 is entered. As there is still much debate about the most suitable test system for determining the PUF value to be supplied to environmental fate models, it is important for an assessment to be carried out to determine the effects that uncertainty could have on the prediction of pesticide fate by these models.

The only previous known study of this kind was conducted by EFSA PPR panel where 3 FOCUS substances were compared across 2 locations using PEARL. A key table among their outputs shows the PEC$_{GW}$ (Predicted Environmental Concentration in the Groundwater, µg/L) of each scenario with the PUF values that were used in the model simulations being 0,0.5, and 1. For each scenario a single PEC$_{GW}$ value is derived, which is the 80th percentile of the mean concentration within the soil pore water at a depth of 1m. There is a short discussion of the effect that the plant uptake factor has on the model outputs, which is deduced to be between 24-43% (EFSA, 2013). Our study has conducted an analysis of 5 different plant uptake values across 6 different locations within PEARL. A similar PEC$_{GW}$ table has been added here for the PEARL results obtained within this study (Table 5) to allow for comparison with the EFSA study. The range of percentage change between the cultivated plant uptake is 0-60.2%.

**Table 6.5 Impact of plant uptake factor on the calculated PEC$_{GW}$ (µg/L) using newly defined 'typical' scenarios and compounds used within this paper. Compound A: KOM = 10, DT50 = 100; Compound B: KOM = 100, DT50 = 40, Compound C: KOM = 500, DT50 = 500.**

| | Compound A | | | | Percent change |
|---|---|---|---|---|---|
| **Location** | 0 | 0.5 | 1 | Fallow | |
| Alfold es Eszak | 37.8 | 23.3 | 15.0 | 28.8 | 60.2 |
| Bassin Parisien | 26.7 | 17.1 | 11.4 | 22.5 | 57.2 |
| Centro | 67.9 | 41.7 | 28.7 | 57.5 | 57.7 |
| East Anglia | 23.2 | 17.1 | 13.1 | 20.6 | 43.7 |

| | | | | | |
|---|---|---|---|---|---|
| Macroreguinea Trei | 81.9 | 75.0 | 69.2 | 55.8 | 15.4 |
| Nord-Est | 67.1 | 65.1 | 63.3 | 44.7 | 5.6 |
| | **Compound B** | | | | **Percent change** |
| **Location** | 0 | 0.5 | 1 | Fallow | |
| Alfold es Eszak | 0 | 0 | 0 | 0 | 0.0 |
| Bassin Parisien | 0 | 0 | 0 | 0 | 0.0 |
| Centro | 0.00013 | 0.00010 | 0.00006 | 0.00003 | 50.0 |
| East Anglia | 0 | 0 | 0 | 0 | 0.0 |
| Macroreguinea Trei | 0.116 | 0.094 | 0.079 | 0.00001 | 31.4 |
| Nord-Est | 0.019 | 0.017 | 0.015 | 0 | 22.1 |
| | **Compound C** | | | | **Percent change** |
| **Location** | 0 | 0.5 | 1 | Fallow | |
| Alfold es Eszak | 0.0045 | 0.0033 | 0.0025 | 0.0001 | 44.9 |
| Bassin Parisien | 0 | 0 | 0 | 0 | 0.0 |
| Centro | 0.236 | 0.166 | 0.120 | 0.142 | 49.2 |
| East Anglia | 0 | 0 | 0 | 0 | 0.0 |
| Macroreguinea Trei | 6.35 | 5.20 | 4.29 | 0.03 | 32.4 |
| Nord-Est | 1.27 | 1.15 | 1.04 | 3E-06 | 18.0 |

**Table 6.6. Impact of plant uptake factor on the calculated $PEC_{GW}$ (µg/L) using FOCUS scenarios and compounds used within this paper.** Compound A: $K_{OM}$ = 10, DT50 = 100; Compound B: $K_{OM}$ = 100, DT50 = 40, Compound C: $K_{OM}$ = 500, DT50 = 500. Reproduced from Table 4.3 in Chapter 4.

| | **Compound A** | | | | **Percent change** |
|---|---|---|---|---|---|
| **Location** | 0 | 0.5 | 1 | Fallow | |
| Chateaudun | 270.2 | 172.7 | 126.5 | 172.3 | 53.2 |
| Hamburg | 334.9 | 218.0 | 154.9 | 179.3 | 53.7 |
| Jokioinen | 498.5 | 296.1 | 169.6 | 188.9 | 66.0 |
| Kremsmunster | 195.9 | 140.9 | 102.3 | 134.6 | 47.8 |
| Okehampton | 186.1 | 161.4 | 142.0 | 142.1 | 23.7 |
| Piacenza | 173.8 | 112.0 | 84.3 | 126.4 | 51.5 |
| Porto | 143.3 | 111.8 | 99.4 | 105.6 | 30.6 |
| Sevilla | 89.2 | 21.4 | 12.7 | 114.0 | 85.7 |
| Thiva | 281.4 | 119.7 | 60.6 | 105.6 | 78.5 |
| | **Compound B** | | | | **Percent change** |
| **Location** | 0 | 0.5 | 1 | Fallow | |
| Chateaudun | 0.555 | 0.45 | 0.367 | 0.47 | 33.9 |
| Hamburg | 5.14 | 4.20 | 3.50 | 3.07 | 32.0 |
| Jokioinen | 1.126 | 0.921 | 0.759 | 0.60 | 32.6 |
| Kremsmunster | 2.23 | 1.82 | 1.49 | 1.34 | 33.4 |
| Okehampton | 5.64 | 4.76 | 4.04 | 4.03 | 28.3 |
| Piacenza | 2.46 | 2.10 | 1.80 | 2.60 | 26.7 |

| | | | | | |
|---|---|---|---|---|---|
| Porto | 3.19 | 2.67 | 2.24 | 2.43 | 29.7 |
| Sevilla | 0 | 0 | 0 | 0.04 | 0.0 |
| Thiva | 0.162 | 0.104 | 0.069 | 0.09 | 57.4 |
| | **Compound C** | | | | **Percent change** |
| **Location** | 0 | 0.5 | 1 | Fallow | |
| Chateaudun | 17.37 | 12.73 | 9.44 | 13.4 | 45.7 |
| Hamburg | 45.09 | 33.64 | 25.86 | 25.8 | 42.6 |
| Jokioinen | 1.89 | 1.55 | 1.29 | 1.84 | 31.8 |
| Kremsmunster | 33.2 | 25.8 | 20.3 | 20.7 | 38.8 |
| Okehampton | 41.2 | 32.6 | 25.9 | 26.9 | 37.1 |
| Piacenza | 30.6 | 23.7 | 18.9 | 20.9 | 38.2 |
| Porto | 25.0 | 19.9 | 16.0 | 16.9 | 35.8 |
| Sevilla | 0.104 | 0.061 | 0.036 | 2.52 | 65.4 |
| Thiva | 8.22 | 4.73 | 2.77 | 7.54 | 66.3 |

The range of percentage change within this study is 0% – 60.2% (Table 5), this is somewhat lower than the range found for the FOCUS locations (0%-85.7% ) that were tested in Chapter 4 and have been reproduced here for ease of comparison, with the fallow scenarios added (Table 6). The 0% change occurs when there are levels of leaching from 1m depth are below the $PEC_{GW}$ sensitivity to $1e^{-6}$. It is likely that there would be an effect of plant uptake on the $PEC_{GW}$ in these areas; however, it is not observed as there is not a high enough concentration of pesticide at each location for this to be detected. Therefore, by removing the zero $PEC_{GW}$ values resulting in a zero-percentage change, the range becomes 5.6%-60.2% (Table 5) and 23.7%-85.7% (Table 6). This shows that the smallest observed effects of plant uptake are much lower for the typical scenarios than the FOCUS. Interestingly, this range is much smaller than the EFSA study which showed 24-43% percentage change. A t-test between the percentage change of the results from Chapter 4 and Chapter 6 shows that, despite the lower range of percentage change in the 'typical' scenarios, there is no significant difference between the FOCUS and newly defined typical scenarios (p-value = 0.2). This test was conducted with the zero-percentage change removed.

Current recommendations state that 0.1 µg/L is the stated amount for a safe $PEC_{GW}$ value. As shown (Table 4), Compound A greatly exceeds this value for all locations, whereas Compound B and Compound C show much lower values, with Compound B only exceeding the 0.1 threshold at Macroreguinea Trei for the zero-uptake scenario. In this scenario the zero-uptake compound shows a value of 0.1, the 0.5 uptake and 1 uptake compounds show $PEC_{GW}$ values below this threshold. Despite the higher $K_{OM}$

values of Compound C compared to Compound A and B, the $PEC_{GW}$ values are much higher than those found for Compound B; this is caused by the fact that the persistence of the compound is much higher due to its higher DT50. $PEC_{GW}$ results from this experiment (Table 6.5) show that plant uptake can have an effect on the output concentration with Compound B for MT dropping below the 0.1 $\mu gL^{-1}$ threshold solely due to the PUF value that was supplied.

It should be noted that, although the concentration at 1m depth was much lower for the fallow scenario in most modelled scenarios (see e.g. Figures 6.6, 6.8,6.9), the $PEC_{GW}$ value is higher. This is owing to the higher concentration of compound in the leaching water due to the lower water output (no irrigation).

## 6.5 Conclusion

The aim of this study was to test whether the effect of plant uptake on the leaching from the soil profile for environmental fate modelling scenarios that are more representative of typical wheat growing conditions was comparable to the 'realistic worst-case' FOCUS scenarios. The typical scenarios used information obtained from the MARS Agri4cast database and European soil database. All locations and are treated as random effects that bring variation to the dataset, there were examples of locations that had a much-reduced effect of plant uptake on than what was observed in Chapter 4. This was seen at Macroreguinea Trei and Nord-Est, for example, which had higher silt content than the other locations. This meant that these locations had much lower water retention and needed to be irrigated much more than others. This is likely to be the cause of the very small difference between the concentration at 1m depth ($C_{1m}$) for the CA0 and CA10 compounds at this location. Plant uptake showed similar bell-shaped curves between compounds, with slight changes being seen to the shape of uptake depending on the availability of the compound within solution during the early or late phases of the growing season. The 'typical' scenarios show much lower levels of leaching than those observed in Chapter 4 (for the worst cases FOCUS scenarios), using the same compounds at the FOCUS locations. This, however, does not mean that there is a difference in the effect of PUF on the leaching of the compounds. Percentage change in $PEC_{GW}$ values showed no significant difference when compared statistically. Although $C_{1m}$ values were lower in the typical scenarios, this does not have a significant effect on the percentage change of plant uptake in the $PEC_{GW}$ results.

## 6.6 References

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# 6.7 Appendix A

**Table 6.7. Parameterisation of the scenarios within PEARL**

| **PEARL settings table** | | | |
|---|---|---|---|
| .PRL file | | | |
| **Parameter description** | **Value, Source and Comments** | **Model default, FOCUS default, User input, user choice or N/A** | **Rationale** |
| Calling Program | Set to FOCUSPEARL | NA | |
| Calling Program Version | Set to 4.4.4 | NA | |
| Init Years | Set to 6 | FOCUS default | Allows for soil moisture to reach equilibrium and allow pesticide to make it's way down the profile |
| TimStart, TimEnd | 01/01/1990, 31/12/2015 | User choice | Most recent data when the data was downloaded |
| AmaSysEnd, Stopcondition(kgha-1) | 0 | Model default | |
| ThetaTol (m3.m-3) | 0.001 | Model default | |
| OptDelTimPrn | Other | User choice | Daily output required |
| DelTimPrn (d) | 1 | User choice | Daily output required |
| Repeat Hydrology | No | User choice | To see variation across years of data |
| OptHyd | OnLine | Model default | |
| DelTimSwaMin (d) | 1.00E-07 | User choice | |
| DelTimeSwqMax (d) | 0.2 | User choice | |
| PrintCumulatives | No | User choice | Can do cumulative in R if needed |
| GWTol (m) | 1m | User choice | |

| | | | |
|---|---|---|---|
| MaxItSwa | 30 | User choice | |
| OptHysterisis | No | User choice | |
| PreHeaWetDryMin (cm) | 0.2 | User choice | |
| OptScreen | Yes | User choice | |
| OptPersistency | No | User choice | |
| OptSys | All | User choice | |
| OptPaddy | No | User choice | Paddy drainage not required |
| OptMacropore | No | User choice | Macropore flow not required |
| SoilTypeID | xx_Soil. Prefix of location (xx) followed by _Soil | User choice | Input based on preferred labelling system |
| Location | xxyy, Location abbreviation. Xx = two letter abbreviation for country. Yy abbreviation for adminitrative region, varying length. | User choice | Input based on preferred labelling system |
| Table Soil Profile | Individual for each location. Consists of 1) ThiHor (depth of soil horizon). 2) NumLay (Number of layers within horizon | User choice | 2m soil profile simulated |
| Table horizon SoilProperties | Individual for each location. Consists of 1) Soil horizon number. 2) FraSand (kg.kg-1), mineral sand fraction 3) FraSilt (kg.kg-1) mineral silt fraction. 4) FraClay (kg.kg-1) mineral clay fraction. 5) CntOm (kg.kg-1) Organic matter content. 6) pH (-) | User choice | Selected from the European Soil databse |

| | | | |
|---|---|---|---|
| Table horizon VanGenuchtenPar | Individual for each location. 1) Soil Horizon number. 2) ThetaSet (m3.m-3) Saturated water concent. 3) ThetaRes (m3.m-3) Residual water content. 4) AlphaDry (cm-1) Inverse air entry suction when dry. 5) AlphaWet (cm-1) Inverse of air entry suction when wet. 6) n (-). 7) Ksat (m.d-1), 8) l (-) Lambda. | User choice | Calculated from pedotransfer functions |
| OptRho | Individual for each location. Table of input for Bulk density. ) Horizon number. 2) Rho (kg.m-3) Bulk density value. | User choice | Calculated from pedotransfer functions |
| ZPndMax (m) | 0.002 | FOCUS default | |
| OptSolEvp | Boesten | User choice | Boesten selected |
| FacEvpSol (-) | 1 | FOCUS default | |
| CofRedEvp (cm1/2) | 0.79 | FOCUS default | |
| PrcMinEvp (m.d-1) | 0.01 | FOCUS default | Evp (m.d-1) drainage 0.01 required |
| Table horizon LenDisLiq (m) | 1) Horizon number. 2) LenDisLiq, 0.05 for all layers | FOCUS default | Liq (m) drainage 1) horizon number. 2) lendisliq, 0.05 for all layerst required |
| OptCofDifRel | MillingtonQuirk | Model default | Rel drainage millingtonquirk required |
| ExpDifLiqMilNom (-) | 2 | Model default | Nom (-) drainage 2 required |
| ExpDifLiqMilDen (-) | 0.6667 | Model default | Den (-) drainage 0.6667 required |
| ExpDifGasMilNom (-) | 2 | Model default | |
| ExpDifGasMilDen (-) | 0.6667 | Model default | Den (-) drainage 0.6667 required |
| OptPnd | Constant | Model Default | |
| MeteoStation | Location Code | User input | user input |
| OptEvp | Input. Use ETRef value | User input | PELMO parameterisation from temperature |
| TemLboSta (degC) | 10 | User choice | Set to standard for all locations. |

| | | | |
|---|---|---|---|
| FacPrc (-) | 1 | FOCUS default | Prc (-) drainage 1 required |
| DifTem (-) | 0 | FOCUS default | Tem (-) drainage 0 required |
| FacEvp (-) | 1 | FOCUS default | Evp (-) drainage 1 required |
| OptIrr | Sprinkler_Weekly | User choice | As real life data of soil moisture not known, this was set to keep the same for all locations |
| OptMetInp | Daily | User input | |
| OptTraRes | Laminar | FOCUS default | Res drainage laminar required |
| OptRainfallEvents | No | FOCUS default | Events drainage not required |
| OptSnow | No | FOCUS default | Snow drainage not required |
| ZGrwLevSta (m) | -100 | User choice | No data so set to lower boundary |
| OptLbo | FreeDrain | User choice | Standard for all locations |
| OptDra | No | N/A | Local drainage not considered to keep compound within soil profile and reduce losses |
| OptSurDra | No | N/A | |
| NumDraLev | 0 | N/A | |
| MolMas_subst (g/mol) | 400 | Mid-weight for pesticide | typical molecular weight |
| DT50Ref_xxxx (d) | Ranged between 40-500 depending on compound. CA = 100, CB = 40, CC = 500 | User choice | Varied to see effects on PU. |
| TemRefTRa_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| ExpLiqTra_xxxx | 0.7 | User choice | |
| CntLiqTraRef_xxxx (kg.kg-1) | 1 | User choice | |
| MolEntTra_xxxx | 65.4 | User choice | |
| Table horizon FacZTraHor_xxxx | 1) Horizon number. 2) FacZTra, 1 | User input | |
| Table horizon FacZSorHor_xxxx | 1) Horizon number. 2) FaczSor, -99 | User choice | |
| MolEntSor_xxxx (kJ.mol-1) | 0 | FOCUS default | |
| TemRefSor_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| OptCofFre | pH-independent | User choice | |

| | | | |
|---|---|---|---|
| KomEql_xxxx (L.kg-1) | 5.8-290 | User choice | Varied to see effects on PU. |
| KomEqlMax_xxxx (mg.L-1) | 5.8-290 | User choice | Varied to see effects on PU. |
| ConLiqRef_xxxx (mg.L-1) | 1 | User choice | |
| ExpFre_xxxx | 0.9 | User choice | |
| PreVapRef_xxxx (Pa) | 1.00E-12 | User choice | Set to a low value to reduce loss from vapourisation |
| TemRefVap_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| SlbWatRef_xxxx (mg.L-1) | 3 | User choice | Set to reasonable solubility but not too high so that losses were drastically increased due to high mobility. Higher solubility means greater concentration in liquid phase, increasing plant uptake and movement down the profile. |
| TemRefSlb_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| MolEntSlb_xxxx (kJ.mol-1) | 27 | FOCUS default | |
| MolEntVap_xxxx (kJ.mol-1) | 95 | FOCUS default | |
| CofDesRat_xxxx (d-1) | 0 | User selected | Non equilibrium soprtion not considered so set to 0 |
| FacSorNeqEql_xxxx (-) | 0 | User selected | |
| FacUpt_xxxx (-) | Value of 0,0.1,0.5,1.0 | User choice | Varied to see effects on PU. |
| ThiAirBouLay (m) | 0.01 | FOCUS default | |
| OptDspCrp_xxxx | Lumped | FOCUS default | |
| DT50DspCrp_xxxx (d) | 1,000,000 | FOCUS default | |
| DT50PenCrp_xxxx (d) | 1,000,000 | FOCUS default | |
| DT50VolCrp_xxxx (d) | 1,000,000 | FOCUS default | |
| DT50TraCrp_xxxx (d) | 1,000,000 | FOCUS default | |
| FacWasCrp_xxxx (m-1) | 0.0001 | FOCUS default | |

| | | | |
|---|---|---|---|
| TemRefDif_xxxx (degC) | 20 | User choice | Set to 20 throughout |
| CofDifWatRef_xxxx (m2.d-1) | 4.30E-05 | FOCUS default | |
| CofDifAirRef_xxxx (m2.d-1) | 0.43 | FOCUS default | |
| ZTgt (m) | 1 | FOCUS default | Target layer depth at 1m and is standard for all profiles. |
| DelTimEvt (Years) | 1 | User choice | Application repeated each year |
| table Applications | 01-Emg-01 AppSolSur 1.0 | User choice | FOCUS application schedule, compound applied 1 day prior to emergence of the crop |
| table_TillageDates | No till | User choice | Didn't want to consider tillage for this study |
| Ztil (m) | 0.2 | User choice | N/A as no till selected |
| Table interpolate CntSysEql (mg.kg-1) | 0 | User choice | Set to 0 as no previous applications expected |
| Table interpolate CntSysNeq (mg.kg-1) | 0 | User choice | Set to 0 as no previous applications expected |
| Deposition scheme | No Deposition | User choice | Not considered |
| table FlmDep (kgha-1.d-1) | No Deposition | User choice | Not considered |
| Crop Calendar | Individual for location | User input | |
| Repeat Crops | Yes | User choice | Annual crops to marry with annual application |
| OptLenCrp | Fixed | User choice | Crop linear development. |
| table Crops | Individual for location | User input | |
| table IrrigationPeriods | 01-Jan to 31-Dec | User choice | Irrigation kept for all locations to keep them all the same as site specific information not known |

| | | | |
|---|---|---|---|
| table CrpPar_WCEREALS1<br>0.0 0.0 1.0 0.1 0.0<br>0.5 4.0 1.0 0.3 0.1<br>1.0 0.0 1.0 0.6 0.2 | Table. 1) Development stage (0 = emergence, 1 = Harvest). 2) LAI (m2.m-2). 3) Crop factor for evaporation. 4) Rooting depth (m). 5) Crop height (m). | User input | |
| table RootDensity_WCEREALS1<br>0.00 1.00<br>1.00 1.00 | Root density table 1) Relative rooting depth 2) Relative root density | User input | |
| HLim1_WCEREALS1 (cm) | 0 | User input | none of these parameters are defaults they are taken from FOCUS for winter cereals |
| HLim2_WCEREALS1 (cm) | -1 | User input | |
| HLim3U_WCEREALS1 (cm) | -500 | User input | |
| HLim3L_WCEREALS1 (cm) | -900 | User input | |
| HLim4_WCEREALS1 (cm) | -16,000 | User input | |
| RstEvpCrp_WCEREALS1 (s.m-1) | 70 | User input | |
| CofExtDif_WCEREALS1 (-) | 0.39 | User input | |
| CofExtDir_WCEREALS1 (-) | 1 | User input | |
| CofIntCrp_WCEREALS1 (cm) | 0.0001 | User input | |
| TemSumSta_WCEREALS1 (degC) | 0 | User input | |
| TemSumEmgAnt_WCEREALS (degC) | 0 | User input | |
| TemSumAntMat_WCEREALS (m) | 0 | User input | |
| Ztensiometer_WCEREALS1 (m) | 0.2 | User input | |
| PreHeaIrrSta_WCEREALS1 (cm) | -100 | User input | |
| IrgThreshold_WCEREALS1 (mm) | 15 | User input | |

# Chapter 7 – Discussion and Conclusions

## 7.1 Introduction

This chapter will seek to bring together the discussions of each paper, provide a link between them and conclude the thesis. Although there may be some repetition of the discussion from previous chapters, cross references will be made to the relevant sections for a more in-depth discussion. There were two main branches to the project. The first was 'understanding plant uptake' the experimental side and the aim of this part was to test the main assumptions used within the framework of the experimental methodologies and attempt to advance the realism of the methodology. The second branch is 'quantifying plant uptake' which is done using the environmental fate models, to test the effects of plant uptake on the leaching of the pesticide to 1 metre depth. It is of interest to observe whether this is the same within different models and if the 'realistic worst-case' scenarios of the FOCUS model show a representative effect of plant uptake when compared to typical scenarios.

## 7.2 Methods for measuring plant uptake in laboratory experiments

While much discussion has surrounded the laboratory measurement of plant uptake, there remains no agreed methodology (Hoke et al., 2016). Since the publication of the Brigg's relationship of log $K_{OW}$ against TSCF, there have been several subsequent definitions of a relationship some of which have kept a similar shape to the original bell-curve shape and shifted the position on the graph, or others have defined a new shape of the relationship (see Chapter 1 – Section 1.5.1)The lack of agreement between scientists suggests that there are other factors affecting the TSCF/PUF results from experiments leading to a lack of reproducibility. This could be caused by several reasons, such as methodology, plant species, variation between individual researchers' interpretation of methods and wrong assumptions made for the basis of the experiment. Currently there are 5 main log $K_{OW}$ against TSCF relationships, using 6 different plant species and 2 different methodologies, however in the wider dataset there are 300 measurements of

155 compounds using 34 plant genera found within 41 publications (Bagheri et al., 2019). Recent analysis by Bagheri et al. (2019) suggests that an approach using Lipinski's rule of 5 (see Chapter 1 – Section xx), their derived relationship gave an overall $R^2$ of 0.8, however this suggestion is very new and requires proper scrutiny before adoption.

The observed variation in the data however suggested that a move away from the attempts to establish a universal plant uptake would be needed. Considering this, the testing of the main assumptions made by previous plant uptake experiments became a clear objective. The first of these assumptions is that plant uptake is a consistent value throughout a plant's lifecycle, regardless of growth stage. However, testing PUF at different growth stages proved unsuitable for a PhD project due to the lead up time needed in the plant growth. Another assumption made is that plant uptake is a singular value and does not vary over time (Lamshoeft et al., 2018). Currently, within the environmental fate models, the option of including plant uptake is only available as a single value that represents the fraction of the concentration in solution that will be taken up for each fraction of the solution volume. However, there seemed to be no evidence within peer-reviewed literature where this has been established. Chapter 3 covers the experiment that was conducted with this aim in mind and the data showed that the original assumption was correct, however only whilst the compound did not degrade. To achieve this the sampling period was extended from 8 days to 21 and the solution was topped up each day. Each compound has a specific uptake value and therefore once the compound starts to degrade, the PUF changes. This means that when modelling the uptake of a compound, knowledge of the key metabolites and the degradation pathways must also be known in detail so that environmental fate predictions can be made. Once known, this can easily be inputted into the models. This approach is not currently pursued.

Another key assumption made by the hydroponic experiments is that it is a suitable and realistic surrogate for the soil environment. One key difference between the hydroponic system and a soil profile is the lack of air-filled pore space in the solution culture. To reduce the effect of anoxic conditions, these systems are often bubbled with air (Dettenmaier et al., 2009; Lamshoeft et al., 2018). In Chapter 3 the 'topping-up' of solution meant that by removing the roots each day, the oxygen was replenished, and plant health was not affected. Chapter 4 used sand within a cylindrical column to replicate a soil profile, sand was chosen as it is an inert media that does not bring the sorption characteristics and microbiome associated with soil. Results showed that much faster degradation occurred in this experiment and PUF results could not be determined. It is recommended that to conduct a similar experiment in future that another compound is found that will remain in solution and that degradation is < 5% for approximately 8 days. Despite the result shown in Chapter 4, a sand profile remains an important experiment to conduct as there are several reasons why it could potentially be different to hydroponics. One reason is that, gas diffusion is $10^4$ times slower in liquid than it is in air, this is likely to affect the gas exchange in hydroponic solution and could affect PUF results. Another consideration of a sand/soil system is the hydraulic connectivity, within a hydroponic system the plant roots can theoretically access the whole reservoir at any point and the solution can regularly be mixed. Within a sand/soil profile the air-filled space in the pore spaces and the physical barriers posed by the mineral content mean that the solution is not always connected and there is more potential for ‘hot spots’ of pesticide when pores are emptied. This could mean that PUF is not as even as experienced in hydroponics and transpiration as it becomes limited by the available pore water and susceptible to change based on the difference in concentration of each pore and their connectivity. The above discussion surrounding the testing of the main modelling assumptions shows that there is still much work to be done with regards to having an agreed PUF/TSCF methodology. This discussion of future research and how to combat this will be considered further in Section 7.4.

## 7.3 Measuring plant uptake within environmental fate models

Within peer-reviewed literature there have been several sensitivity analyses conducted into the suite of environmental models, there appears to be little discussion into the effect of plant uptake on leaching values. Predicted Environmental Concentration in the Groundwater ($PEC_{GW}$)was established by FOCUS and represents a singular indication of the leaching potential of a compound in a scenario and allows for easy comparison between model runs. Using $PEC_{GW}$, the European Food Safety Authority (EFSA), conducted a small study using PEARL to model two locations with 3 example compounds. Predicted Environmental Concentration in the Groundwater ($PEC_{GW}$) is the 80th percentile of the mean annual concentration at 1m depth. EFSA’s study showed that a percentage change of 24-43% of $PEC_{GW}$ when PUF was changed from 0 – 1 (EFSA, 2013). Chapter 4 built on this work by comparing 9 FOCUS locations with 3 example compounds using both PEARL and PELMO. When considering the results from EFSA, the percentage change using the same locations and model in this study showed a percentage change of 32-53%. However, when considering the data from both models, all compounds and locations plant uptake has an effect of 0 – 85%. The greater variation could possibly be down to the greater number of locations used within the study in Chapter 4. The EFSA study on used two locations (Hamburg and Kremsmünster) and the $PEC_{GW}$ values produced within Chapter 4 showed comparable levels of plant uptake percentage change when varied from 0 - 1. This shows that plant uptake can have a very significant effect on the leaching outputs of a compound and a pesticide showing leaching levels near the threshold of 0.01, could leach below these levels if it has a high PUF.

One consideration with the FOCUS scenarios is that they are designed to be 'realistic worst-case' scenarios where the leaching is expected to be high, therefore, there is potential that the effect of plant uptake would be exaggerated at these locations (FOCUS, 2009). Therefore, 'typical' scenarios were defined to determine whether a greater effect was seen within the FOCUS scenarios or in the new locations (Chapter 6). Within this study the effect of plant uptake on $PEC_{GW}$ was 0-60.2%. The zero values represent locations where all $PEC_{GW}$ values were zero, however, the lowest percentage change where leaching was seen was 5.6%. This represents locations where much lower effects of leaching were seen than the FOCUS locations, where the lowest value was 23.7%. Despite this, results showed that although there were slightly lower levels of leaching, there was no significant difference between the $PEC_{GW}$ values of the FOCUS scenarios and the 'typical' scenarios.

Chapter 5 focussed on the effect of climate on the plant uptake within the models, results from this showed the two climatic inputs (based on the input files for PEARL) were potential evapotranspiration ($ET_{REF}$) and maximum temperature ($T_{MAX}$). This is an important finding as these inputs can be highlighted in the future when looking at areas where plant uptake could become significant. However, this experiment was only modelled at one location and this caveat should be stated when considering this in future work. Within this chapter, more recent climate files (1999-2018) were also run against the original FOCUS files (1992-2002), which showed that leaching levels were reduced when more recent data is used and that this was statistically significant. This result suggests that the FOCUS scenarios should be updated periodically to allow environmental fate predictions to be the most up to date. To the authors' knowledge, although plant uptake is much discussed with regards to the effect that it can have on leaching and reducing uncertainty within PUF estimate (Vanclooster et al., 2000). There weren't many peer-reviewed papers that could be found discussing the effects that it has on the outputs. Therefore, the results from Chapters 4-6 have been discussed within the context of the EFSA study and between themselves but little else could be found to compare this with. This means that there are many different avenues that are available for future areas of research into the effects of plant uptake which will be discussed below.

## 7.4 Considerations of future direction of research

There are two main assumptions that are made by the environmental fate model in regards plant uptake assessments that prior to this project had not been tested explicitly. The first is that PUF/TSCF is consistent over time and maintains a linear relationship, this was discussed in detail in Chapter 2. The second assumption is that the PUF is the same for young plants as it is for old. The majority of plant uptake assessments are conducted using young plants that are in the early stages of their development, this helps to reduce the lead-up time taken for the experiment (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990; Lamshoeft et al., 2018). However, in the early stages of development, plants are consistently increasing the amount they transpire each day and biomass is increasing (See chapter 2 – section 2.4.3). As the plant enters the later stages of their development, their water demands (often represented as $K_C$) begins to reduce and their growth slows (Hong et al., 2017). When we model the plant uptake of pesticides, we supply one value as a PUF and expect this to be consistent over time, however, we cannot be sure that this is the case without testing the hypothesis (Lamshoeft et al., 2018; Michalski et al., 2004). One of the reasons that it is possible that this may change is that plant roots change how they take up water as they get older, with the tip accounting for the majority of uptake and the older parts of the root becoming suberized, which is accepted may still absorb some water (Taiz and Zeiger, 2010). Suberisation is the process that also gives rise the Casparian strip and has been shown to be impermeable to many water solutes (Grebe, 2011). This means that as the plants move into the maturity stage prior to harvest, there is a chance that plant uptake may change, and this assumption be incorrect. The application strategy of several pesticides is many and varied, meaning that this could be particularly important when modelling the environmental fate of a pesticide that is applied at the late growth stage of a crop.

Increased realism is one part of plant uptake where there has been very little development since the early plant uptake experiments (Briggs et al., 1982; Lamshoeft et al., 2018). Early experiments were conducted in hydroponic solution as it made it easier to expose the plant roots and extract the plant for harvesting (Briggs et al., 1982). Despite the development of the ‘de-topping’ method following this, this does not represent an advancement of realism as the plant roots are still grown within a hydroponic system (Hsu et al., 1990). Chapter 3 represents an attempt to conduct a plant uptake assessment in a new system and provides interesting insights into the blueprint of how this system could work. The results were not comparable to the hydroponic system due to degradation of the compound meaning that there was no confidence in the PUF being of [$^{14}$C]-1,2,4-Triazole. If it is shown to be the case that the hydroponic system is an accurate surrogate for the soil environment, then it may that hydroponics are taken forward for the methodology due to the ease of setup, however this remains to be proven first. As a result, the priorities of future research must be to continue advancing the realism of the laboratory test system.

Aside from the changes to testing the assumptions made by researchers and improving the realism of the test system, it is important to point out that this project only focussed on one agricultural crop. Wheat (Triticum aestivum, L.) is an important agricultural crop due to high production across Europe and was selected for this reason, however it would be remiss to ignore the potential for variability in plant species. This may be one of the key reasons behind the absence of agreement from the research community with regards to a universal plant uptake vs log $K_{OW}$ relationship, within the currently derived relationships alone, 5 different species have been used (Briggs et al., 1982; Burken and Schnoor, 1998; Dettenmaier et al., 2009; Hsu et al., 1990; Lamshoeft et al., 2018). Therefore, before any universal relationship can be derived or applied in future studies, it is paramount that this is tested with multiple plant species to ensure there is no species-specific uptake. It should also be considered a universal relationship may not be possible due to the aforementioned variability and in which case, plant uptake values should be applied on a compound and species specific basis (Lamshoeft et al., 2018).

One area of future work that was mentioned in Chapter 5, is to conduct analysis of the future effect of anthropogenic climate change, to test how this would affect the leaching of pesticides from the soil profile and the effect of plant uptake within this. This would give a sense of the future direction of the study of environmental fate and quantify the influence of plant uptake. There are however many different forecasts for future climates. It is also uncertain as to how much effect positive feedback loops will have in future climates and whether they will continue to accelerate or maintain their current rate (Masson-Delmotte et al., 2018). This means that many different datasets could be added to the environmental fate models and several studies could be conducted.

Another uncertainty surrounding future climates that would be more difficult to model is the response of plants to changes to the atmosphere as a result of potential future burning of fossil fuels. Experiments have been conducted in elevated $CO_2$ to assess the fertilisation effect that this may have, yield increasing as a result. However, it has also been suggested that this may reduce the transpiration rate of the plants, which in turn could affect the plant uptake of pesticides (Mcgrath and Lobell, 2013). Whether this would be observed in practice remains to be seen, as it may be competing with many other factors that have a stronger influence over plant uptake. It is however something to consider when modelling future climatic scenarios.

Finally, within Chapter 5, the climatic input data was tested to determine which climatic input parameter had the strongest linear relationship with uptake. The results showed that potential evapotranspiration ($ET_{REF}$) and maximum temperature ($T_{MAX}$) showed the best relationships. This experiment was only conducted on one location (Okehampton) and using 4 years of climate data. This helped to reduce the dataset; however, it would be interesting to see whether $ET_{REF}$ and $T_{MAX}$ show the best linear relationships at all locations or whether it is just the case that the climate in the United Kingdom is most limited by these factors.

## 7.5 Comments on personal development

Broadly, a PhD programme is a development programme for a researcher and therefore it felt important to include a short section on the development and training that has been observed whilst working on this project. Although not all the work that has been conducted has made the final draft of the thesis, many methods and techniques have been attempted during the method development phase. This has allowed for the main author to work on a broad range of instruments and extraction procedures which will stand them in good stead moving forward. What also became clear during this project is that much of the knowledge of plant uptake assessment is held within industry rather than peer-reviewed literature. Therefore, any project that is taken on following this research should be in conjunction with a strong partnership to industry or perhaps should even be based in industry with a link to academia.

It may have been a slight misjudgement to not pick up the modelling aspect of the project earlier and run this alongside the experimental program. Much of the early work in this project was conducted on the experimental side of the project as it was important to establish an uptake methodology so that this can be taken and applied to the future experiments when needed. It became apparent that the final uptake experiments would need to be conducted at the industrial partner's facilities as their laboratory enabled them to carry out radiolabelled experiments. While this work was being finalised, an accident and subsequent injuries meant that the experimental programme had to be abandoned in favour of the modelling aspect of the project. As less groundwork had been put into this aspect, much personal training and development had to be conducted into creating and analysing the model outputs that effectively paused progress in the project for a period. On reflection, although this accident was unavoidable, the project would've progressed a lot smoother and faster if the initial training had been conducted earlier.

Working as a part of this project has allowed for a lot of development into the data analysis and presentation part of research. The statistical program R has been invaluable tool that has allowed for greater variability in the type of plots that have been produced in this thesis and has also allowed for whole datasets of outputs from the environmental fate models to be analysed within minutes. One of the key advantages of R is that a script can produce outputs within seconds, which can make it very valuable when re-running models due to changes to the inputs files or errors being spotted. Whilst R has been available for many years, one of the big stumbling blocks with using it, is the time needed to learn the coding language versus being able to load your data into programs such as Excel and produce a plot. As a PhD allows for training time within the timeline, it makes it possible for such skills to be learned and refined, making it beneficial to learn R and advance your skills over the years. A comparison of the scripts written in the early part of this project and the latest scripts that have been written in the final stages would show the advancement in coding language and the value of R as a tool in data presentation and communication.

## 7.6 Concluding remarks

There is still much work to be done until researchers can be confident that a laboratory derived PUF represents how the PPPs behave in the environment. A lack of agreement with regards to researchers on the ‘true’ position of the log $K_{OW}$ against TSCF relationship, means that there is lack of clarity with regards to this dataset and universal relationship going forward. Therefore, it was decided that the main assumptions made for plant uptake experiments and modelling should be tested to check whether they stand up to experimental rigour. The assumption that was tested was how stable PUF remained over time. The results from this experiment showed that PUF was stable and displayed a strong positive linear relationship whilst the target compound remained stable in solution. Another key area that the authors felt required development was the realism of the hydroponic solution experimental system, it is assumed that this is a realistic surrogate for the soil profile when measuring plant uptake, however it has yet to be experimentally shown. The experiment mirrored that of the hydroponic system in the previous chapter to allow for easy comparison, however much faster degradation of the target compound meant that a linear relationship for PUF could not be derived. The recovery of total radioactivity from this experiment however, showed that it would be possible to obtain such values, however selection of a target compound that remained stable in solution over a longer period, may produce an easier to interpret result.

PUF or TSCF values are placed into environmental fate models which require a single value across the whole growing season. However, only a small amount of work has been published that discusses the effects that plant uptake has on the leaching outputs of the profile. The first experiment was conducted to test the effect of varying plant uptake and the physical-chemical properties of 3 example compounds to see how they performed in two environmental fate models, PEARL and PELMO using the FOCUS locations. Results showed that there wasn’t a significant difference in the leaching between the two models, however there was a slight difference in the plant uptake. Whilst looking at the leaching, it is also important to determine what is likely to affect the plant uptake within the models, the next chapter was an experiment to show which climatic inputs had the best linear relationship with plant uptake using the Okehampton FOCUS location in PEARL. The strongest relationship was with potential evapotranspiration and maximum temperature; therefore, these values should be considered when looking at locations where plant uptake could play a big part. Within the same chapter a more recent climate dataset was tested to see whether changes in the climatic inputs had a significant effect on leaching and the plant uptake within that. The effect of plant uptake did not change significantly between the datasets; however, the leaching levels were significant lower in the more recent dataset. The final experiment led to the defining of some new scenarios to represent a more 'typical' wheat growth scenario in comparison to the 'worst-case' devised by FOCUS. Although this dataset showed a slightly lower effect of plant uptake on leaching, this was not statistically significant.

Whilst plant uptake within a regulatory context has long been discussed from an experimental point of view, there has been relatively slow advancement of the experimental system. This is likely due to a lack of consensus within the scientific community, but it is something that researchers should look towards advancing. Based on the environmental fate modelling results within this chapter, it is clear that plant uptake can have a significant effect on the leaching outputs of the models and this is something that should be further investigated, particularly when it comes to determining how great this effect could be in future climates.

## 7.7 References

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# Appendix 1 – Additional documents

## A1.1 Viva presentation

### QUANTIFYING AND UNDERSTANDING THE UPTAKE OF PLANT PROTECTION PRODUCTS FROM SOIL INTO PLANTS

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Image credit: www.hargravesnursery.au

**Benjamin Jones**

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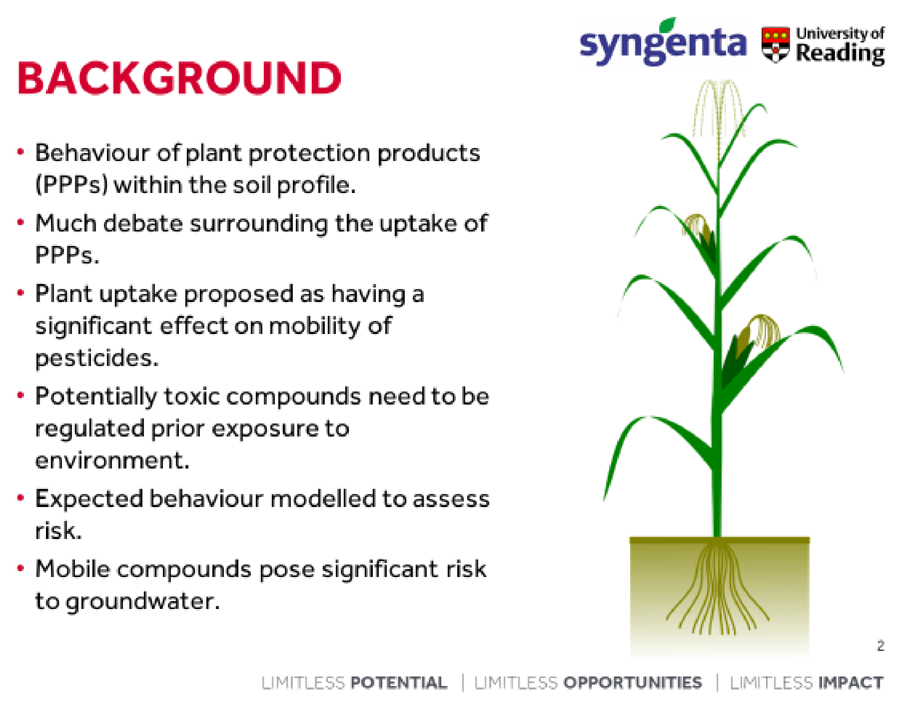

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### BACKGROUND

- Behaviour of plant protection products (PPPs) within the soil profile.
- Much debate surrounding the uptake of PPPs.
- Plant uptake proposed as having a significant effect on mobility of pesticides.
- Potentially toxic compounds need to be regulated prior exposure to environment.
- Expected behaviour modelled to assess risk.
- Mobile compounds pose significant risk to groundwater.

# AIMS AND OBJECTIVES

1. Test whether the main assumptions within the laboratory methodology framework hold true for PUF. Namely whether PUF can remain stable over time.
2. Test the suitability of the hydroponic system as a surrogate for the soil profile by increasing realism.
3. Test the effect that plant uptake has on the leaching output within environmental fate models
4. Test the scenarios where plant uptake may become more or less significant.

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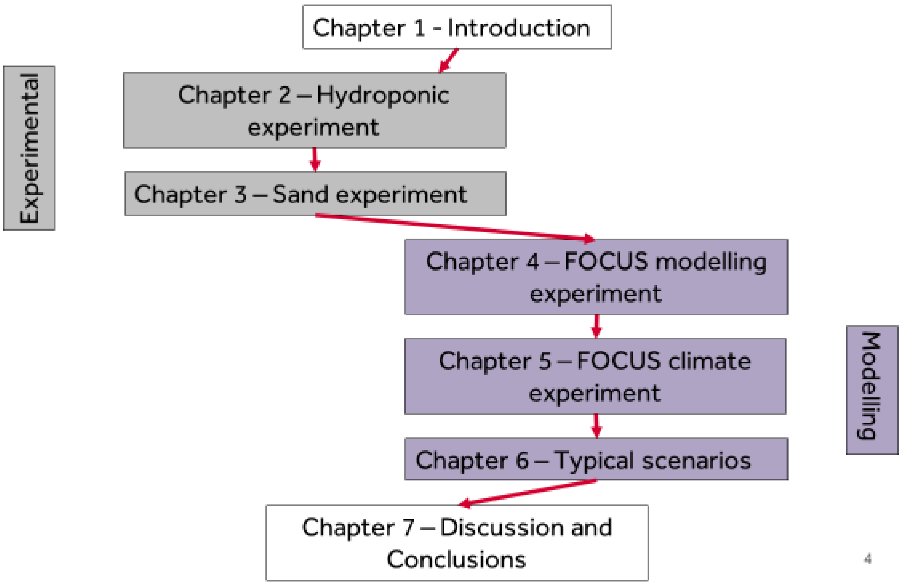
# STRUCTURE OF THESIS



Chapter 1 - Introduction

Chapter 2 – Hydroponic experiment

Chapter 3 – Sand experiment

Chapter 4 – FOCUS modelling experiment

Chapter 5 – FOCUS climate experiment

Chapter 6 – Typical scenarios

Chapter 7 – Discussion and Conclusions

Experimental

Modelling

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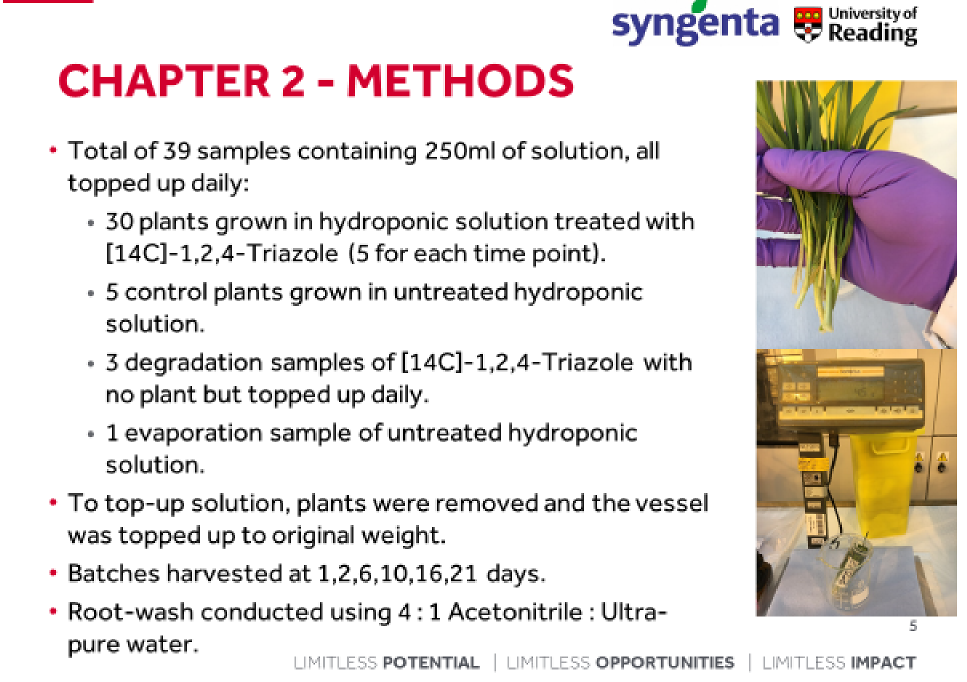

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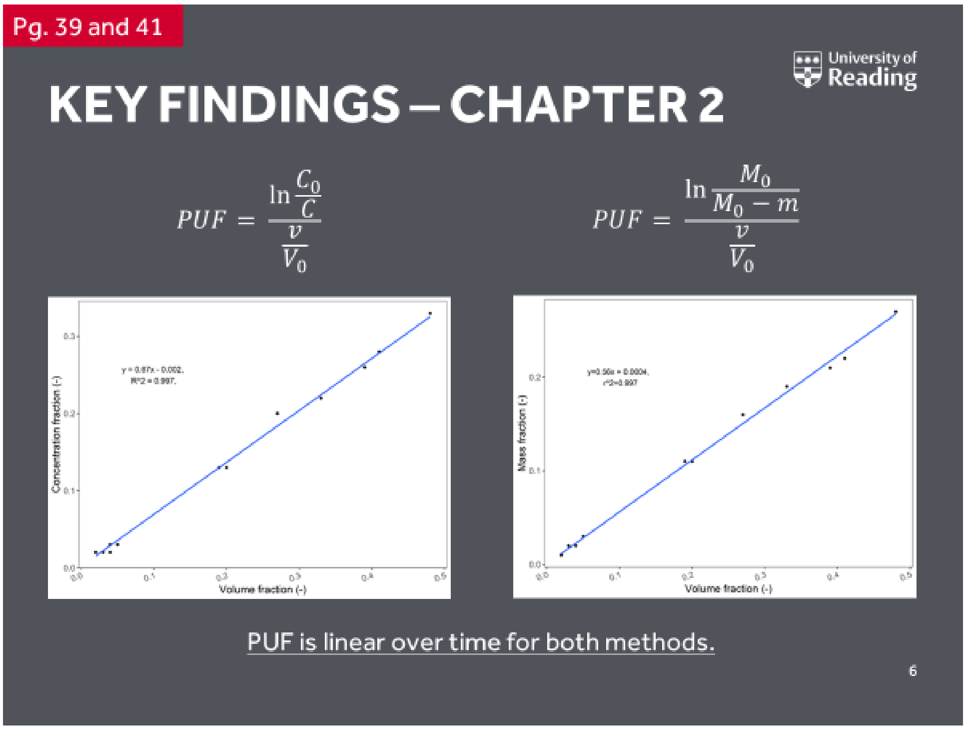
# CHAPTER 2 - METHODS

- Total of 39 samples containing 250ml of solution, all topped up daily:
  - 30 plants grown in hydroponic solution treated with [14C]-1,2,4-Triazole (5 for each time point).
  - 5 control plants grown in untreated hydroponic solution.
  - 3 degradation samples of [14C]-1,2,4-Triazole with no plant but topped up daily.
  - 1 evaporation sample of untreated hydroponic solution.
- To top-up solution, plants were removed and the vessel was topped up to original weight.
- Batches harvested at 1,2,6,10,16,21 days.
- Root-wash conducted using 4 : 1 Acetonitrile : Ultra-pure water.

LIMITLESS **POTENTIAL** | LIMITLESS **OPPORTUNITIES** | LIMITLESS **IMPACT**

Pg. 39 and 41



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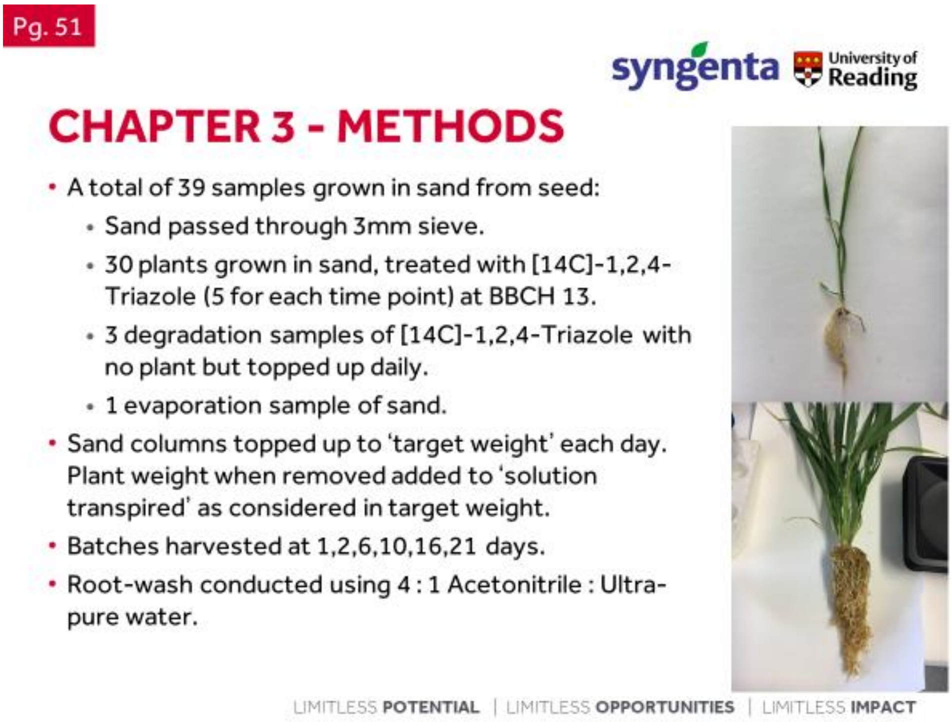
# KEY FINDINGS – CHAPTER 2

$$PUF = \frac{\ln \frac{C_0}{C}}{\frac{v}{V_0}}$$

$$PUF = \frac{\ln \frac{M_0}{M_0 - m}}{\frac{v}{V_0}}$$

PUF is linear over time for both methods.



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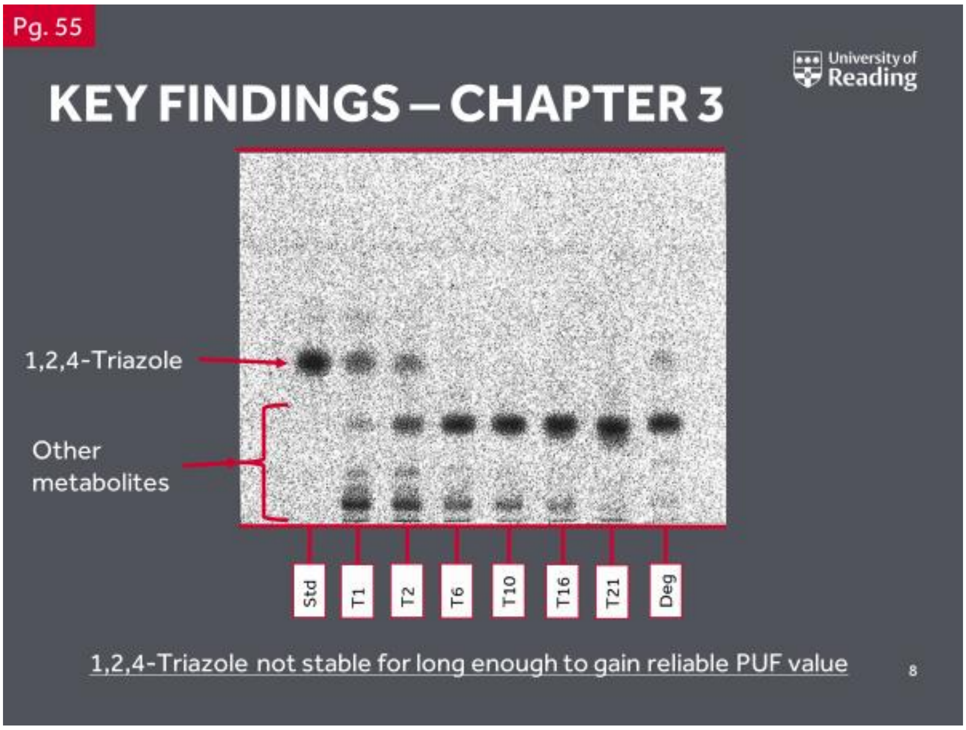
## CHAPTER 3 - METHODS

- A total of 39 samples grown in sand from seed:
  - Sand passed through 3mm sieve.
  - 30 plants grown in sand, treated with [14C]-1,2,4-Triazole (5 for each time point) at BBCH 13.
  - 3 degradation samples of [14C]-1,2,4-Triazole with no plant but topped up daily.
  - 1 evaporation sample of sand.
- Sand columns topped up to 'target weight' each day. Plant weight when removed added to 'solution transpired' as considered in target weight.
- Batches harvested at 1,2,6,10,16,21 days.
- Root-wash conducted using 4 : 1 Acetonitrile : Ultra-pure water.

LIMITLESS **POTENTIAL** | LIMITLESS **OPPORTUNITIES** | LIMITLESS **IMPACT**



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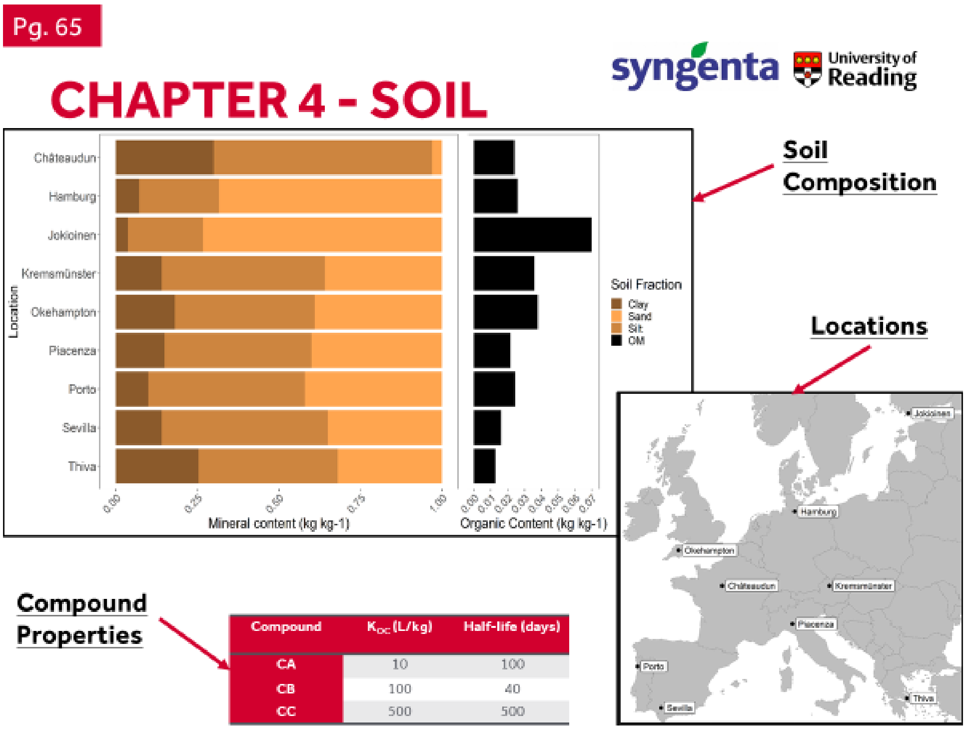
## KEY FINDINGS – CHAPTER 3

1,2,4-Triazole not stable for long enough to gain reliable PUF value

8



Pg. 65

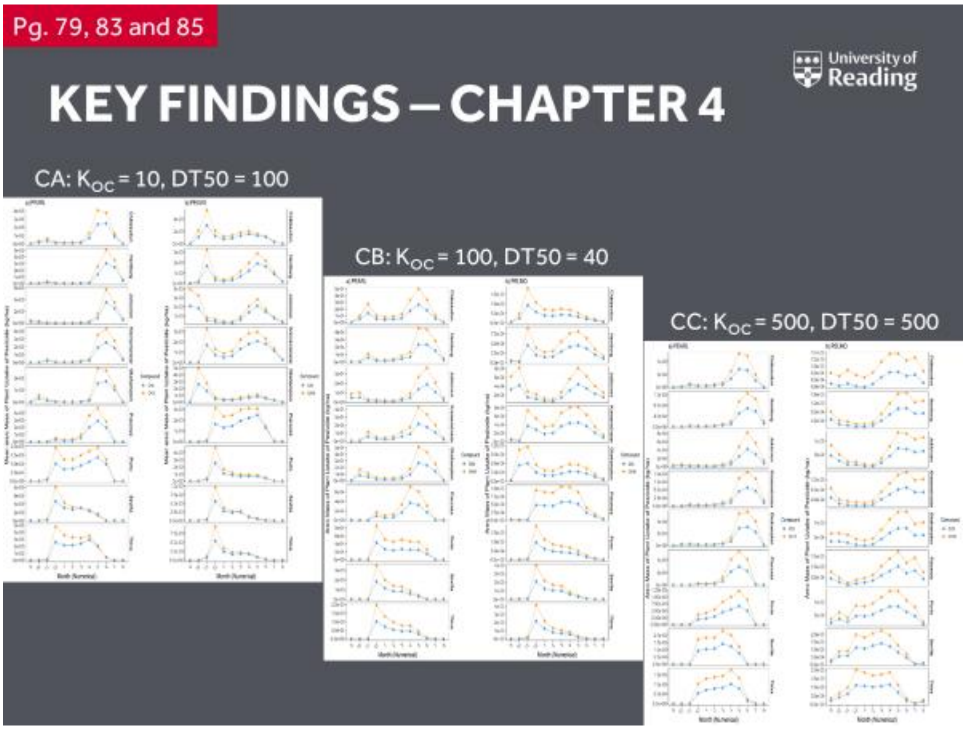
# CHAPTER 4 - SOIL

**Soil Composition**

**Locations**

**Compound Properties**

| Compound | $K_{OC}$ (L/kg) | Half-life (days) |
|---|---|---|
| CA | 10 | 100 |
| CB | 100 | 40 |
| CC | 500 | 500 |



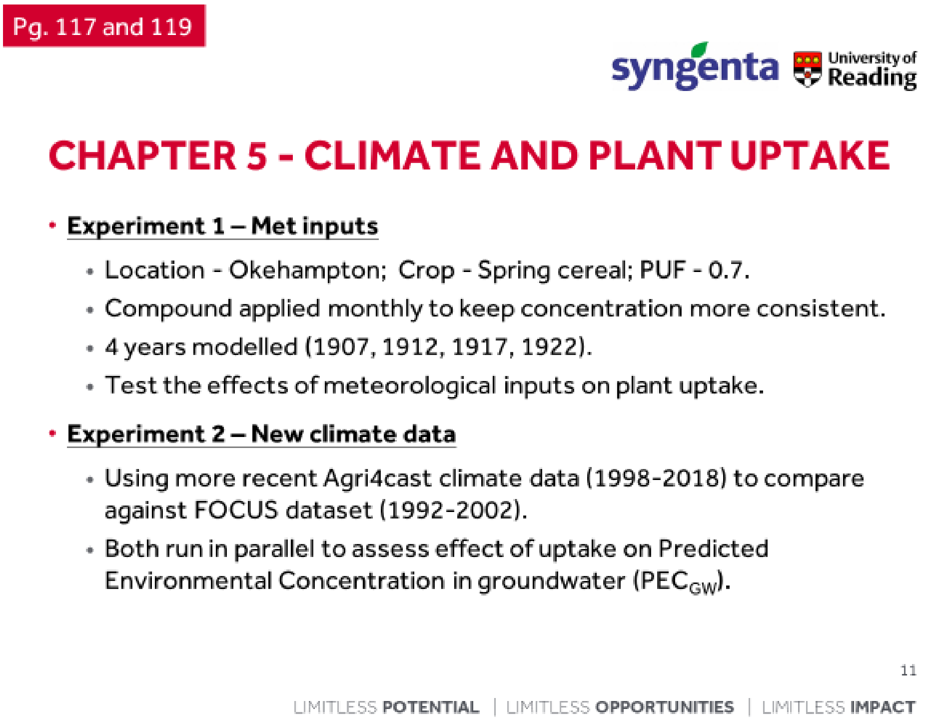
Pg. 79, 83 and 85

# KEY FINDINGS – CHAPTER 4

CA: $K_{OC}$ = 10, DT50 = 100

CB: $K_{OC}$ = 100, DT50 = 40

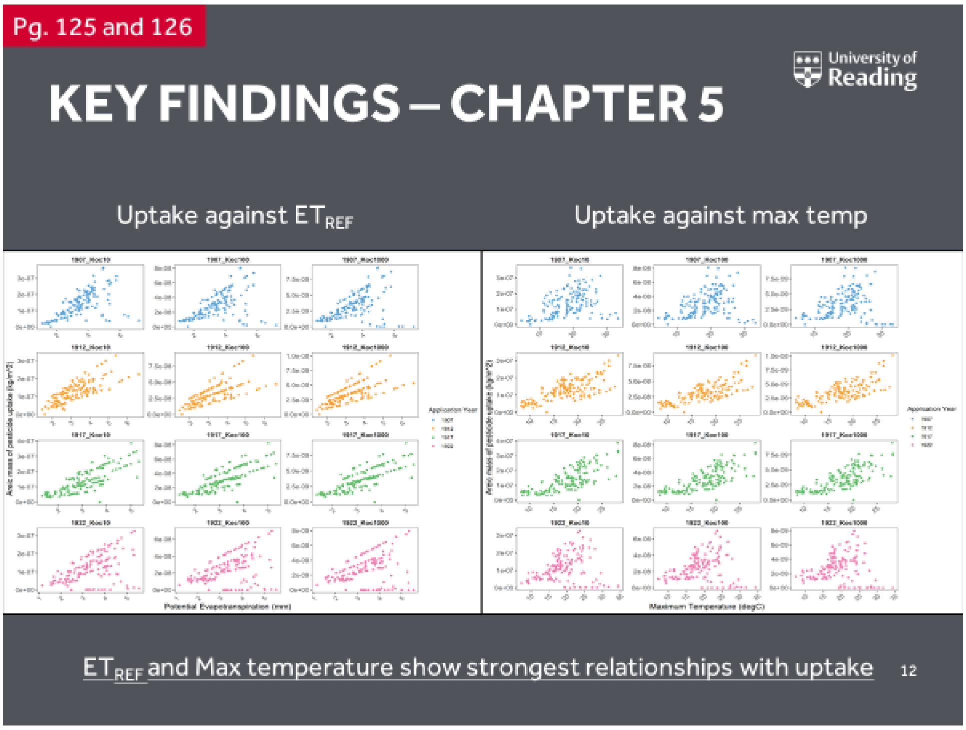
CC: $K_{OC}$ = 500, DT50 = 500



Pg. 117 and 119

# CHAPTER 5 - CLIMATE AND PLANT UPTAKE

- **Experiment 1 – Met inputs**
  - Location - Okehampton;  Crop - Spring cereal; PUF - 0.7.
  - Compound applied monthly to keep concentration more consistent.
  - 4 years modelled (1907, 1912, 1917, 1922).
  - Test the effects of meteorological inputs on plant uptake.
- **Experiment 2 – New climate data**
  - Using more recent Agri4cast climate data (1998-2018) to compare against FOCUS dataset (1992-2002).
  - Both run in parallel to assess effect of uptake on Predicted Environmental Concentration in groundwater ($PEC_{GW}$).



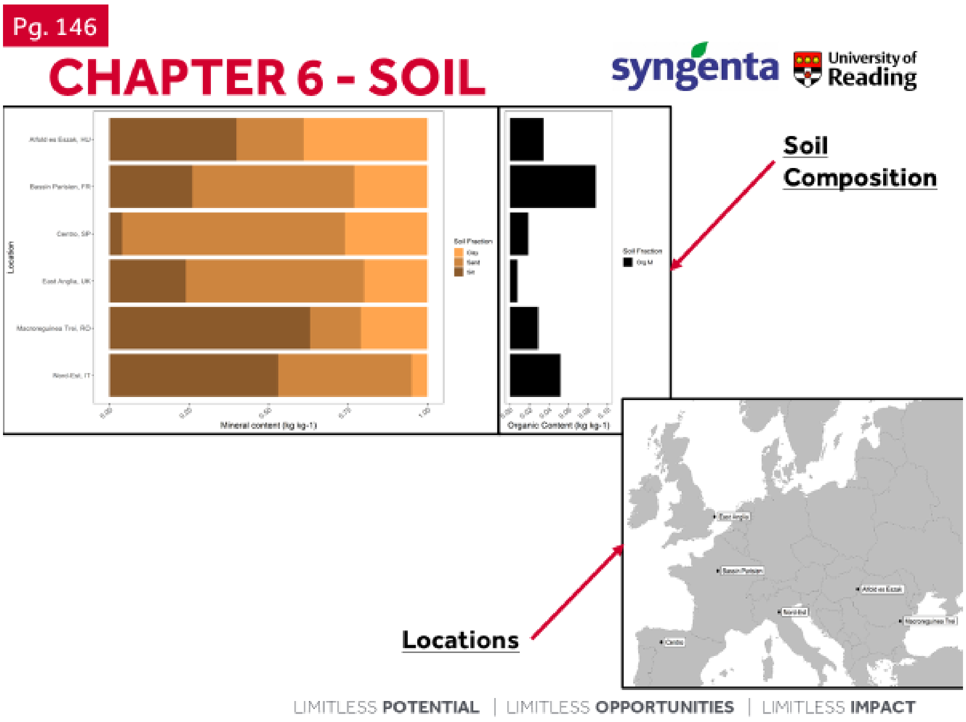
Pg. 125 and 126

# KEY FINDINGS – CHAPTER 5

Uptake against $ET_{REF}$

Uptake against max temp

$ET_{REF}$ and Max temperature show strongest relationships with uptake



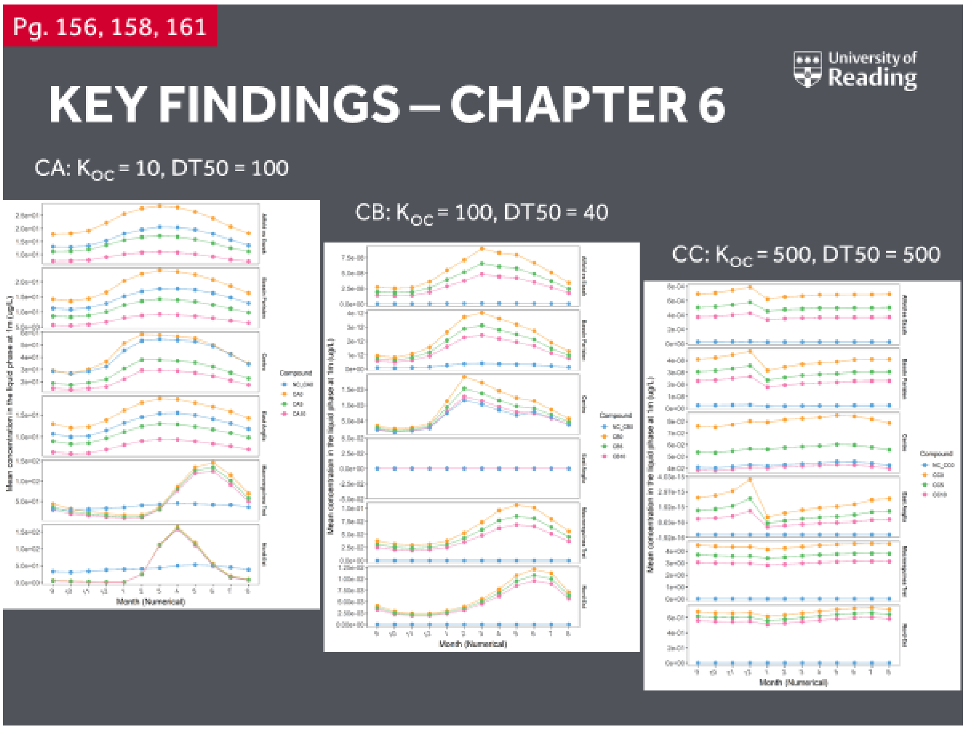
Pg. 146

## CHAPTER 6 - SOIL

Soil Composition

Locations

LIMITLESS POTENTIAL | LIMITLESS OPPORTUNITIES | LIMITLESS IMPACT



Pg. 156, 158, 161

## KEY FINDINGS – CHAPTER 6

CA: $K_{OC}$ = 10, DT50 = 100

CB: $K_{OC}$ = 100, DT50 = 40

CC: $K_{OC}$ = 500, DT50 = 500

# CONCLUDING REMARKS

- PUF is consistent over time, this was demonstrated by the strong linear relationship shown in experiment with [$^{14}$C]-1,2,4-Triazole.
- Recovery of radiolabelled compound was good in sand experiment but quick degradation of [$^{14}$C]-1,2,4-Triazole meant that PUF could not be derived.
- Environmental fate models PEARL and PELMO showed good agreement on leaching levels of applied compound but exhibited varied uptake behaviour during growing season.
- Potential evapotranspiration and maximum temperature showed the strongest relationship with plant uptake in PEARL.
- No greater effect of plant uptake in updated climate files but lower levels of leaching observed.
- 'Typical' scenarios showed a slightly lower effect of plant uptake in $PEC_{GW}$ but this was not statistically significant.

Image credit: Phil Jones

# ANY QUESTIONS?

# A1.2. SETAC 2019 Helsinki Poster

Department of Geography and Environmental Science

University of Reading

syngenta

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## Understanding plant root uptake of pesticides from hydroponic solution

Benjamin Jones | Sarah Webb | Anne Verhoef | Paul Sweeney | Christopher Collins

### Introduction

**Plant uptake is proposed to be a passive uptake process. Plant Uptake Factor (PUF) has been proposed as and alternative to Transpiration Stream Concentration Factor (TSCF)[1]. PUF measures plant root uptake as a rate of concentration change over the rate of volume change[2]. Previous PUF studies have been conducted over one time point and produced a single PUF value[2,3]. This study aims to test whether PUF remains constant over time by conducting harvests over a 21-day period using a topping up method. PUF will be calculated from both the solution and the plant material.**

### Methodology

Wheat (*Triticum aestivum* L.) seeds were germinated in sharp sand until BBCH 11, conditioned in 50% Hoagland's solution until BBCH 13, and treated with 1,2,4-Triazole in 100% Hoagland's solution. Each sample was topped up to the original weight daily with untreated solution.

**Figure. 1 Top left: Wheat prior to treatment, Bottom left: Plants prior to harvest on day 21. Right: Control plant following harvest on day 21.**

During harvest, plants were removed and cut at the seed prior to recording their weight. 1ml aliquots were taken of the remaining treatment solution in duplicate to analyse by Liquid Scintillation Counting (LSC). A root-wash was conducted using 25ml 4:1 Acetonitrile:Water. PUF was calculated using the below equations (Table 1), radioactivity detected in the root-wash was not considered to have been taken up by the plant and was therefore added back into the concentration of hydroponic solution (C, Table 1). Mass within the plant was calculated by combustion of the plant material. Purity of 1,2,4-Triazole was determined through Thin Layer Chromatography (TLC).

**Table 1. PUF was calculated using the below equations (Table 1). Where $C_0$ = Concentration in solution at the beginning of the experiment; C = Concentration in solution at the time point; v = total volume transpired by the plant at the measurement interval; $V_0$ = Constant volume; $M_0$ = Mass in the starting concentration, m = Total mass of substance in the plant**

| | |
|---|---|
| 1) PUF from solution | $PUF = \frac{\ln\left(\frac{C_0}{C}\right)}{\frac{v}{V_0}}$ |
| 2) PUF from plant material | $PUF = \frac{\ln\left(\frac{M_0}{M_0 - m}\right)}{\frac{v}{V_0}}$ |

### Results and Discussion

PUF measured from the remaining solution had an overall mean of 0.72, whereas PUF measured from plant material had an overall mean of 0.55. PUF measured from combustion of plant material had a reduced PUF over time, possibly due to metabolism of the treatment compound by the plant. Recovery of total radioactivity dropped below 95% for samples taken on Day 16 and 21, however the same effect was not observed in degradation controls, which were kept in the same conditions without a plant.

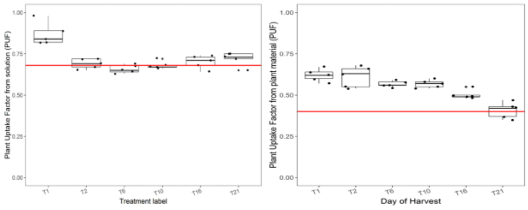

**Figure 1. Left: PUF calculated from solution. Right: PUF calculated from plant material. For both plots, the red line is the slope of the linear relationship of all samples within the dataset**

As purity of 1,2,4-Triazole reduced after for samples on days 16 and 21, the optimum period for measuring PUF was found to be from Day 1 – Day 10. Plant uptake across this period was deemed to be linear and therefore a linear regression was drawn between the fraction of change in the concentration ($C_0$/C or $M_0$/$M_0$-m, Table 1) and fraction of change in the volume (v/$V_0$, Table 1) over the treatment period. The slope of this relationship then becomes the PUF for the dataset.

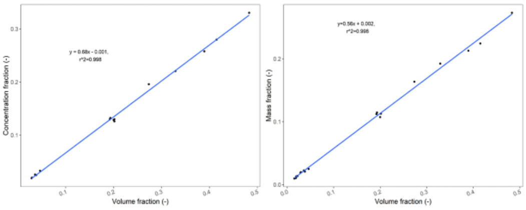

**Figure. 2. Left: PUF plotted as a linear relationship from analysis of radioactivity in solution. Right: PUF plotted as a linear relationship from analysis of radioactivity in plant material.**

### Conclusions

- Plant Uptake showed a linear relationship when measured over time.
- Optimum period for PUF measurement of 1,2,4-Triazole was between day 1-10 based on results from this study.

#### References

1. Author's name, *Book title*, (Publisher: Year) pp. XX-YY
2. Author's name, 'Article title', *Journal title*, publication info, pp. AA-BB
3. Researcher's name, Institution

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