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BEHAVIOUR OF PESTICIDES AND THEIR METABOLITES IN WATER, SOIL AND PLANTS - TRANSPORT PROCESSES INVESTIGATED USING LYSIMETER EXPERIMENTS

Dissertation

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DECLARATION

I hereby declare that the work in this dissertation is the result of my own investigation, except where otherwise stated. This dissertation, neither parts of it, have previously been accepted nor are simultaneously being submitted for any other degree.

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Wien, October 2020

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ABSTRACT

Pesticides are used in agriculture to assure sufficient food and feed supply. At the same time the environment is contaminated with potentially hazardous compounds. Pesticide pollution as a result of agricultural application is of growing concern.

Pesticides and their metabolites that show the greatest threats to groundwater were selected for this study. To investigate the environmental fate of bentazone, chloridazon, clothianidin, S-metolachlor and terbuthylazine and their main metabolites N-methyl-bentazone, desphenyl-chloridazon, methyl-desphenyl-chloridazon, metolachlor-ESA, metolachlor-OA, desethyl-terbuthylazine and 2-hydroxy-terbuthylazine different lysimeter experiments with a loamy sandy soil at the research station in Wagna (Styria, Austria) were conducted. The emphasis lies on the degradation in soil at different depths, the uptake from soil into maize and the leaching into groundwater.

The QuEChERS (quick, easy, cheap, effective, rugged and safe) method for analysing pesticide residues in soil and maize (leaves, roots and kernels) was adapted and validated. The quantification of pesticide concentrations in soil, maize and leachate was performed using a previous validated liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) which provides low detection limits.

A weighable, monolithic lysimeter and a backfilled, gravitation lysimeter were used for the pre-emergence application of chloridazon in 2010. The application of bentazone and terbutyhazine were performed post-emergence in 2010 and S-metolachlor and terbuthylazine were also applied post-emergence at the weighable, monolithic lysimeter in 2012. In 2012 clothianidin-coated maize seeds were planted on the surface of the weighable, monolithic lysimeter. In addition, repeated applications for S-metolachlor were performed in 2013 and 2014. All pesticides were applied on the lysimeter surfaces in form of commercial formulations. Soil and maize samples were collected and analysed at predetermined time intervals. Leachate was collected due to natural precipitation.

After the single application of chloridazon at the surface of the weighable, monolithic lysimeter and the backfilled, gravitation lysimeter, chloridazon and especially its polar metabolites desphenyl-chloridazon, methyl-desphenyl-chloridazon were continuously detected in the leachate over five years. The results obtained suggest persistence of chloridazon and its degradation products in soil. The parent compound chloridazon and its metabolite desphenyl-chloridazon were also detected in maize. Slight variations in concentrations amonst the different types of lysimeters were observed.

As expected, high concentrations, especially of the metabolites metolachlor-ESA and metolachlor-OA were detected in leachate. In a second terbuthylazine application, concentrations of desethyl-terbuthylazine were detected more frequently in leachate. In soil, bentazone degraded faster than terbuthylazine and S-metolachlor, whereas the metabolization of terbuthylazine after the second application resulted in an enhanced formation of desethyl-terbuthylazine and a highly increased hydroxylation of terbuthylazine. Clothianidin was detected in soil during the growing season of the treated seeds and remained in leachate years beyond the maize growing season.

Data of chloridazon and S-metolachlor as well as their metabolites obtained from the weighable, monolithic lysimeter were used for the PEARL model. The results of the simulation with PEARL show a good agreement with the measured water flow. A comparison of the simulated results and the leaching rates yielded adequate results for desphenyl-chloridazon, methyl-desphenyl-chloridazon, but rather poor correlation for chloridazon. While the simulated metolachlor-ESA leaching is quite close to the measured data, the metolachlor-

OA simulation results significantly overestimate the measured data. The dynamic of measured S-metolachlor leaching is not simulated very well. Discrepancies may be associated with preferential flow effects or sorption and degradation processes.

The validated analytical methods were successfully applied to determine the pesticides and metabolites in leachate, soil and maize. The results obtained suggest persistence and high dispersion of chloridazon and S-metolachlor, and especially their metabolites, in leachate and soil. Finally, it should be noted that the leaching of the pesticide and metabolites might be influenced by dry periods and rainfall events. The present investigations show the importance of analysing both parent compounds and metabolites in a long-term study under field conditions to better understand the transport of pesticides in soil-plant-water systems.

Keywords: Pesticides, Metabolites, Lysimeter, Soil, Leachate

KURZFASSUNG

In der Landwirtschaft werden Pflanzenschutzmittel eingesetzt um den steigenden Nahrungsmittelbedarfs zu decken. Dadurch gelangen aber auch giftige Wirkstoffe in die Umwelt. Die Besorgnis, dass durch die Aufbringung von Pflanzenschutzmitteln auf landwirtschaftliche Nutzflächen die Umwelt negativ beeinträchtigt wird, wächst.

Die vorliegende Dissertation beinhaltet ausgewählte Pestizide und Metaboliten die bereits im Grundwasser nachgewiesen werden konnten. Um das Verhalten von Bentazon, Chloridazon, Clothianidin, S-Metolachlor und Terbuthylazin und deren Metaboliten Bentazon-methyl, Chloridazon-desphenyl, Chloridazon-methyl-desphenyl, Metolachlor-ESA, Metolachlor-OA, Terbuthylazin-desethyl und Terbuthylazin-2-hydroxy zu untersuchen wurden verschiedene Lysimeterversuche an der Forschungsstation in Wagna (Steiermark, Österreich) durchgeführt. Von besonderem Interesse sind neben dem Verhalten der Pestizidwirkstoffe in unterschiedlichen Bodenschichten, die Aufnahme vom Boden in die Pflanzen sowie die Verlagerung ins Grundwasser.

Zur Bestimmung von Pflanzenschutzmittelrückständen im Boden und Mais (Grünanteil, Wurzelmaterial und Maiskörner) wurde die QuEChERS Probenvorbereitungsmethode adaptiert und validiert. Die Quantifizierung der Pestizide in Boden-, Mais- und Sickerwasserproben erfolgte mittels Flüssigchromatographie-Elektrospray-Ionisation-Tandemmassenspektrometrie (LC-ESI-MS/MS), wodurch niedrige Konzentrationsbereiche nachgewiesen werden können.

Im Frühjahr 2010 wurde der Pestizidwirkstoff Chloridazon direkt nach der Aussaat auf den Boden des wägbaren Präzisionslysimeter und des Gefäßlysimeters aufgetragen. Die Versuche mit Bentazon und Terbuthylazin wurden im Nachauflauf auch im Frühjahr 2010 durchgeführt. Im Frühjahr 2012 wurde ein mit Clothianidin gebeizter Mais am Präzisionslysimeter angebaut und es erfolgte die Aufbringung von S-Metolachlor und Terbuthylazin im Nachauflauf. Wiederholte Aufbringungen von S-Metolachlor fanden in den Jahren 2013 und 2014 statt. Alle Pestizidwirkstoffe wurden in Form von kommerziell erhältlichen Produkten auf die Lysimeterfläche aufgetragen. Im Laufe der Lysimeterstudie wurden Boden- und Pflanzenproben zu definierten Zeitpunkten entnommen. Sickerwasserproben wurden abhängig vom Niederschlag für die Analysen gesammelt.

Chloridazon und dessen polare Metababoliten Chloridazon-desphenyl und Chloridazon-methyl-desphenyl konnten durchgehend über fünf Jahre im Sickerwassser des Präzisionslysimeter und des Gefäßlysimeters nachgewiesen werden, obwohl der Wirkstoff nur einmal aufgebracht wurde. Die Ergebnisse zeigen die Langlebigkeit von Chloridazon und dessen Metaboliten im Boden. Rückstände von Chloridazon und Chloridazon-desphenyl wurden sogar im Mais nachgewiesen. Die gemessenen Konzentrationen zeigen Unterschiede aufgrund der Lysimetertypen auf.

Erwartungsgemäß wurden hohe Konzentrationen vor allem von den Metaboliten, Metolachlor-ESA und Metoalchlor-OA im Sickerwasser nachgewiesen. Durch die zweite Aufbringung von Terbuthyazin im Jahr 2012 konnten vermehrt Konzentrationen von Terbuthyazin-desethyl im Sickerwasser nachgewiesen werden. Im Boden wurde Bentazon schneller abgebaut als Terbuthylazin und S-Metolachlor, wobei die Metabolisierung von Terbuthylazin nach der zweiten Aufbringung ein häufigers Auftreten von Terbuthylazin-desethyl und einen vermehrten Anstieg der Hydoxylierung von Terbuthylazin zeigte. Clothianidin wurde im selben Jahr, in dem die gebeizten Samen gesät wurden, im Boden nachgewiesen und konnte Jahre über die Vegetationsperiode von Mais hinaus im Sickerwasser detektiert werden.

Die Messdaten von Chloridazon und S-Metolachlor als auch von deren Metaboliten wurden für die Modellierung mit PEARL verwendet. Die Simulationsergebnisse von PEARL zeigen eine gute Übereinstimmung mit dem gemessenen Wasserfluss. Ein Vergleich der Berechnungsergbnisse mit den gemessenen Konzentrationen im Sickerwasser zeigt eine angemessene Übereinstimmung für Chloridazon-desphenyl und Chloridazon-methyl-desphenyl, allerdings weichen die Ergebnisse für Chloridazon voneinander ab. Während die berechneten Konzentrationen von Metolachlor-ESA nahe an den gemessenen liegen, wird der Austrag von Metolachlor-OA eindeutig überschätzt. Die Ergebnisse von S-metolachlor zeigen keine gute Übereinstimmung. Abweichungen können auf präferentiellem Fluss sowie Sorptions- und Abbauprozesse zurückgeführt werden.

Die validierten analytischen Methoden zur Bestimmung der Pestizide und Metaboliten im Sickerwasser, Boden und Mais konnten erfolgreich angewandt werde. Die Ergebnisse weisen auf eine Persistenz und weitgehende Verteilung von Chloridazon und S-Metolachlor und vor allem deren Metaboliten im Sickerwasser und Boden hin. Anzumerken ist, dass Trockenperioden und Regenereignisse für die Verlagerung von Pestiziden und Metaboliten eine entscheidende Rolle spielen. Die vorliegenden Versuche zeigen deutlich, dass die Durchführung von Langzeitstudien mit Wirkstoffen und Metaboliten unter realen Bedingungen für ein besseres Verständnis von Transportprozessen unumgänglich ist.

Schlagworte: Pestizide, Metaboliten, Lysimeter, Boden, Sickerwasser

PUBLICATIONS IN CONTEXT WITH THIS STUDY

The thesis is based on four articles already published in scientific journals (peer-reviewed and SCI[1]-listed).

I. Fuhrmann A, Gans O, Weiss S, Haberhauer G, Gerzabek MH (2014) Determination of bentazone, chloridazon and terbuthylazine and some of their metabolites in complex environmental matrices by liquid chromatography-electrospray ionization-tandem mass spectrometry using a modified QuEChERS method: An optimization and validation study. Water Air Soil Pollut 225:1944-1959. DOI 10.1007/s11270-014-1944-7.

II. Schuhmann A, Gans O, Weiss S, Fank J, Klammler G, Haberhauer G, Gerzabek MH (2016) A long-term lysimeter experiment to investigate the environmental dispersion of the herbicide chloridazon and its metabolites - comparison of lysimeter types. J Soils Sediments 16:1032-1045. DOI 10.1007/s11368-015-1311-3.

III. Kupfersberger H, Klammler G, Schuhmann A, Brückner L, Kah M (2018) Modeling subsurface fate of S-metolachlor and metolachlor ethane sulfonic acid in the Westliches Leibnitzer Feld aquifer. Vadose Zone J 17:170030. DOI 10.2136/vzj2017.01.0030.

IV. Schuhmann A, Klammler G, Weiss S, Gans O, Fank J, Haberhauer G, Gerzabek MH (2019) Degradation and leaching of bentazone, terbuthylazine and S-metolachlor and some of their metabolites: A long-term lysimeter experiment. Plant Soil Environ 65:273–281. DOI.org/10.17221/803/2018-PSE.

Further publications and dissemination of the results during the dissertation period (papers not included in the thesis):

- Fuhrmann A, Gans O, Weiss S, Fank J, Gerzabek MH (2012) Lysimeter experiments to investigate the fate of chloridazon in leachate samples. 4th International Congress EUROSOIL 2012, 02 - 06 July 2012 in Bari, Italy.
- Fuhrmann A, Gans O, Weiss S, Fank J, Haberhauer G, Gerzabek MH (2014) Verhalten ausgewählter Pestizide im System Wasser-Boden-Pflanzen in den Lysimetern der Forschungsstation Wagna. Mitteilungen der Fachgruppe Umweltchemie und Ökotoxikologie, Gesellschaft Deutscher Chemiker, 20. Jahrgang, 3:65-67, ISSN 1618-3258.
- Fuhrmann A, Gans O, Weiss S, Fank J, Haberhauer G, Gerzabek MH (2014) Lysimeter experiments to study the behaviour of pesticides in soil, plant and leachate samples. – CASEE conference, 25 – 27 May 2014 at the University of Novi Sad, Serbia.

[1] Science citation index

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1 INTRODUCTION

1.1 Motivation

The contamination of soils and groundwater by organic pollutants, particularly pesticides, is still one of the most critical environmental problems. Although the environmental hazard associated with the observed concentrations is poorly defined, it is generally accepted that there is a need for action to minimize leaching losses within agricultural fields.

Since 2009 the Austrian Umweltbundesamt had worked on the special monitoring programme, which was carried out in the framework of the National Groundwater Monitoring System Ordinance on the monitoring of the status of water bodies (GZÜV 2006) to investigate the real groundwater pollution in Austia. In this study, selected groundwater and some river monitoring sites which are potentially threatened by pesticide contamination were monitored for active components of pesticides and their metabolites. The most frequently detected pesticides amongst others were bentazone, terbuthylazine, desethyl- terbutyhlazine, 2-hydroxy- terbuthylazine, chloridazon, desphenyl-chloridazon, methyl-desphenyl-chloridazon, S-metolachlor, metolachlor-ESA and metolachor-OA (Umweltbundesamt - Austria 2011). The findings especially of pesticide metabolites in groundwater have been unexpected and were related to parent pesticides that had been in use for several decades already.

The results of the special monitoring programme, the particular problem in Upper Austria caused by the pesticide bentazone as well as the discussion about the legislative situation in Europe in water and soil were essential for the selection of the pesticides bentazone, chloridazon and terbuthylazine, and some of their metabolites for the lysimeter experiment in 2010. According to the results of this lysimeter experiment and the ongoing discussion about the metabolites of metolachlor and metazachlor metabolites in Austria, the pesticides S-metolachlor and terbuthylazine were chosen for the lysimeter experiment in 2012. The upcoming topics of neonicotinoids such as imidacloprid, clothianidin and thiamethoxam, which are classified as being particularly harmful to bees lead to the decision that the lysimeter was seeded with clothianidin-treated maize in 2012.

The groundwater regulation in Austria refers to the European drinking water limit (0.1 µg L^{-1}) for pesticides and their relevant metabolites (EC 1998). In the meantime, an ordinance was issued by the Ministry of Health (BMG 2014) stating that metabolites need to be differentiated between relevant and non-relevant metabolites in drinking water according to the respective DG SANTE guidance (EC 2003). Thereupon action values in the range between 0.75 and 10 µg L^{-1} for non-relevant metabolites were determined. Amongst the selected pesticides the metabolites of chloridazon and metoalchlor were classified as non-relevant metabolites with an action value of 3 µg L^{-1} (BMG 2014). Official monitoring data of non-relevant metabolites of pesticides in groundwater in Germany and Austria have shown that the metabolites of chloridazon were occasionally measured at concentrations > 4.5 µg L^{-1} in groundwater (LUBW 2014; Umweltbundesamt-Austria 2011). Even through desphenyl-chloridazon, methyl-desphenyl-chloridazon are so-called non-relevant metabolites, the occurrence of these pesticide metabolites in ground and surface waters concerns regarding their environmental fate including toxicity, pesticide persistence and metabolite characteristics. Most agricultural areas in Austria are made up of silty loamy and loamy sandy soils. It is therefore interesting to investigate how the selected pesticides behave in the

agricultural soils used for crop production at different depth level and the possibility of contaminating ground water.

1.2 Aim of the study

The objective of this dissertation is to develop, adapt and validate analytical methods to determine bentazone, chloridazon, clothianidin, S-metolachlor and terbuthylazine and some of their metabolites in leachate, soil and maize. To further investigate the fate of the selected pesticides in leachate, soil and maize, different lysimeter experiments at the research station in Wagna were conducted.

The determination of pesticides and metabolites in soil and maize using the QuEChERS method as well as the quantification of the pesticide and metabolite residues in QuEChERS extracts and leachate is presented in Publication I (Fuhrmann et al. 2014). In this context, the QuEChERS method was optimized and validated to determine the pesticides and metabolites in soil and maize samples. The QuEChERS extracts obtained from soil and maize matrices and leachate samples were analysed by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS). In order to gain more knowledge about the selected pesticides and the soil of the lysimeter surfaces, batch sorption experiments for bentazone, chloridazon and terbuthylazine were performed. Publications II (Schuhmann et al. 2016) and IV (Schuhmann et al. 2019) addressed the quantification of the selected pesticides in soil, maize and leachate from differnet lysimeter experiments. In publication III (Kupfersberger et al. 2018) the detected leachate concentrations of S-metolachlor and metolachlor-ESA from the lysimeter experiments were used to calibrate the PEARL model.

The environmental fate of chloridazon and its metabolites using different types of lysimeters is presented in Schuhmann et al. (2016). It includes a detailed investigation of the herbicide chloridazon and its main metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon in leachate, soil and maize. The leaching through soil profiles, the distribution of the herbicides by downward movement in the different soil layers, and the translocation from soil into maize were studied. In addition, the PEARL model was used to simulate soil water dynamics and the pesticide fate using the field data from the weighable, monolithic lysimeter. Schuhmann et al. (2019) includes the degradation and leaching of bentazone, terbuthylazine and S-metolachlor and some of their metabolites in leachate and soil after different application rates at the weighable, monolithic lysimeter.

To provide an outline of the following thesis, first the properties of the selected pesticides are presented (chapter 1). Chapter 2 focused on the developed analytical methods for the determination of bentazone, chloridazon, clothianidin, S-metolachlor and terbuthylazine and some of their main metabolites N-methyl-bentazone, desphenyl-chloridazon, methyl-desphenyl-chloridazon, metolachlor-ESA, metolachlor-OA, desethyl-terbuthylazine and 2-hydroxy-terbuthylazine in leachate, soil and maize using LC–ESI–MS/MS. An overview of the sorption experiments for bentazone, chloridazon and terbuthylazine is given in chapter 3. In chapter 4, the lysimeter setups in Wagna, the application of the pesticides and the designed sampling regimes are described. The peer reviewed articles themselves (Publications I-IV) are included in the results and discussion chapter 5 together with the unpublished supplementary data. Supplementary data of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon measured in the leachate from 2010 to 2015, the lysimeter experiment with

clothianidin and the modeling approach with S-metolachlor and its metabolites is given. Following the final conclusions (chapter 6), limitations, further research challenges and outlook (chapter 7) the indexes (chapter 8) are included.

1.3 Pesticides and metabolites

Approximately 1 to 2.4 million tons of active substances are released into the environment worldwide each year (US EPA 2011). A large number of pesticides with a wide range of physico-chemical properties were introduced into the market. In Austria about 300 pesticides releasing approximately 700 metabolites of different persistence and mobility in soil were registered (BMLFUW 2013). Although the authorisation of plant protection products by the EU regulation 1107/2009 (EC 2009) is only granted if unacceptable effects of an active substance on the environment are excluded, the application can have undesirable consequences for the environment. At the time of the initial authorization, information on all possible transformation products is often not known.

Pesticides can be classified according to their target pest, their mode or period of action, or their chemical structure. Moreover, pesticides are considered by the EU as priority pollutants as they are highly noxious, long-term persistent, highly mobile throughout the environment and most of them also present carcinogenic properties (EC 1998). Once in the environment, metabolites were formed through transformation processes depending on properties inherent to the pesticide as well as physical, chemical and biological characteristics of the soil, climatic conditions and cultivation practices. The properties of the metabolites can greatly differ from those of the parent compound. Metabolites are often more polar, thermo-labile and less volatile and hence a greater risk for groundwater contamination (Fenner et al. 2013) due to their high water solubility and low adsorption to soils.

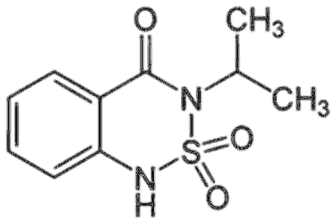
Agricultural soil is the first recipient of pesticides after their application. The fate of pesticides is generally governed by a variety of complex dynamic physical, chemical and biological processes, including sorption–desorption, volatilization, chemical and biological degradation, uptake by plants, run-off, and leaching (Arias-Estévez et al. 2008). These processes directly control the transport of pesticides within the soil and their transfer from the soil to water, air or food. The relative importance of these processes varies with the chemical nature of the pesticide (e.g. polarity, water solubility, volatility) and environmental properties (e.g. soil constituents, soil pH, in situ microorganisms, rain events, and climate). Some pesticides are degraded in the soil within a certain time. On the other hand, some degrade only slowly or are sequestered within soil particles by being inaccessible for microbial degradation. The mobility is decreased and long-term bound residues may be formed (Gevao et al. 2000). For many pesticides or their metabolites, soils became the prevalent source of pesticide pollution of groundwater through leaching and/or surface runoff. In general, the more mobile a pesticide is (high water solubility, low sorption potential), the higher is the groundwater contamination potential.

The different pesticides used in this study were selected based on their different physico-chemical properties such as mobility and polarity which are mainly responsible for persistence in soil and leaching into groundwater. Investigations are necessary to assess the fate of bentazone, chloridazon, clothianidin, S-metolachlor and terbuthylazine in the environment. In the following the pesticides of this study are shortly introduced.

1.3.1 Bentazone

Bentazone is a weakly acidic herbicide and belongs to the thiodiazine family. Bentazone is widely used for post-emerge control of sedges and broadleaf weeds in soybeans, rice, maize, peanuts, mint and peas. Bentazone is approved for use in the EU. The pesticide is used, either in combination with other active ingredients or alone, formulated as a soluble concentrate or granules, mixed with water and applied as a spray (Lewis et al. 2016). Bentazone is highly mobile and moderate persistent herbicide (Table 1.1). The sorption of bentazone in agricultural topsoil has been described using either a linear isotherm (Li et al. 2003) or the Freundlich isotherm (Gaston et al. 1996; Boivin et al. 2005).

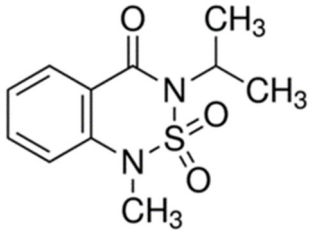
Table 1.1: Structure and physiochemical properties of bentazone (Lewis et al. 2016)

Common name	Bentazone
IUPAC name	3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
CAS number	25057-89-0
Chemical structure	
Molecular formula	$C_{10}H_{12}N_2O_3S$
Molecular weight (g mol^{-1})	240.28
Solubility in water at 20°C (mg L^{-1})	7112
Octanol-water partition coefficient (pH 7, 20°C)	3.47 x 10^{-1}
Dissociation constant (pK_a)	3.5
K_{oc} (mL g^{-1})	55.3
K_{foc} (mL g^{-1})	59.6
DT_{50} soil - lab at 20°C [days]	20
DT_{50} soil - field [days]	7.5
DT_{50} water - sediment [days]	716
DT_{50} water [days]	80

Bentazone is biodegraded mainly by the hydroxylation of the 6- or 8-position of the phenyl ring to form 6-OH-bentazone or 8-OH bentazone. It is difficult to identify these metabolites, because both are further metabolized rapidly (Huber and Otto 1994). Known metabolites in soil are 2-amino-N-isopropylbenzamide (AIBA, CAS 30391-89-0) and 2-aminobenzoic acid. AIBA is produced by hydrolysis of the sulphamide function, while 2-aminobenzoic acid is a product of AIBA. The most stable metabolite of bentazone in soil is N-methyl-bentazone which is very prone to microbially-mediated degradation (Wagner et al. 1996; Table 1.2).

Table 1.2: Structure and physiochemical properties of N-methyl-bentazone (Lewis et al. 2016)

Common name	N-methyl-bentazone
IUPAC name	n.a.

CAS number	61592-45-8
Chemical structure	
Molecular formula	$C_{11}H_{14}N_2O_3S$
Molecular weight (g mol^{-1})	254.31
Solubility in water at 20°C (mg L^{-1})	n.a.
Octanol-water partition coefficient (pH 7, 20°C)	n.a.
Dissociation constant (pK$_a$)	n.a.
K$_{oc}$ (mL g^{-1})	n.a.
K$_{foc}$ (mL g^{-1})	257.5
DT$_{50}$ soil - lab at 20°C [days]	55.8
DT$_{50}$ soil - field [days]	n.a.
DT$_{50}$ water - sediment [days]	n.a.
DT$_{50}$ water [days]	n.a.

n.a. not available

1.3.2 Chloridazon

Chloridazon is used as a selective systemic herbicide which inhibits photosynthesis and it is used for pre-planted, pre-emergence, and early post-emergence for weed control, particularly on sugar beet crops, fodder beet and beetroot (EPA 2005). Choridazon is approved for use in the EU. Chloridazon has a moderate aqueous solubility and is moderately persistent in soil (Table 1.3). In addition, chloridazon is absorbed predominantly by the roots, with translocation to all plant parts (Lewis et al. 2016).

The persistence of chloridazon in the environment has been reported to range from 8.6 to 187.6 days (half-lives) depending on soil type, moisture content and temperature (EFSA 2007). In soil, microbial degradation generates the two major metabolites desphenyl-chlordiazon and methyl-desphenyl-chloridazon (Table 1.4 and Table 1.5). The dephenylated degradation product, desphenyl-chloridazon, is found as the major degradation product (EPA 2005; Buttiglieri et al. 2009). Weber et al. (2007) identified methyl-desphenyl-chloridazon as another degradation product. Both metabolites hava a great potential to leach and pollute surface and groundwater.

Table 1.3: Structure and physiochemical properties of chloridazon (Lewis et al. 2016)

Common name	Chloridazon
IUPAC name	5-amino-4-chloro-2-phenylpyridazin-3(2H)-one
CAS number	1698-60-8

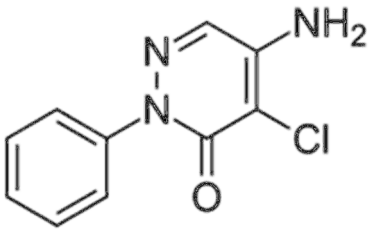
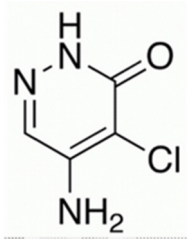
Chemical structure	
Molecular formula	$C_{10}H_8ClN_3O$
Molecular weight (g mol^{-1})	221.65
Solubility in water at 20°C (mg L^{-1})	422
Octanol-water partition coefficient (pH 7, 20°C)	1.55 x 10^1
Dissociation constant (pK_a)	3.38
K_{oc} (mL g^{-1})	120
K_{foc} (mL g^{-1})	199
DT_{50} soil - lab at 20°C [days]	43.1
DT_{50} soil - field [days]	34.7
DT_{50} water - sediment [days]	137
DT_{50} water [days]	51.5

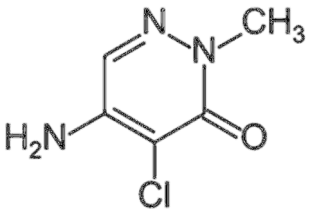
Table 1.4: Structure and physiochemical properties of desphenyl-chloridazon (Lewis et al. 2016)

Common name	Desphenyl-chloridazon
IUPAC name	5-amino-4-chloro-pyridazine-3-one
CAS number	6339-19-1
Chemical structure	
Molecular formula	$C_4H_4ClN_3O$
Molecular weight (g mol^{-1})	145.55
Solubility in water at 20°C (mg L^{-1})	n.a.
Octanol-water partition coefficient (pH 7, 20°C)	n.a.
Dissociation constant (pK_a)	n.a.
K_{oc} (mL g^{-1})	n.a.
K_{foc} (mL g^{-1})	50
DT_{50} soil - lab at 20°C [days]	106.3
DT_{50} soil - field [days]	235.5

DT_{50} water - sediment [days]	n.a.
DT_{50} water [days]	n.a.

n.a. not available

Table 1.5: Structure and physiochemical properties of methyl-desphenyl-chloridazon (Lewis et al. 2016; Dechene et al. 2014[a])

Common name	Methyl-desphenyl-chloridazon
IUPAC name	5-amino-4-chloro-2-methylpyridazin2-3-one
CAS number	17254-80-7
Chemical structure	
Molecular formula	$C_5H_6ClN_3O$
Molecular weight (g mol^{-1})	159.6
Solubility in water at 20°C (mg L^{-1})	730[a]
Octanol-water partition coefficient (pH 7, 20°C)	4.17 x 10^{-2}
Dissociation constant (pK_a)	n.a.
K_{oc} (mL g^{-1})	n.a.
K_{foc} (mL g^{-1})	92
DT_{50} soil - lab at 20°C [days]	143.8
DT_{50} soil - field [days]	n.a.
DT_{50} water - sediment [days]	n.a.
DT_{50} water [days]	n.a.

n.a. not available

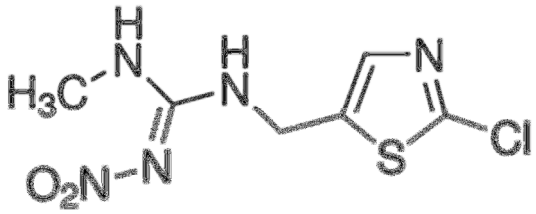
1.3.3 Clothianidin

Clothianidin is a nitroguanidine neonicotinoid pesticide used in many crops to control various insects. As a systemic insecticide, clothianidin can be used as a soil or foliar spray or as a seed treatment. The use of clothianidin as a seed treatment in maize has gained wide acceptance in an effort to protect crops against the maize rootworm and European maize borer. Clothianidin is moderately soluble and volatile but has a high potential for leaching to groundwater (Lewis et al. 2016; Table 1.6).

In 2013 environmental risk assessments of three neonicotinoids by the European Food Safety Authority resulted in the European Union placing restrictions on the use of thiamethoxam, clothianidin and imidacloprid (EFSA 2013) because of the potential risk for honey bees and other pollinators. Recently these restrictions were extended indefinitely following reassessment of these compounds (EFSA 2018).

Clothianidin is the main metabolite of thiamethoxam which metabolizes quickly into clothianidin in plants (Nauen et al. 2003; Klein 2003), and soil (Bonmartin et al. 2015). Clothianidin and thiamethoxam are structurally similar. Clothianidin has a higher lipophilicity than thiamethoxam based on water solubility and the partition coefficient. In addition, clothianidin has been shown to be resistant to hydrolysis at environmental pH-values and temperatures, and metabolic degradation occurred very slowly in aerobic soil (EPA 2003). Clothianidin metabolism in plants has been evaluated in a variety of crops, including maize and sugar beet, apples, and tomatoes (EFSA 2010).

Table 1.6: Structure and physiochemical properties of clothianidin (Lewis et al. 2016)

Common name	Clothianidin
IUPAC name	(E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CAS number	210880-92-5
Chemical structure	
Molecular formula	$C_6H_8ClN_5O_2S$
Molecular weight (g mol^{-1})	249.7
Solubility in water at 20 °C (mg L^{-1})	340
Octanol-water partition coefficient (pH 7, 20°C)	8.04 x 10^0
Dissociation constant (pK_a)	11.1
K_{oc} (mL g^{-1})	123
K_{foc} (mL g^{-1})	160
DT_{50} soil - lab at 20°C [days]	545
DT_{50} soil - field [days]	121.2
DT_{50} water - sediment [days]	56.4
DT_{50} water [days]	40.3

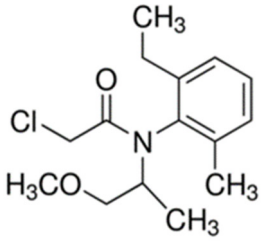
1.3.4 Metolachlor

Metolachor is a selective chloroacetamide herbicide used in agriculture to control broadleaf weeds and annual grasses, primarily in maize, soybean and sorghum. Metolachlor was first introduced into the market as a racemic product which contained four isomers, an R isomer pair and an S isomer pair (typically 50:50, S:R). Current formulations are based primarily on the S-metolachlor isomer (at least 80:20, S:R) and the racemic metolachlor product was banned by the European Union in 2002 (EC 2002). At the same application level S-metolachlor is more active on a gram for gram basis due to the enrichment with the S-isomer and thus reduces the load of herbicides applied to the field (Shaner et al. 2006; Spindler et al. 1998; Zemolin et al. 2014). Most analytical techniques measure all isomers together because

only the use of specific equipment, such as a chiral column, makes it possible to distinguish between them (Klein et al. 2006).

Metolachlor has the potential to leach to groundwater because of its relatively high water solubility (Lewis et al. 2016; Table 1.7; Table 1.8). The prominent metabolites metolachlor ethane-sulfonic acid (ESA) and oxanilic acid (OA) are well known groundwater contaminants (Kalkhoff et al. 1998; Kolpin et al. 2000; Reemtsma et al. 2013) and differ in their formation, chemical properties and environmental persistence (Table 1.9 and Table 1.10).

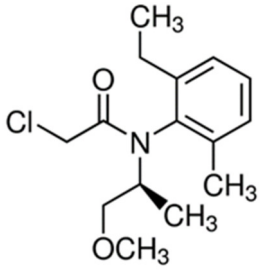
Table 1.7: Structure and physiochemical properties of metolachlor (Lewis et al. 2016)

Common name	Metolachlor
Compound number	CGA 24705
IUPAC name	2-chloro-N-(6-ethyl-o-tolyl)-N-[(1RS)-2-methoxy-1-methylethyl]acetamide
CAS number	51218-45-2
Chemical structure	
Molecular formula	$C_{15}H_{22}ClNO_2$
Molecular weight (g mol^{-1})	283.79
Solubility in water at 20°C (mg L^{-1})	530
Octanol-water partition coefficient (pH 7, 20°C)	2.51 x 10^3
Dissociation constant (pK_a)	n.a.
K_{oc} (mL g^{-1})	120
K_{foc} (mL g^{-1})	163
DT_{50} soil - lab at 20°C [days]	15
DT_{50} soil - field [days]	21
DT_{50} water - sediment [days]	365
DT_{50} water [days]	88

n.a. not available

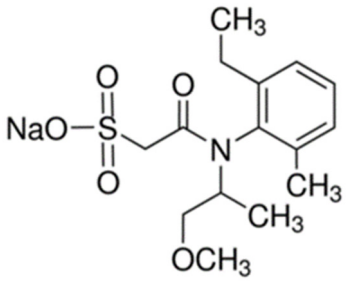
Table 1.8: Structure and physiochemical properties of S-metolachlor (Lewis et al. 2016)

Common name	S-metolachlor
Compound number	CGA 77102
IUPAC name	2-Chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl]acetamide
CAS number	87392-12-9
Chemical structure	

	
Molecular formula	$C_{15}H_{22}ClNO_2$
Molecular weight (g mol^{-1})	283.79
Solubility in water at 20°C (mg L^{-1})	480
Octanol-water partition coefficient (pH 7, 20°C)	1.12 x 10^3
Dissociation constant (pK_a)	n.a.
K_{oc} (mL g^{-1})	n.a.
K_{foc} (mL g^{-1})	200.2
DT_{50} soil - lab at 20°C [days]	51.8
DT_{50} soil - field [days]	23.17
DT_{50} water - sediment [days]	43.3
DT_{50} water [days]	9.0

n.a. not available

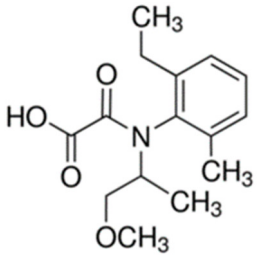
Table 1.9: Structure and physiochemical properties of metolachlor-ESA (Lewis et al. 2016)

Common name	Metolachlor - ESA
Compound number	CGA 354743
IUPAC name	2-[2-ethyl-N-(1-methoxypropan-2-yl)-6-methylanilino]-2-oxoethanesulfonic acid
CAS number	171118-09-5
Chemical structure	
Molecular formula	$C_{15}H_{23}NO_5S$
Molecular weight (g mol^{-1})	329.41
Solubility in water at 20°C (mg L^{-1})	212,461
Octanol-water partition coefficient (pH 7, 20 °C)	1.29 x 10^{-2}
Dissociation constant (pK_a)	n.a.

K_{oc} (mL g^{-1})	9
K_{foc} (mL g^{-1})	n.a.
DT_{50} soil - lab at 20°C [days]	235
DT_{50} soil - field [days]	n.a.
DT_{50} water - sediment [days]	n.a.
DT_{50} water [days]	n.a.

n.a. not available

Table 1.10: Structure and physiochemical properties of metolachlor-OA (Lewis et al. 2016)

Common name	Metolachlor-OA
Compound number	CGA 51202
IUPAC name	N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-oxalamic acid
CAS number	152019-73-3
Chemical structure	
Molecular formula	$C_{15}H_{21}NO_4$
Molecular weight (g mol^{-1})	279.33
Solubility in water at 20°C (mg L^{-1})	360000
Octanol-water partition coefficient (pH 7, 20°C)	n.a.
K_{oc} (mL g^{-1})	17
K_{foc} (mL g^{-1})	18.3
Dissociation constant (pK_a)	n.a.
DT_{50} soil - lab at 20°C [days]	325
DT_{50} soil - field [days]	n.a.
DT_{50} water - sediment [days]	n.a.
DT_{50} water [days]	n.a.

n.a. not available

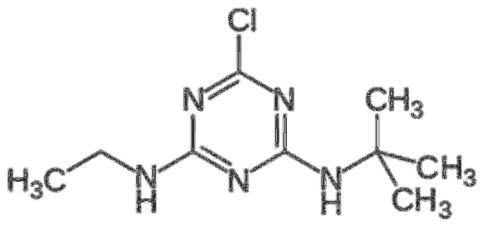
1.3.5 Terbuthylazine

Terbuthylazine is an s-triazine herbicide widely used in agriculture to control grass in maize and sorghum cultures. Terbuthylazine was introduced as a hydrophobic and less mobile alternative to atrazine, which has been banned by the Austrian government in 1995 (BMLFUW 2012). However, a similar potential for terbuthylazine as groundwater contaminant has been identified (Dousset et al. 1997; Gerstl et al. 1997). Terbuthylazine is

weakly basic, and under circumneutral soil pH conditions present as neutral, moderately hydrophobic (Table 1.11) species capable of hydrophobic interactions with soil.

The major degradation products of terbuthylazine are desethyl-terbuthylazine (Table 1.12), 2-hydroxy-terbuthylazine (Table 1.13) and 2-hydroxy-desethyl-terbuthylazine. Microbial degradation of triazines proceeds mainly via dealkylation, hydroxylation and ring cleavage of the parent compound. Terbuthylazine and its metabolite desethyl-terbuthylazine are highly mobile and have been frequently detected in surface and ground water (Bozzo et al. 2013; Stipicevic et al. 2015; Guzzella et al. 2003). The European Food Safety Authority has reported that terbuthylazine poses high long-term risks for organisms (EFSA 2011).

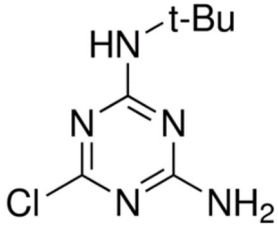
Table 1.11: Structure and physiochemical properties of terbuthylazine (Lewis et al. 2016)

Common name	Terbuthylazine
IUPAC name	*N*2-tert-butyl-6-chloro-*N*4-ethyl-1,3,5-triazine-2,4-diamine
CAS number	5915-41-3
Chemical structure	
Molecular formula	$C_9H_{16}ClN_5$
Molecular weight (g mol^{-1})	229.71
Solubility in water at 20°C (mg L^{-1})	6.6
Octanol-water partition coefficient (pH 7, 20°C)	2.51 x 10^3
Dissociation constant (pK_a)	1.9
K_{oc} (mL g^{-1})	n.a.
K_{foc} (mL g^{-1})	231
DT_{50} soil - lab at 20°C [days]	72
DT_{50} soil - field [days]	21.8
DT_{50} water - sediment [days]	70
DT_{50} water [days]	6.0

n.a. not available

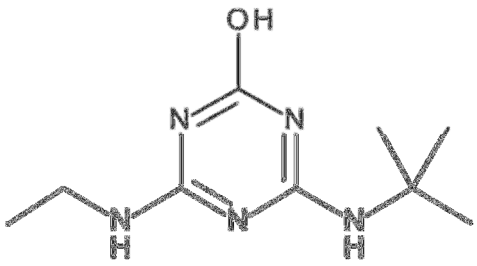
Table 1.12: Structure and physiochemical properties of desethyl-terbuthylazine (Lewis et al. 2016)

Common name	Desethyl-terbuthylazine
IUPAC name	N-tert-butyl-6-chloro-[1,3,5]triazine-2,4-diamine
CAS number	30125-63-4

Chemical structure	
Molecular formula	$C_7H_{12}ClN_5$
Molecular weight (g mol^{-1})	201.68
Solubility in water at 20°C (mg L^{-1})	327.1
Octanol-water partition coefficient (pH 7, 20°C)	2.0 x 10^2
Dissociation constant (pK_a)	n.a.
K_{oc} (mL g^{-1})	n.a.
K_{foc} (mL g^{-1})	78
DT_{50} soil - lab at 20°C [days]	54
DT_{50} soil - field [days]	28.6
DT_{50} water - sediment [days]	n.a.
DT_{50} water [days]	n.a.

n.a. not available

Table 1.13: Structure and physiochemical properties of 2-hydroxy-terbuthylazine (Lewis et al. 2016; 2016a; Kaune et al. 1998[b])

Common name	2-hydroxy-terbuthylazine
IUPAC name	4-tert-butylamino-6-ethylamino-[1,3,5]triazin-2-ol
CAS number	66753-07-9
Chemical structure	
Molecular formula	$C_9H_{17}N_5O$
Molecular weight (g mol^{-1})	211.33
Solubility in water at 20°C (mg L^{-1})	7.19
Octanol-water partition coefficient (pH 7, 20°C) (log P)	1.5[b]
Dissociation constant (pK_a)	n.a.
K_{oc} (mL g^{-1})	n.a.
K_{foc} (mL g^{-1})	187

DT_{50} soil - lab at 20°C [days]	559
DT_{50} soil - field [days]	n.a.
DT_{50} water - sediment [days]	n.a.
DT_{50} water [days]	n.a.

n.a. not available

2 ANALYTICAL METHODS

2.1 Determination of bentazone, chloridazon and terbuthylazine and some of their metabolites in complex environmental matrices by liquid chromatography-electrospray ionization-tandem mass spectrometry using a modified QuEChERS method: An optimization and validation study (Publication I)

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Clarification

The publication was written and prepared by Andrea Schuhmann (maiden name Fuhrmann). Comments of co-authors were included in the revised manuscript.

Determination of bentazone, chloridazon and terbuthylazine and some of their metabolites in complex environmental matrices by liquid chromatography-electrospray ionization-tandem mass spectrometry using a modified QuEChERS method: An optimization and validation study

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Abstract In a study on the behaviour of pesticides in a soil-plant-water system, the QuEChERS (quick, easy, cheap, effective, rugged and safe) method for analysing pesticide or metabolite residues in soil and maize (leaves, roots and kernels) was optimized and validated. The pesticides bentazone, chloridazon and terbuthylazine and their metabolites bentazone-methyl, chloridazon-desphenyl, chloridazon-methyl-desphenyl, terbuthylazine-desethyl and terbuthylazine-2-hydroxy were selected in this study. The QuEChERS extracts obtained from soil and maize matrices and the collected leachate were analysed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) using a HPLC and an UHPLC analytical column. As expected, shorter run times and higher sensitivity were achieved with the UHPLC column. Validation studies focused on recovery, repeatability, matrix effects, limits of detection and quantification. Recoveries (and repeatability RSD) of the spiked samples were in the range of 55 to 98% (7.4-18) in soil, 23 to 101% (1.7-20) in maize and 82 to 105% (4.4-25) in leachate. Quantification limits were lower than 3.0 µg kg^{-1} in soil, 7.3 µg kg^{-1} in maize and 0.080 µg L^{-1} in leachate.

Keywords Pesticides; Metabolites; Soil; Leachate; Maize; QuEChERS

1 Introduction

Pesticides are ingredients of plant protection products used in agriculture to increase productivity. The use of pesticides for weed control on agricultural fields often leads to the contamination of soil, plants and water. Residues of commonly used pesticides and their metabolites can be detected in the environment for years. Following their application, pesticides undergo a variety of transformations that give rise to a complex pattern of metabolites. The presence of metabolites raises particular concern, as they can exist at higher levels than the parent pesticides (Andreu and Picó 2004). A good example is the parent compound atrazine and its associated metabolites (Kolpin et al. 1998).

To monitor pesticides in soil and plant material an appropriate sample preparation method is required which assures the comprehensive extraction of the pesticides of interest. Traditionally, soxhlet extraction (Prados-Rosales et al. 2002; US EPA 1996) or alternatively

pressurized liquid extraction (PLE) (Henriksen et al. 2002; Dagnac et al. 2005) are used to analyse pesticides in soils. QuEChERS (an acronym for quick, easy, cheap, effective, rugged and safe) is a sample preparation method based in dispersive liquid-liquid partitioning with acetonitrile followed by a dispersive SPE clean up, first introduced by Anastassiades et al. (2003) for a broad range of pesticide residues in fruits and vegetables. Since then, the acetate buffering version has gained the distinction of becoming the AOAC Official Method 2007.01 (Lehotay 2007) and the citrate buffering version was released by the European Committee for Standardization as Standard Method EN 15662 (CEN 2008). The QuEChERS multiresidue procedure replaces previously complicated analytical steps, increasing sample throughput and reducing material costs. The method is frequently used for the extraction of a wide variety of compounds in different matrices as modifications can be implemented easily. Lehotay (2007) stated that, except those relatively few that contain carboxylic acid groups, nearly all pesticides can be monitored by the QuEChERS method. The effectiveness of the method for extracting pesticides from different food matrices is well documented (Cunha et al. 2007; Garrido-Frenich et al. 2008; Lehotay et al. 2005; Lehotay 2007; Lesueur et al. 2008; Payá et al. 2007). The QuEChERS method has also been applied to the analysis of veterinary drugs (Stubbings and Bigwood 2009), mycotoxins (Sospedra et al. 2010; Vaclavik et al. 2010; Zachariasova et al. 2010) plus soil analysis for pesticides (Lesueur et al. 2008; Rashid et al. 2010; Zhang et al. 2012), phenols (Padilla-Sánchez et al. 2010) and chlorinated compounds (Pinto et al. 2010).

Analytical methods to determine pesticides and/or metabolites have improved, making it possible to detect low residue levels in complex environmental matrices. In water analysis, liquid-liquid extraction (LLE) (Galeano-Díaz et al. 2008) or the alternative solid-phase-extraction (SPE) (Kuster et al. 2006) is widely used as a pre-concentration step to provide the sensitivity required for LC-MS/MS or GC-MS/MS. Due to the high sensitivity and selectivity of tandem mass spectrometers, various classes of pesticides can be determined by direct injection. The use of direct injection LC-MS/MS is now widely accepted for pesticide analysis in water (Kuster et al. 2006; Reemtsma et al. 2013). Recently, an attractive alternative to using high performance liquid chromatography (HPLC) known as ultra high performance liquid chromatography (UHPLC) has been developed, whereby the diameter and the particle size of the chromatographic columns are decreased, the run time reduced and the resolution enhanced. Compared to conventional HPLC, the instrumentation is operated at high pressures and mobile phases at high velocities are used (Kmellár et al. 2011; Kowal et al. 2009; Wode et al. 2012). The potential for matrix effects when using HPLC/UHPLC connected to a tandem mass spectrometer via an electrospray ionization interface (ESI) should be considered. Matrix effects induce the suppression or enhancement of the analyte response due to co-eluting compounds. The influence of the co-eluting compounds occurs during the analyte ionization process, before the analyte ion reaches the high vacuum of the mass analyser (Kruve et al. 2008; Niessen et al. 2006).

The pesticides selected for this study were bentazone, chloridazon and terbuthylazine as well as their metabolites bentazone-methyl, chloridazon-methyl-desphenyl, chloridazon-desphenyl, terbuthylazine-desethyl and terbutylazine-2-hydroxy. Bentazone, chloridazon and terbuthylazine are in widespread agricultural use. Their metabolites are usually more polar, and thus pose a greater potential risk of groundwater contamination (Loos et al. 2010). The selection has a wide range of physico-chemical properties (Table 1) and some of them are particularly challenging to analyse (e.g. chloridazon-desphenyl).

The present study focuses on the optimization and validation of the QuEChERS method for the determination of the pesticides and metabolites described above in soil and maize. The quantification of the pesticide and metabolite residues in QuEChERS extracts and leachate samples was performed using HPLC-MS/MS and UHPLC-MS/MS with electrospray ionization (ESI). To the best of our knowledge, QuEChERS has not previously been used to extract the selected pesticides or metabolites from the extremely pertinent matrices of soil and maize.

2 Material and Methods

2.1 Chemicals and Standards

Pesticide standards (bentazone, bentazone-methyl, chloridazon, chloridazon-desphenyl, chloridazon-methyl-desphenyl, terbuthylazine, terbuthylazine-desethyl, terbuthylazine-2-hydroxy) and isotopically labelled internal standards (bentazone-d_6, chloridazon-d_5, chloridazon-desphenyl-$15N_2$, terbuthylazine-d_5, terbuthylazine-desethyl-d_9) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). All organic solvents were of high performance liquid chromatography (HPLC) grade. Acetonitrile (ACN) and water (HPLC) were obtained from LGC PromoChem (Wesel, Germany). Methanol (MeOH) was purchased from VWR (Vienna, Austria). Formic acid (98-100%), acetic acid and ammonium acetate, all of analytical grade, were obtained from Merck (Darmstadt, Germany). Ultra-pure water was produced in the laboratory with a Milli-Q gradient system produced by Millipore (Vienna, Austria). Anhydrous magnesium sulphate and sodium citrate dibasic sesquihydrate in powder form were obtained from Sigma Aldrich (Vienna, Austria). Sodium citrate dehydrate, sodium chloride and calcium chloride were purchased from Merck (Darmstadt, Germany). Primary secondary amine (PSA) sorbent was obtained from Varian (Palo Alto, CA, USA) and C_{18} from J.T. Baker was purchased from Bartelt (Vienna, Austria). Disposable syringe filters (Chromafil PTFE 0.45 µm) were purchased from Macherey-Nagel (Düren, Germany) and syringes (2 ml) from B. Braun (Melsungen, Germany).

A solution of 5% formic acid (v/v) was prepared in ACN. The salts used for the initial extraction step were prepared by mixing 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g trisodium citrate dihydrate (Na_3Citrate x $2H_2O$) and 0.5 g disodium hydrogencitrate sesquihydrate (Na_2HCitrate x 1.5 H_2O). Several sorbent combinations were filled in 15 ml centrifuge tubes for the clean up: 150 mg PSA and 950 mg anhydrous $MgSO_4$, 300 mg PSA and 300 mg $CaCl_2$, 150 mg PSA, 900 mg anhydrous $MgSO_4$, and 150 mg C_{18}.

Table 1 Physico-chemical properties of the selected pesticides

Compound	Bentazone	Bentazone-methyl	Chloridazon	Chloridazon-deshenyl	Chloridazon-methyl-desphenyl	Terbuthylazine	Terbuthylazine-desethyl	Terbuthylazine-2-hydroxy
CAS number	25057-89-0	61592-45-8	1698-60-8	6339-19-1	17254-80-7	5915-41-3	30125-63-4	66753-07-9
Molar mass [g mol^{-1}]	240.28	240.28	221.65	145.55	159.51	229.71	201.66	211.27
Chemical formula	$C_{10}H_{12}N_2O_3S$	$C_{11}H_{14}N_2O_3S$	$C_{10}H_8ClN_3O$	$C_4H_4ClN_3O$	$C_5H_6ClN_3O$	$C_9H_{16}ClN_5$	$C_7H_{12}ClN_5$	$C_9H_{17}N_5O$
Solubility in water at 20 °C [mg L^{-1}]	570		400			8.5	327.1[a]	7.19[a]
Dissociation constant (pK_a)	3.28		3.38			2		
Octanol-water partition coefficient (log K_{ow})	0.77		1.14		-1.38[a]	3.21	2.3[a]	1.50[b]
log K_{oc}	55.3		120			-		
DT50 in soil [days]	716		137	108[a]	145[a]	70	70.5[a]	453[a]
DT50 in water phase [days]	80		51.5			6		

[a] PPDB (2013)
[b] Kaune et al. (1998)

Stock solutions of the individual standards were prepared in MeOH. Solutions were stored in 4 ml amber glass vials at 4°C. A working standard solution in MeOH containing all target pesticides at a concentration of 1 µg ml^{-1} was prepared from stock solutions. An internal standard mixture solution was made at a concentration of 1 µg ml^{-1} in MeOH. These solutions were used for fortification of the samples and for the preparation of the analytical calibration curves. Calibration solutions ranging from 0.075 to 20 ng ml^{-1} were prepared by adding equal aliquots of working standard solution (100 ng ml^{-1} in H_2O or ACN) and internal standard mixture (100 ng ml^{-1} in H_2O or ACN) into individual vials for the analysis of soil and maize extracts. Each solution was made up to a final volume of 1500 µl. For the analysis of the leachate, calibration solutions with analyte concentrations ranging from 0.01 to 20 ng ml^{-1} were obtained by adding aliquots of working standard solution into individual vials. An internal standard mixture (15 µl) was added to each vial to a final volume of 1500 µl.

2.2 Sample Sources and Preparation

Samples were taken from an experimental site located in Wagna (Styria, Austria). The soil is classified as sandy loam Dystric Cambisol with 51.8% sand, 33.5% silt and 14.6% clay. Further characteristics are a pH of 6.6 ($CaCl_2$), an organic carbon content (OC) of 2.7% and a cation exchange capacity (CEC) of 11.53 cmolc kg^{-1} at a depth of 0-25 cm. Soil samples were air-dried, sieved (< 2 mm) and stored at room temperature until required. Maize samples were divided into the green part (leaves and stems), roots and kernels. Samples were lyophilized, homogenised and ground using a cutting mill (SM 2000, Retsch, Haan, Germany) for the leaves and stems, and a centrifugal mill (ZM 200, Retsch, Haan, Germany) for root samples. Maize kernels were milled to a flour consistency using a vibratory tungsten carbide disc mill (KHD Humboldt Wedag, Germany). Leachate samples were collected in flasks and were stored at -18°C prior to analysis.

2.3 Liquid Chromatography – Tandem Mass Spectrometry

Analyses were performed on an HP1200 HPLC system and an HP1290 UHPLC system (Agilent Technologies, Vienna, Austria) connected to a 4000 QTRAP triple-stage quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany) and controlled by Analyst 1.6.1 software. Qualification and quantification data was obtained with the electrospray probe operated in the positive and negative ion mode. The HPLC and UHPLC systems were equipped with a membrane degasser, a binary high-pressure pump, an automatic sampler and a column heater. Different gradient methods for the positive and negative ion mode of the mass spectrometer were used. Soil and leachate samples were analysed with both the HPLC and UHPLC systems. All maize extracts were analysed using the UHPLC system. When working with the HPLC system, the eluents for the positive ion mode were water modified with 0.01% formic acid (A) and MeOH comprising 2mM ammonium acetate (B). Separation was performed on a 2x150 mm Luna C18 column (Phenomenex, Aschaffenburg, Germany) with 5 µm particle size attached to a security guard cartridge C18 4x2 mm (Phenomenex, Aschaffenburg, Germany) at 30°C. Gradient elution was used starting with 2% B at 0 minutes, held for 3 minutes, increased to 18% B within 2 minutes, increased to 35% B within 1 minute, increased to 98% B within 18 minutes, held for 10 minutes, decreased to 2% B within 1 minute. After 46 minutes the system was ready for injection again. Flow was set to

200 µL/min. The injection volume was 50 µL for soil samples and 100 µl for leachate samples. The gradient method for the negative ion mode was operated with the eluents water modified with 0.2% acetic acid (A) and MeOH comprising 0.2% acetic acid (B). The compounds were separated with a 2.1x150 mm Zorbax Eclipse Plus C18 column (Agilent Technologies, Vienna, Austria) with 3.5 µm particle size attached to a security guard cartridge C18 4x2 mm (Phenomenex, Aschaffenburg, Germany) at 40°C. Gradient elution was used starting with 20% B at 0 minutes, held for 2 minutes, increased to 98% B within 16 minutes, held for 3 minutes, decreased to 20% B within 1 minute. After 30 minutes the system was ready for injection again. Flow was set to 300 µL/min. The injection volume was 50 µL for soil and leachate samples.

When working with the UHPLC system, the eluents for the positive ion mode composed of 0.01% formic acid and 2mM ammonium acetate in water (A) and 2mM ammonium acetate in methanol (B) for soil and maize samples. Analyzing leachate samples, water modified with 0.01% formic acid was used as eluent (A). Separation was achieved on a Kinetex column (C8, 2.6 µm particle size, 2.1x100 mm, Phenomenex, Aschaffenburg, Germany) with a security guard cartridge (C8, 2.1x4.6 mm, Phenomenex, Aschaffenburg, Germany) at 30°C. For soil and leachate samples, gradient elution was used starting with 2% B at 0 minutes, held for 2 minutes, increased to 40% B within 2 minutes, increased to 95% B within 4 minutes, held for 3 minutes, decreased to 2% B within 1 minute. For the maize samples, gradient elution was used starting with 5% B at 0 minutes, held for 2 minutes, increased to 40% B within 2 minutes, increased to 95% B within 4 minutes, held for 3 minutes, decreased to 5% B within 1 minute.

After 15 minutes the system was ready for injection again. Flow was set to 400 µL/min. The injection volume was 40 µL for soil and leachate samples and 3 µl for maize samples. The gradient method for the negative run was operated with the eluents water modified with 0.04% acetic acid (A) and ACN (B). The UHPLC column Zorbax Eclipse Plus C18 2.1x50 mm (Agilent Technologies, Vienna, Austria) with 1.8 µm particle size at 40°C was used for separation. Gradient elution was used starting with 20% B at 0 minutes, held for 2 minutes, increased to 50% B within 1 minute, increased to 95% B within 1 minute, held for 1 minute, decreased to 20% B within 1 minute. After 9 minutes the system was ready for injection again. Flow was set to 300 µL/min. The injection volume was 10 µL for soil samples, 20 µl of leachate samples and 5 µl for maize samples.

When working with the HPLC system, an ionization voltage of 5500 and a temperature of 700°C were used in the positive ion mode. In the negative ion mode an ionization voltage of -4500 and a temperature of 500°C were operated. For the UHPLC system, an ionization voltage of 4200 and a temperature of 700°C were used in the positive ion mode. In the negative ion mode an ionization voltage of -4200 and a temperature of 500°C were operated. Nitrogen was provided by a nitrogen generator (CMC instruments, Eschborn, Germany) and used as nebulizer, curtain and collision cell gas. Numerous experiments using solutions of the individual analytes were performed to determine the optimal MRM transition, collision energies and declustering potentials for each individual compound. A syringe at constant flow was used to infuse the standard solutions directly into the instrument.

2.4 Leachate

Samples were analysed by direct injection after the addition of an internal standard mixture as injection standard. 10 µl of internal standard mixture (100 ng ml^{-1} in MeOH) containing bentazone-d_6, chloridazon-d_5, chloridazon-desphenyl-$15N_2$, terbuthylazine-d_5 and terbuthylazine-desethyl-d_9 was added to 1 ml leachate sample.

2.5 QuEChERS Procedures

The original QuEChERS method, according to Anastassiades et al. (2003) and CEN EN 15662 (2008), was developed for the extraction of samples with more than 75% water content and consists of the following steps: (1) weigh 10 g sample into 50 ml centrifuge tubes; (2) add 10 ml ACN and shake the sample vigorously for 1 min; (3) add 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3Citrate x $2H_2O$ and 0.5 g Na_2HCitrate x 1.5 H_2O and shake immediately for 1 min, (4) centrifuge the extract for 5 min at 3000 U/min; (5) take an aliquot into a 15 ml centrifuge tube containing $MgSO_4$ and sorbent; (6) shake the sample for 30 sec and centrifuge for 5 min at 3000 U/min; (7) take an aliquot and add 5 % formic acid in ACN prior to the determination by GC-MS and LC-MS.

In this study, the original QuEChERS procedure was adapted for the dry matrices of soil and maize (leaf/stem, root and kernel). Several procedures and QuEChERS compositions were tested through recovery studies. The studied steps were: (i) the addition of water for matrix swelling and the acidification of the extraction solvent, (ii) the extraction time, and (iii) different clean up procedures.

An experiment to compare three acid variations, namely 1% (v/v) acetic acid, 1% (v/v) formic acid or 5% (v/v) formic acid, in combination with ACN as an extraction solvent was investigated. The extraction solvent experiment was carried out in triplicate using 5 g soil and 5 ml of water added for swelling.

The influence of extraction time was evaluated in maize kernels testing. Samples of maize kernels (2.5 g) were spiked with the target compounds (40 µg kg-1) and either shaken for 1 min on the vortex, or placed for 1 hour on a wrist shaker after the addition of 10 ml water and 10 ml ACN containing 5% (v/v) formic acid. The presence of fats requires the additional clean-up of freezing out to obtain appropriate extracts. Briefly, after the initial extraction step with 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3Citrate x $2H_2O$ and 0.5 g Na_2HCitrate x $1.5H_2O$, aliquots of 8 ml were taken from the ACN phase, placed into 15 ml centrifuge tubes and stored for 2 hours in a freezer (-20°C). Three replicates were analysed at each extraction condition.

To effectively remove co-extracts and to identify interactions between the pesticides and sorbents, a comparison of different sorbents for the dispersive-SPE clean up for maize samples of leaves and stems was performed. After the first centrifugation, 6 ml of the upper ACN extract was transferred into 15 ml centrifuge tubes containing either 150 mg PSA, 900 mg $MgSO_4$ and 150 mg C_{18} or 300 mg PSA and 300 mg $CaCl_2$ (CUVA 2009). Three replicates for each sorbent mixture were tested.

After QuEChERS extraction, solvent exchange of the ACN extracts to water were examined for all soil and maize (leaf/stem, root and kernel) matrices. 1 ml of the ACN extract was transferred into an auto-sampler vial. The extract was evaporated to 0.5 ml under a stream of nitrogen at a temperature of 30°C. Following the addition of 0.5 ml HPLC-water, the extracts

were again reduced to 0.5 ml at 30°C. The extract obtained was filled up with HPLC water to 1 ml. In the case of the maize matrices, extracts in ACN and water were measured.

2.6 Method Validation

A validation study of the optimized extraction procedures was carried out in terms of recovery, repeatability, matrix effects and analytical limits including method limits of quantification (LOQs) and instrument limits of detection (LODs). Basic validation for each pesticide was carried out with SQS 2000 to determine the instrumental limits of quantification (LOQ). Solvent-based calibration standards were measured three-times at each concentration level to provide data for the basic validation.

For leachate, method LOQ in µg L^{-1} was equivalent to the instrumental LOQ resulting from the basic validation. The method LOD was determined by dividing the method LOQ by 2. The internal standard mixture was added prior to the instrumental analysis to compensate for matrix and instrument variations. To determine the recovery rates, the peak areas of the isotopically labelled internal standards (bentazone-d_6, chloridazon-d_5, chloridazon-desphenyl-$15N_2$, terbuthylazine-d_5 and terbuthylazine-desethyl-d_9) and those obtained from the solvent-based standards were used.

The method LOQ for soil and maize was calculated from the instrumental LOQ values of the mass spectrometer, multiplied by the extraction factor, divided by the lowest weighed sample, and corrected with the mean recovery, minus the standard deviation of the corrosponding deuterated internal standards. In the case of chloridazon-desphenyl in soil, the value was multiplied by the dilution factor. The method LOD was determined by dividing the method LOQ by 2.

Extractions from non-spiked soil and maize samples were performed to check the absence of the selected pesticides and the chromatographic interferences that precluded the correct detection and quantification of the analytes. The internal standard mixture was added (at the same concentration level as the pesticide standard) before extraction to keep track of possible losses occurring during the sample preparation and chromatographic analysis. Recoveries were determined at the concentration levels of 3 µg kg^{-1} for soil, 5 µg kg^{-1} for root, 10 µg kg^{-1} for leaf/stem and 40 µg kg^{-1} for maize kernel. The pesticide concentration measured by performing the complete procedure was compared with the pesticide concentration initially added to the individual blank matrices. The overall recovery of each pesticide was calculated as the mean recovery of the spiked samples extracted on different days using the same method and the same equipment. Repeatability is expressed as relative standard deviation (% RSD). Matrix effects in soil were examined by comparing the concentration derived from standard additions into sample extract to concentrations in pure aqueous solution. A post-extraction spiked experiment was carried out in four replicates by adding 10 µl of appropriate standard solution to 200 µl of soil extract and 200 µl of water.

3 Results and Discussion

3.1 Chromatographic Optimization

The optimized conditions of the selected (MRM) transitions of the eight pesticides are summarised in Table 2. In comparison with HPLC, the application of UHPLC-MS/MS

improved the quantitative response and reduced the analysis time. The flow rate was increased and the injection volume reduced. Under the chromatographic conditions described above, the total analytical time for instrumentation using UHPLC was reduced from 46 to 15 min in the positive mode and from 30 to 9 min in the negative mode. Fig. 1 presents a typical ion chromatogram of the eight pesticides or metabolites (all of them at 1 ng ml^{-1} concentrations) which were obtained from a standard sample in the positive mode using the UHPLC method. The chloridazon metabolites chloridazon-desphenyl and chloridazon-methyl-desphenyl did not allow proper peak recognition when the extracts of soil and maize samples were injected in ACN. The solvent exchange of QuEChERS extracts from ACN to water and additionally, a dilution of 1:5 (v/v), improved the detection of chloridazon-desphenyl in soil. Chromatograms of chloridazon-desphenyl in soil matrix are given in Fig. 2.

Table 2 Analytical conditions of the studied pesticides

Compound	**Ion mode**	**MRM transitions (m/z)**	**Collision energy (ev)**	**Dwell time (ms) HPLC**	**Dwell time (ms) UHPLC**
Bentazone	ESI-	238.9> 131.8	-34	50	20
		238.9> 174.9	-26		
Bentazone-methyl	ESI+	254.9> 212.9	17	50	20
		254.9> 149.0	31		
Chloridazon	ESI+	222.1> 92.0	39	50	20
		222.1> 65.1	63		
Chloridazon-desphenyl	ESI+	145.9> 66.0	53	200	20
		145.9> 116.8	31		
Chloridazon-methyl-desphenyl	ESI+	160.0> 88.1	43	20	20
		160.0> 117.0	31		
Terbuthylazine	ESI+	230.1> 174.1	25	50	10
		230.1> 103.9	47		
Terbuthylazine-desethyl	ESI+	202.1> 146.1	23	50	10
		202.1> 79.1	41		
Terbuthylazine-2-hydroxy	ESI+	212.2> 155.9	23	50	10
		212.2> 114.0	35		

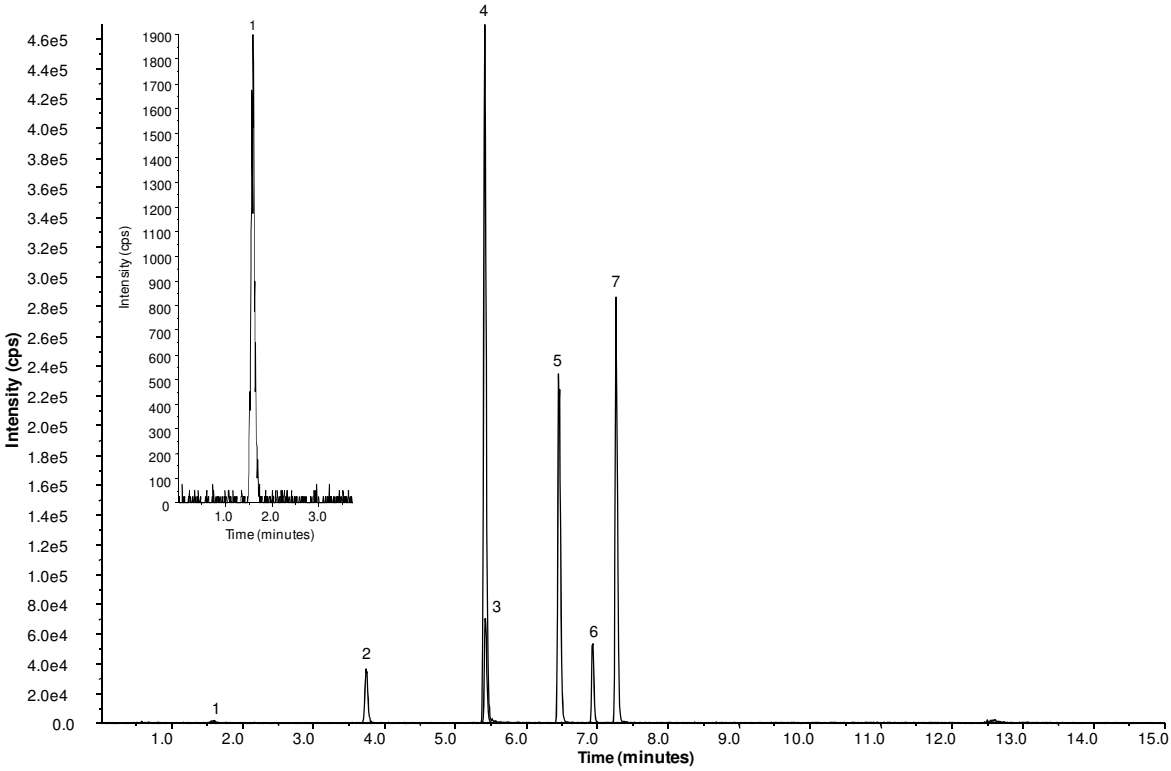
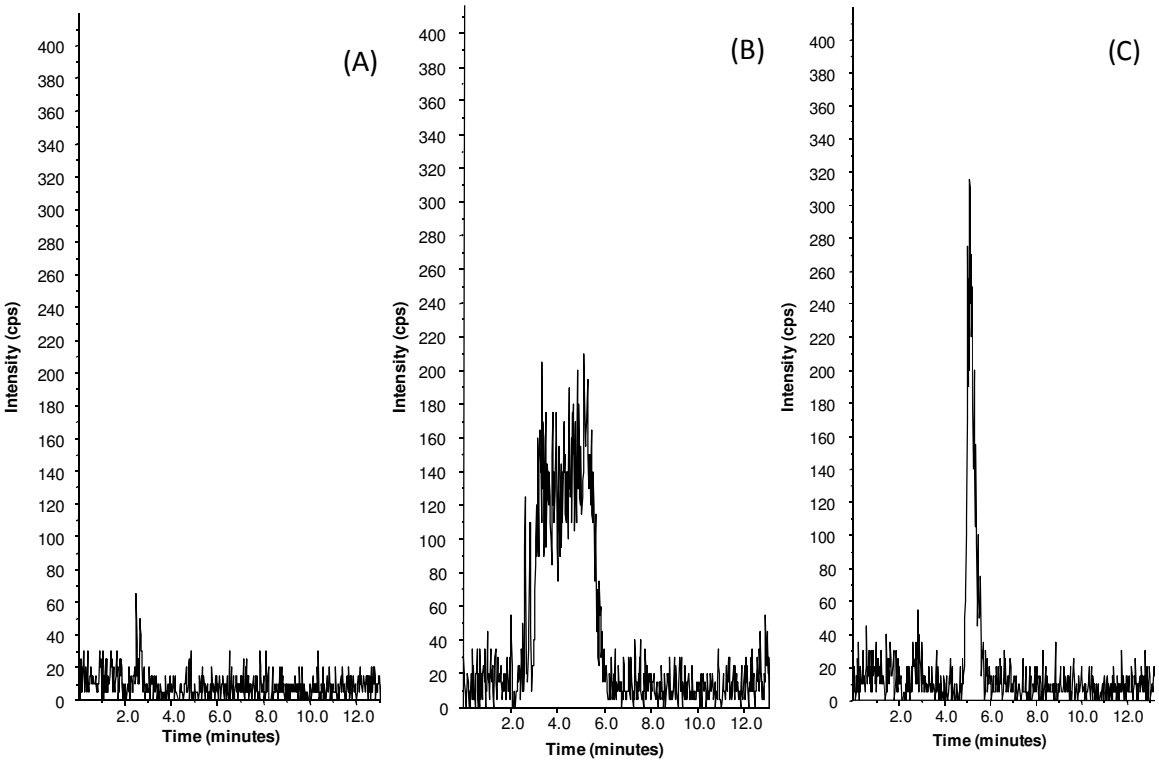


Fig. 1 Extracted ion chromatogram from the positive mode UHPLC-MS/MS of 1 ng/ml (1) chloridazon-desphenyl, (2) chloridazon-methyl-desphenyl, (3) chloridazon, (4) terbuthylazine-2-hydroxy, (5) terbuthylazine-desethyl, (6) bentazone-methyl and (7) terbuthylazine from a standard sample in water



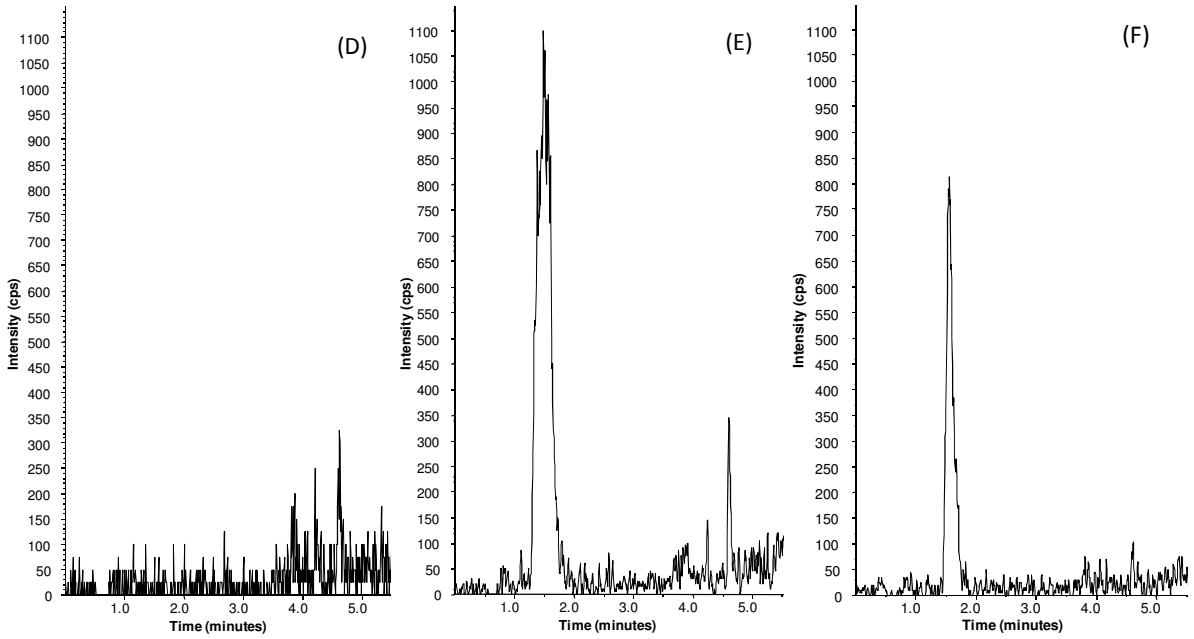


Fig. 2 Examples of ion suppression on chloridazon-desphenyl in water: (A) a control soil matrix, (B) soil matrix at 3 µg kg^{-1} and (C) 1:5 (v/v) dilution of B from HPLC and (D) a control soil matrix, (E) soil matrix at 3 µg kg^{-1} and (F) 1:5 (v/v) dilution of E from UHPLC

3.2 Matrix Effect

The effect of ion suppression, or in rare cases, enhancement from using an ESI source is well known, and environmental samples contain a large amount of compounds that can interfere with the analytical signal, producing matrix effects.

In leachate, matrix effects for chloridazon-desphenyl were evaluated using the method of standard addition at five concentration levels. Results indicated that no significant suppression or enhancement was observed for chloridazon-desphenyl in leachate (1.2%). An isotopically labelled internal standard was added just before instrumental analysis, thereby compensating run to run variation in instrument response and improving the precision. However, isotopically labelled internal standards are often not commercially available for recently found metabolites.

In soil, matrix effects were determined by a post-extraction spiked experiment. Results indicated a strong ionization suppression of chloridazon-desphenyl in soil extracts. An possible explanation is that chloridazon-desphenyl is the first eluting compound in the chromatogram where interference of the sample matrix with the solvent can occur. Kruve et al. (2008) documented that the ionization efficiency of polar pesticides is more affected by co-eluting compounds. Soil extracts were diluted with water (1:5 v/v and 1:10 v/v) to reduce the amount of matrix components introduced into the LC-MS/MS system. Soil samples diluted 1:5 (v/v) provided an overall recovery of 60% for chloridazon-desphenyl, whereas a complete elimination of matrix effects was observed when analysing the samples with a 1:10 (v/v) dilution. The main drawback of using the dilution approach to minimize matrix effects is the increase of the detection limit (Niessen et al. 2006; Sancho et al. 2002). Thus, all soil extracts were diluted (1:5, v/v) prior to injection to overcome the matrix effect of chloridazon-desphenyl (Fig. 2). No visible precipitation of matrix compounds was noticed and the peak shapes of polar pesticides (chloridazon-desphenyl and chloridazon-methyl-desphenyl) were

clearly improved. In addition, the isotopically labelled internal standard chloridazon-desphenyl-$15N_2$ was added in every sample before the extraction and clean up stages to compensate matrix effects and thus, improve the accuracy and precision of the method.

In maize, direct injection of small volumes (3 µl and 5µl) of the crude QuEChERS extracts in ACN and water were used to avoid matrix effects. Choi et al. (2001), Niessen et al. (2006) and Lacina et al. (2010) documented the effect of injection volume on matrix signal suppression. The risk of a rapid contamination of the sample cone of the mass spectrometer resulting in a significant decrease in the sensitivity of the analyte detection is also obviated by using small injection volumes.

3.3 Extraction Procedure

Several parameters were studied to optimize the performance of the extraction methods, such as the ratio of sample mass to extraction solvent volume, the extraction solvent, the extraction time and different clean-up procedures before the validation experiments.

3.3.1 Soil

The first optimization experiments used parts of the original QuEChERS and CEN method for soil. The best overall results were achieved using 5 g soil, 5 ml water and 10 ml ACN including 5% formic acid for the first extraction step. Recoveries increased to 24%, 30% and 67% for chloridazon-desphenyl and 70%, 78% and 85% for chloidazon-methly-desphenyl using 1% acetic acid, 1% formic acid and 5% formic acid. Chloridazon, terbuthylazine and terbuthylazine-desethyl achieved recoveries of 91, 92, and 91%, 88, 89 and 84%, and 92, 95 and 85% for 1% acetic acid, 1% formic acid and 5% formic acid, respectively. Based on these results, all subsequent experiments were carried out with 5 g soil, 5 ml water and 10 ml ACN including 5 % formic acid.

The freezing out step was not included in the final procedure as no precipitation of co-extracts and no improvement of recoveries was observed. The extraction method was further optimized by using sorbent combination of 150 mg PSA and 950 mg anhydrous $MgSO_4$ in the dispersive SPE clean up. The resulting soil extracts were taken for the solvent exchange prior to the LC-MS/MS analysis. Good recoveries were achieved for this optimized method (Table 3).

Table 3 Mean recoveries (%), repeatability (%RSD), limits of detection (LOD) and limits of quantification (LOQ) in µg kg^{-1} of the 8 compounds derived from the spiked extractions experiments for soil and maize

Compound	**Matrix**	**LOD (µg kg^{-1})**	**LOQ (µg kg^{-1})**	**Recovery (%, n=10) RSD (%) Spiked level (3 µg kg^{-1})**	**LOD (µg/kg)**	**LOQ (µg/kg)**	**Recovery (%, n=10) RSD (%) Spiked level (3 µg kg^{-1})**
		H_2O	H_2O	H_2O	ACN	ACN	ACN
Bentazone	Soil	0.025	0.050	87 (9.7)	-	-	b
Bentazone-methyl		0.070	0.14	86 (7.4)	-	-	b
Chloridazon		0.17	0.34	80 (8.0)	-	-	b
Chloridazon-desphenyl		1.5	3	67 (13)[a]	-	-	b
Chloridazon-methyl-desphenyl		0.19	0.37	73 (10)	-	-	b
Terbuthylazine		0.043	0.085	64 (18)	-	-	b
Terbuthylazine-desethyl		0.13	0.25	55 (13)	-	-	b
Terbuthylazine-2-hydroxy		0.39	0.78	98 (17)	-	-	b
Compound	**Matrix**	**LOD (µg kg^{-1})**	**LOQ (µg kg^{-1})**	**Recovery (%, n=6) RSD (%) Spiked level (5 µg kg^{-1})**	**LOD (µg/kg)**	**LOQ (µg/kg)**	**Recovery (%, n=6) RSD (%) Spiked level (5 µg kg^{-1})**
		H_2O	H_2O	H_2O	ACN	ACN	ACN
Bentazone	Root	0.44	0.88	60 (4.4)	-	-	b
Bentazone-methyl		1.4	2.8	52 (8.7)	1.0	2.0	69 (5.9)
Chloridazon		-	-	c	-	-	c
Chloridazon-desphenyl		-	-	c	-	-	c
Chloridazon-methyl-desphenyl		0.39	0.77	74 (14)	-	-	c
Terbuthylazine		0.50	1.0	30 (9.6)	1.1	2.2	47 (7.3)
Terbuthylazine-desethyl		0.71	1.4	48 (10)	0.85	1.7	50 (8.2)
Terbuthylazine-2-hydroxy		0.46	0.92	70 (9.7)	0.76	1.5	57 (7.1)

Compound	Matrix	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Recovery (%, n=10) RSD (%) Spiked level (10 µg kg^{-1})	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%, n=8) RSD (%) Spiked level (10 µg kg^{-1})
		H_2O	H_2O	H_2O	ACN	ACN	ACN
Bentazone	Leaf/Stem	1.0	2.0	68 (20)	1.0	2.1	53 (7.1)
Bentazone-methyl		1.5	3.1	88 (10)	1.3	2.6	101 (5.7)
Chloridazon		0.71	1.4	76 (7.0)	1.7	3.4	78 (8.0)
Chloridazon-desphenyl		3.7	7.3	41 (14)	-	-	c
Chloridazon-methyl-desphenyl		0.61	1.2	82 (5.9)	-	-	c
Terbuthylazine		1.1	2.2	24 (5.1)	2.2	4.5	50 (9.7)
Terbuthylazine-desethyl		1.5	3.0	45 (9.3)	2.1	4.3	40 (6.8)
Terbuthylazine-2-hydroxy		0.78	1.6	83 (12)	1.1	2.2	79 (10.5)
Compound	**Matrix**	**LOD (µg kg^{-1})**	**LOQ (µg kg^{-1})**	**Recovery (%, n=6) RSD (%) Spiked level (40 µg kg^{-1})**	**LOD (µg/kg)**	**LOQ (µg/kg)**	**Recovery (%, n=6) RSD (%) Spiked level (40 µg kg^{-1})**
		H_2O	H_2O	H_2O	ACN	ACN	ACN
Bentazone	Maize kernel	0.35	0.70	60 (3.5)[d]	0.32	0.64	69 (9.0)[d]
Bentazone-methyl		2.2	4.5	23 (1.7)	0.58	1.2	92 (5.7)
Chloridazon		0.90	1.8	31 (9.0)	1.5	3.0	47 (15)
Chloridazon-desphenyl		1.1	2.3	41 (6.0)	-	-	c
Chloridazon-methyl-desphenyl		0.21	0.41	96 (6.0)	-	-	c
Terbuthylazine		-	-	c	0.53	1.1	70 (3.5)
Terbuthylazine-desethyl		-	-	c	0.41	0.82	73 (3.2)
Terbuthylazine-2-hydroxy		0.29	0.57	84 (6.9)	0.45	0.90	74 (5.7)

n - number of samples.
[a] dilution of the extract (1:5).
[b] No injection was made into the LC-MS/MS.
[c] No results were obtained.
[d] n=3

3.3.2 Root

The optimized method used followed the main steps and proportions of the original QuEChERS and CEN methods together with the optimizations already investigated for soil. To obtain the best homogenization and dispersion between the root and the extraction solvent, the ratio of 2 g sample, 8 ml of water for swelling and 10 ml ACN (5% formic acid) were used for all further root extractions. The resulting root extracts were ready for injection into UHPLC-MS/MS and an aliquot of 1 ml from the ACN extract was taken for the solvent exchange. The recovery results (Table 3) indicate that further investigation will be necessary to achieve better pesticide recoveries from root matrices.

3.3.3 Leaf and Stem

The extraction method reviously used for soil and root was further optimized for maize leaves and stems. The volume of the leaves and stems necessitated a reduction of the sample amount to ensure sufficient ACN for the collection of the supernatant that followed. In addition, the effect of different sorbents in the dispersive SPE clean up was investigated to improve purification and recoveries for maize leaves and stems. Recovery yields for the pesticides studied were satisfactory with each sorbent combination used. The recoveries obtained in water extracts were 26 and 41% for chloridazon-desphenyl, 82% for chloridazon-methyl-desphenyl, 76% for chloridazon, 28 and 24% for terbuthylazine, 66 to 45% for terbuthylazine-desethyl, 84 and 83% for terbuthylazine-2-hydroxy and 100 and 88% for bentazone-methyl using 150 mg PSA, 900 mg $MgSO_4$ and 150 mg C_{18} or 300 mg PSA and 300 mg $CaCl_2$. A visual observation of the initial and final extracts showed less coloured extracts using 300 mg PSA and 300 mg $CaCl_2$ and therefore, these sorbents were selected for subsequent validation experiments.

The resulting extracts were ready for injection into UHPLC-MS/MS and an aliquot of 1 ml from the ACN extract was taken for the solvent exchange. Satisfactory recoveries were achieved for leaves and stems using the optimized method (Table 3).

3.3.4 Maize Kernel

The extraction method was further optimized for maize kernels by reducing the sample amount, examining the appropriate extraction time, performing the freezing-out step and using a different sorbent combination in the dispersive SPE clean up. The sample amount was reduced to a 2.5 g sample, following Mastovska et al. (2010). The samples were extracted with 10 ml water and 10 ml ACN (5% formic acid).

The influence of extraction time was studied for maize kernels using both 1 minute and 1 hour. In the same experiment the freezing-out step for maize kernels was also examined in order to reduce the intrusive effect of the maize starch in the initial extracts. Recoveries showed no differences between the extraction times. Results indicated cleaner extracts using the freezing-out step with no significant effects on pesticide recoveries. Recoveries obtained in ACN extracts were 65 and 69% for chloridazon, 67 and 66% for terbuthylazine, 70 and 75% for terbuthylazine-desethyl, 67 and 70% for terbuthylazine-2-hydroxy and 87 and 82% for bentazone-methyl, with or without the freeze-out step.

As a result, an extraction time of 1 minute was chosen in order to simplify the optimized method as far as possible. A freeze-out step of 2 hours was carried out and C_{18} associated to

PSA and $MgSO_4$ in the dispersive SPE was used to minimize the presence of interfering compounds in the extract.

The resulting extracts were ready for injection into UHPLC-MS/MS and an aliquot of 1 ml from the ACN extract was taken for the solvent exchange. Adequate recoveries were obtained using the optimized method shown in Table 3.

3.4 Method Performance

Recovery and repeatability were determined for each pesticide or metabolite in different environmental matrices using the above described methods.

In leachate, results reveal that the recoveries for all compounds were satisfactory, ranging from 82% to 105% with RSD values lower than 25% in all cases (Table 4). The method LOD and LOQ values obtained for the selected pesticides are shown in Table 4.

The extractions carried out with the non-spiked samples detected a contamination of the metabolite terbuthylazine-2-hydroxy in soil (0.73 µg kg^{-1}) and maize leaves and stems (7.5 µg kg^{-1}) and root samples contained concentrations of chloridazon (62 µg kg^{-1}) and chloridazon-desphenyl (51 µg kg^{-1}). The initial pesticide concentrations were considered in the calculation of the recoveries. Detailed recovery and repeatability data for all pesticides and metabolites analysed in soil and maize are given in Table 3. All recoveries given were overall recoveries including matrix effects.

In soil, the compounds bentazone, bentazone-methyl and chloridazon gave excellent recoveries in the range of 80%-87% and a good repeatability of less than 10% was obtained with the RSDs. The recoveries of terbuthylazine (64%) and terbuthylazine-desethyl (55%) were lower in the validation experiments compared with those obtained in the method development stage. Results of the post-extraction spiked experiment showed matrix effects of 19% for terbuthylazine and 26% for terbuthylazine-desethyl. The large RSD values of terbuthylazin-2-hydroxy can be linked to the initial concentrations of the pesticide in blank samples.

The metabolites chloridazon-desphenyl and chloridazon-methyl-desphenyl were the most problematic compounds, due to their polar characteristics. These polar transformation products required the solvent exchange of the extracts to water to improve retention on the HPLC column as well as the peak shape. Thus, the matrix effects are minimized with overall recoveries of 67% and 73% in soil (Table 3).

Table 4 Validation parameters of the optimized methods for leachate

Compound	**Matrix**	**LOD ($\mu g\ L^{-1}$)**	**LOQ ($\mu g\ L^{-1}$)**	**Internal standard**	**Recovery (%, n=207) RSD (%) HPLC, 100 µl**	**Recovery (%, n=220) RSD (%) UHPLC, 40 µl**
Bentazone	Leachate	0.015	0.030	Bentazone-d_6	91 (24)	100 (23)
Bentazone-methyl		0.015	0.030	[a]	-	-
Chloridazon		0.010	0.020	Chloridazon-d_5	[a]	91 (4.4)[c]
Chloridazon-desphenyl		0.040	0.080	Chloridazon-desphenyl-$15N_2$	102 (25)[b]	82 (11)
Chloridazon-methyl-desphenyl		0.025	0.050	[a]	-	-
Terbuthylazine		0.010	0.020	Terbuthylazine-d_5	105 (14)	93 (8.6)
Terbuthylazine-desethyl		0.015	0.030	Terbuthalyazine-d_9	102 (14)	89 (7.1)
Terbuthylazine-2-hydroxy		0.015	0.030	[a]	-	-

[a] No internal standard available.
[b] n=21
[c] n=110

The final solvent used led to differing matrix effects of the selected pesticides in maize, and thus influenced the overall recoveries. Some pesticides indicated less matrix effects in the ACN extracts, whereas others had better overall recoveries in water extracts. Bentazone-methyl, chloridazon and terbuthylazine indicated better recoveries in ACN extracts for all maize matrices. Recoveries obtained for terbuthylazine-desethyl in the ACN extracts were higher in roots and maize kernels and slightly lower in leaves and stems. Higher recoveries for bentazone in the ACN extracts were only achieved in maize kernels. In roots, and leaves and stems, the overall recoveries of bentazone increased to 60% and 68% using the water extracts. Comparing the results of terbuthylazine-2-hydroxy, the water extracts provided the better recoveries for all maize matrices.

As described above, chloridazon-desphenyl and chloridazon-methyl-despenyl were obtained in water extracts due to their polarity. Good recovery values were found for chloridazon-methyl-desphenyl, ranging from 74% in roots, 82% in leaf and stem, and 96% in maize kernels, whereas chloridazon-desphenyl only reached 41% in leaf and stem as well as in maize kernels. Recoveries for chloridazon and chloridazon-desphenyl in root samples could not be calculated because the root samples used were highly contaminated with these compounds. Thus, the optimized method for root samples could not be properly evaluated.

This illustrates the difficulties in developing a single method for the determination of compounds with a wide range of physical-chemical properties. It can be observed that RSD values were lower than 20% for all the compounds investigated in water and ACN of all maize samples. The detection limits for the majority of the pesticides indicates that the optimized HPLC and UHPLC-MS/MS method is capable of sensitive quantitation of pesticides from environmental samples (Table 3 and 4).

High LOQ values were found for chloridazon-desphenyl in comparison to the other selected pesticides. This is due to the stronger matrix effects and difficulties in chromatographic separation, resulting in a higher uncertainty of measurements. Despite the described difficulties, the performance of the optimized methods was found to be useful for the investigation of pesticide behaviour in a lysimeter experiment. The proposed methods were applied for research into the transfer of pesticides in soil, water and plants.

4 Conclusion

The proposed methods were optimized and validated for the determination of bentazone, chloridazon, terbuthylazine and their known main metabolites in different environmental samples by LC-MS/MS. The extraction procedures described showed sufficient recoveries and precision. The different properties of the selected pesticides were challenging especially the chloridazon metabolites. A solvent exchange in water was necessary to ensure the correct quantification of the chloridazon metabolites in soil and maize. An LC-MS/MS method using HPLC and UHPLC in both positive and negative mode is available for the quantitative determination of the eight selected pesticides in soil, maize and water. The shorter injection cycle time and the improved sensitivity have led to the increasing adoption of UHPLC-MS/MS. The validated methods were successfully applied to determine the behaviour of bentazone, chloridazon, terbuthylazine and some of their metabolites in the complex system soil, plant and water using lysimeter experiments. Results will be published separately (Fuhrmann et al. in prep).

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2.1.1 Appendix A. Supplementary data: clothianidin and metolachlor

The analytical methods described in Fuhrmann et al. (2014) were extended for the pesticides clothianidin and metolachlor as well as the metabolites metolachor-ESA and metolachlor-OA in leachate and soil. The effectiveness of the QuEChERS method for soil without the cleanup step for the determination of the acidic metabolites metolachlor-ESA and metolachlor-OA was investigated. Recovery and repeatability were determined for each pesticide in leachate and soil. Additionally, the detection conditions for UHPLC-ESI-MS/MS were established and suitable chromatographic conditions for clothianidin, metolachlor, metolachor-ESA and metolachlor-OA had to be identified.

Pesticide standards (clothianidin, metolachlor CGA24705, metolachlor-ESA CGA354743, metolachlor-OA CGA51202) and isotopically labelled internal standards (clothianidin-d_3, metolachlor-d_6) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). All solvents, chemicals and solutions as well as soil and leachate samples used are described in Fuhrmann et al. (2014).

The QuEChERS procedure for soil as described in Fuhrmann et al. (2014) comprises a salting out extraction step followed by a dispersive SPE cleanup procedure. The dispersive SPE cleanup step uses a sorbent combination of 950 mg anhydrous magnesium sulphate and 150 mg primary secondary amine (PSA). Magnesium sulphate is used to remove water and PSA is the frequently used dispersive SPE sorbent, which can remove various polar organic acids, polar pigments, some sugars, and fatty acids that may be present in soil matrix (Lehotay et al. 2010). Due to the acidic nature of the metabolites metolachlor-ESA and metolachlor-OA the extracts were analysed with and without the dispersive SPE cleanup step.

Recoveries of metolachlor-ESA and metolachlor-OA without the cleanup ranged from 68 to 104 % and from 79 to 95 %, respectively. Recoveries obtained with the cleanup were 55 to 77 % for metolachlor-ESA and 71 to 91 % for metolachlor-OA. Based on these results, soil extracts for metolachlor-ESA and metolachlor-OA were directly analysed without the dispersive SPE cleanup step.

The analytical method used is based on UHPLC-ESI-MS/MS method described in Fuhrmann et al. (2014). Analytical conditions of the pesticides are given in Table 2.1.

Table 2.1: Analytical conditions of clothianidin, metolachlor, metoloachlor-ESA and metolachlor-OA

Compound	Ion mode	MRM transitions (m/z)	Collision energy (ev)	Dwell time (ms)
Clothianidin	ESI+	249.8> 169.1	19	20
		249.8> 132.0	25	
Metolachlor	ESI+	283.6> 251.9	31	10
		283.6> 176.1	31	
Metolachlor-ESA	ESI-	328.4> 79.9	-62	20
		328.4> 120.7	-32	
Metolachlor-OA	ESI-	278.0> 205.8	-16	20
		278.0> 174.0	-24	

In leachate, results reveal that the recoveries for clothianidin (87 %) and metolachlor (98 %) were satisfactory. The method LOD and LOQ values obtained for the selected pesticides are shown in Table 2.2.

Table 2.2: Mean recoveries (%), repeatability (%RSD), limits of detection (LOD) and limits of quantification (LOQ) of the pesticides in leachate

Compound	**LOD (µg L^{-1})**	**LOQ (µg L^{-1})**	**Internal standard**	**Recovery (%, n=110) RSD (%)**
Clothianidin	0.025	0.050	Clothianidn-d_3	87 (9.7)
Metolachlor	0.015	0.030	Metolachlor-d_6	98 (15)
Metolachlor-ESA	0.025	0.050		-
Metolachlor-OA	0.025	0.050		-

In soil, average recovery was in the range of 80–87 % and the relative standard deviations were below 20 % (Table 2.3). High LOQ values were found for clothianidin in comparison to the metolachlor pesticides. This is due to the stronger matrix effects and difficulties in chromatographic separation, resulting in a higher uncertainty of measurements.

Table 2.3: Mean recoveries (%), repeatability (%RSD), limits of detection (LOD) and limits of quantification (LOQ) of the pesticides derived from the spiked extractions experiments in soil

Compound	**LOD (µg kg^{-1})**	**LOQ (µg kg^{-1})**	**Recovery (%, n=10) RSD (%)**
			Spiked level (3 µg kg^{-1})
Matrix	H_2O	H_2O	H_2O
Clothianidin	0.65	1.3	80 (18)
Metolachlor	0.060	0.12	87 (15)
Metolachlor-ESA	0.13	0.25	84 (17)
Metolachlor-OA	0.10	0.20	86 (4.8)

The development of analytical methods that include metabolites is highly demanded, regarding their high persistence in the environment. The method described provides reliable quantitative analysis of clothianidin, metolachlor and its metabolites in leachate and soil. Thus, the proposed method was successfully applied to determine these pesticides in leachate and soil from lysimeter experiments. Although S-metolachlor was applied on the lyimeter surface, metolachlor (R and S isomers) were quantified by UHPLC-ESI-MS/MS. In this thesis, the quantification of metolachlor was performed without any distinction being made of specific isomers.

3 SORPTION EXPERIMENTS

3.1 Introduction

Soils have multiple functions to filter and buffer contaminants through interactions with reactive soil interfaces or microbal degradation. To understand the fate of pesticides released by agriculture it is important to investigate their medium to long-term behaviour in soil. Sorption of pesticides to soils is thought to be influenced by the structure of the pesticide, by the properties of the soil such as pH, organic matter ionic strength and by competition with other solutes (Pignatello and Xing 1996).

Sorption reactions at solid-water interfaces decrease the solute mobility and therewith control degradation, bioavailability and transport at the soil surface and within the soil profile. Pesticide adsorption is therefore an important issue for understanding and predicting the fate of pesticides in soils. Batch experiments were often used to determine equilibrium sorption data to predict herbicide reactivity such as leaching through a soil profile or sorbing to or from aged pesticide residues (Boivin et al. 2005; González-Pradas et al. 2005; Köhne et al. 2006).

Studies regarding the behaviour of pesticides in the soil are usually carried out considering individual molecules. In agriculture, pesticides are often added as a mixture, not as an individual compound. Little is known about herbicide mixture effects in the soil (Bonfleur et al. 2015; Mendes et al. 2016). The pesticides may compete for sorption sites and thus reducing their individual sorption. The competition between pesticides for sorption places might in turn cause a higher bioavailability of the mixture of pesticides, compared to that of an individual compound. A decrease in sorption could also provoke an increase in leaching potential of the pesticide (Xing et al. 1996; De Wilde et al. 2008).

In this study, we conducted sorption studies with bentazone, chloridazon and terbuthylazine to find out appropriate time periods for obtaining equilibrium. Therefore, the experimental setup was kept as simple as possible with five data points spread over 24 hours. Particular attention was given to competitive sorption process to get a rough estimation about the sorption behaviour of the pesticides applied alone and mixed. Because at agricultural sites, soils are mainly treated with pesticide mixtures.

3.2 Material and methods

Standard solutions of bentazone, chloridazon and terbuthylazine were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and $CaCl_2$ from Merck (Darmstadt, Germany). Methanol (MeOH) was purchased from VWR (Vienna, Austria).

The soil samples originate from the long-term experimental site in Wagna, state of Styria, Austria. The soil is classified as loamy sandy Dystric Cambisol with 52 % sand, 34 % silt and 15 % clay. Further characteristics are a pH of 6.6 ($CaCl_2$), an organic carbon content (OC) of 2.7 % and a cation exchange capacity (CEC) of 11.53 cmolc kg^{-1} at a depth of 0-25 cm. Surface soil (0-20 cm) was collected, air-dried, sieved < 2 mm and homogenised for the study.

The sorption behaviour of the pesticides was studied using a batch equilibration method according to the OECD guideline 106 (OECD 2000). All experiments were performed in duplicate using 50 mL polypropylene centrifuge tubes including blank samples without soil.

Four soil:solution ratios (1:1, 1:5, 1:25 and 1:100) were tested to select the appropriate ratio for the batch adsorption experiment. Air-dried soil (25 g, 5 g, 1 g, 0.25 g) was weighed into the vials and background solution was added keeping the headspace at minimum. The background solution contained 0.01 mol L^{-1} $CaCl_2$ in order to keep ionic strength similar to natural soil solutions. The tubes were shaken (200 rpm) overhead to break up soil macro-aggregates and to pre-equilibrate the soil suspensions. After 24 h, an aliquot from a methanol stock solution of bentazone, chloridazon and terbuthyalzine was spiked to the solutions (0.01 mg L^{-1}). Tubes were shaken overnight and then centrifuged at 3000 g for 10 min. An aliquot of the supernatant was filtered (<0.45 µm) prior to analysis.

Samples were filled with 2 g of soil and 10 mL of 0.01 M $CaCl_2$ aqueous solution (pH = 5.7). After the pre-equilibration of 24 h, a methanol stock solution containing a mixture of pesticides (bentazone, chloridazon and terbuthylazine) or the individual compounds was added to the tubes to yield a concentration of 0.01 mg L^{-1}. The samples were then placed at 20°C in the dark on a horizontal shaker (200 rpm). Tubes filled with pesticide solutions but without soil were analysed to determine the pesticide sorption on surfaces of the tubes and filters. Duplicate samples were obtained at specific time intervals (1, 2, 4, 6 and 24 hours). After shaking for the pre-determined times, the samples were centrifuged for 10 minutes at 3000 g, the supernatants decanted and filtered (< 0.45 µm). The pH-values of the equilibrium solutions were recorded prior to analysis.

All samples were analyzed by an HP1200 HPLC system (Agilent Technologies, Vienna, Austria) connected to a 4000 QTRAP triple-stage quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany) and controlled by Analyst 1.6.1 software. Data was obtained with the electrospray probe operated in the positive and negative ion mode. The eluents for the positive ion mode to detect chloridazon and terbuthylazine were water modified with 0.01% formic acid (A) and MeOH comprising 2 mM ammonium acetate (B). Separation was performed on a 2 x 150 mm Luna C18 column (Phenomenex, Aschaffenburg, Germany) with 5 µm particle size attached to a security guard cartridge C18 4 x 2 mm (Phenomenex, Aschaffenburg, Germany) at 30°C. Gradient elution was used starting with 2 % B at 0 minutes, held for 3 minutes, increased to 18 % B within 2 minutes, increased to 35 % B within 1 minute, increased to 98 % B within 18 minutes, held for 10 minutes, decreased to 2 % B within 1 minute. After 46 minutes the system was ready for injection again. Flow was set to 200 µL min^{-1}. The injection volume was 50 µL. The gradient method for the negative ion mode to detect bentazone was operated with the eluents water modified with 0.2 % acetic acid (A) and MeOH comprising 0.2 % acetic acid (B). The compounds were separated with a 2.1 x 150 mm Zorbax Eclipse Plus C18 column (Agilent Technologies, Vienna, Austria) with 3.5 µm particle size attached to a security guard cartridge C18 4 x 2 mm (Phenomenex, Aschaffenburg, Germany) at 40°C. Gradient elution was used starting with 20 % B at 0 minutes, held for 2 minutes, increased to 98 % B within 16 minutes, held for 3 minutes, decreased to 20 % B within 1 minute. After 30 minutes the system was ready for injection again. Flow was set to 300 µL min^{-1}. The injection volume was 50 µL.

The amount of sorbed substance (C_s) was calculated by the difference between the initial substance concentration (C_{in}) and the substance concentration in the aqueous phase (C_w) according to the equation:

$$C_s = (C_{in} - C_w)\frac{V}{m}, \qquad (1)$$

where V is the volume of the liquid phase (in mL) and m the mass of sorbent (in g). C_s is expressed in mg kg^{-1}; C_{in} and C_w are expressed in mg L^{-1}. Monitoring the blanks revealed that solute loss due to adsorption onto glass walls and septa was negligible.

The sorption coefficient, K_d, describes an apparent sorption constant that is time, soil and pesticide dependent. However, the K_d for a specific pesticide and soil type is continuous across a range of equilibrium concentrations and is thus a linear relationship calculated with the following equation:

$$K_d = \frac{C_s}{C_w}, \qquad (2)$$

3.3 Results and discussion

The soil:solution ratio selected for the sorption study of bentazone, chloridazon and terbuthylazine was 1:5. Blanks did not reveal any interfering peaks or changes due to adsorption or degradation for all pesticides.

Sorption data showed initial rapid losses from the aqueous to the solid phases followed by slower rates of sorption for all pesticides (Figure 3.1). The soil sorbs greater amounts of terbuthylazine and chloridazon than bentazone. As expected, the sorption order of the pesticides conforms to the inverse order of their solubilities (6.6 mg L^{-1} for terbuthylazine, 340 mg L^{-1} for chloridazon and 500 mg L^{-1} for bentazone). As can be seen, the classically recommended equilibration time of 24 hours yielded equilibrium sorption for bentazone, chloridazon and terbuthylazine.

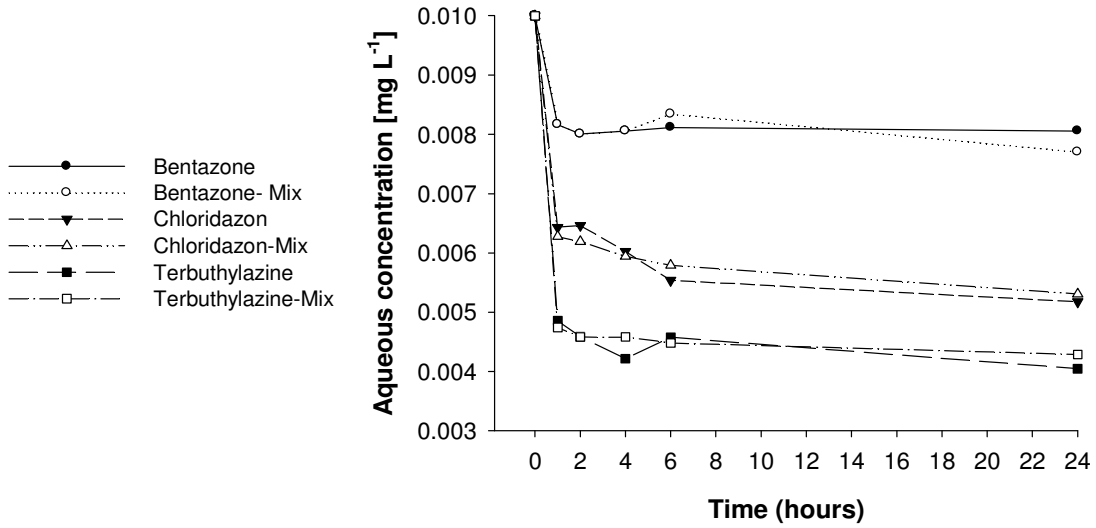

Figure 3.1: Decrease over time of bentazone, chloridzaon and terbuthylazine concentrations in the aqueous phase (C_w)

The selected pesticides differ chemically and thus, adsorb in different ways. Bentazone sorption was weak, as indicated by low K_d values (Figure 3.2) which is in line with existing results (Li et al. 2003; Boivin et al. 2004; Larsbo et al. 2009). The sorption of bentazone mainly depends on soil pH (Boivin et al. 2005; Rodrígues-Cruz et al. 2008). (Clausen and Fabricius 2001) also reported that at pH values above 6 the adsorption of bentazone is negligible. In our experiment, the pH values ranged from 5.7 to 5.8 which can be an explanation for weak sorption of bentazone. In addition, the sorption of bentazone might be affected by the presence of chloridazon and terbuthylazine. Results showed lower K_d values for bentazone mixed with chloridazon and terbuthylazine in comparison to the results when applied alone (Figure 3.2).

Chloridazon sorbed to a lesser extent than terbuthylazine on the sandy loamy soil. Sorption results of chloridazon applied alone and mixed with bentazone and terbuthylazine showed K_d values ranging from 2.7 to 4.7 L kg^{-1} and 3.0 to 4.4 L kg^{-1} respectively. Whether applied alone or mixed, chloridazon retention in the soil was similar. Terbuthylazine, due to its low water solubility, might have a higher affinity for soil organic matter than bentazone and chloridazon. Pesticides which are of low polarity and have low solubility, organic matter is the most important sorbent, with hydrophobic interaction as driving force (Wauchope et al. 2002).

The sorption behaviour of terbuthyalzine was partly affected by the presence of the other two herbicides. After an equilibration time of 4 hours, the K_d value of terbuthylazine applied alone (6.9 L kg^{-1}) was greater than the K_d value obtained for terbuthylazine mixed (5.9 L kg^{-1}) with chloridazon and bentazone. Whereas after 24 hours, the K_d value of terbuthylazine applied alone (5.3 L kg^{-1}) was clearly lower than the K_d value obtained for terbuthylazine mixed (6.7 L kg^{-1}) with chloridazon and bentazone.

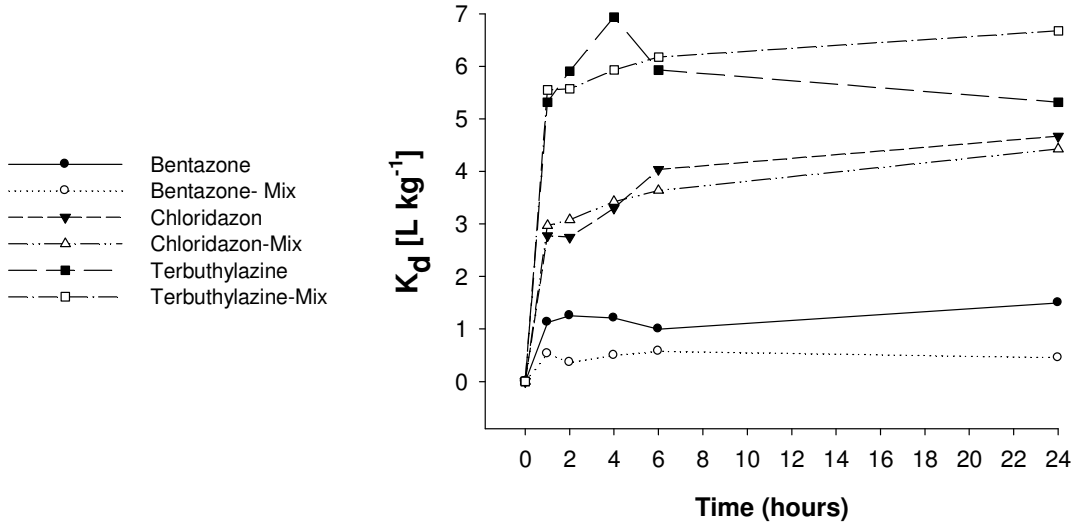

Figure 3.2: K_d values (L kg^{-1}) of bentazone, chloridzaon and terbuthylazine increasing with time

The difference between the sorption behaviour of bentazone, chloridazon and terbuthylazine applied alone or in a mixture can be due to competitive sorption processes. The phenomenon

of competition for sorption sites has been described for triazine herbicides (Xing et al. 1996) and other contaminants (Martins and Mermound 1998).

3.4 Conclusions

Sorption of bentazone, chloridazon and terbuthylazine was performed in the laboratory using a batch method. Results showed that the sorption of bentazone was generally lower than the sorption of chloridazon and terbuthylazine. In addition, sorption seems to be affected by applying each pesticide alone or in a mixture. The sorption behaviour of bentazone and terbuthylazine was obviously influenced while chloridazon was almost unaffected by the presence of bentazone and terbuthylazine. This difference might be attributed to the various physiochemical properties of the selected pesticides. Overall, the sorption of these herbicides in the sandy loamy soil is relatively low, indicating leaching potential, which will be further studied in the following lysimeter experiments.

4 LYSIMETER EXPERIMENTS

In this study, we performed different lysimeter experiments at the experimental site in Wagna using two types of lysimeters and various pesticide formulations.

4.1 Description of the site

The test site Wagna with a total area of 4.4 ha is located within the Mur Valley between Graz and Bad Radkersburg. The test site consists of 32 test plots with approx. 1000m^2 each and was built in the early 1990ies for researching soil water movement and solute transport through the unsaturated zone into the groundwater. It is situated on a gravel terrace of Würm glaciation, which is covered with clayey-sandy Cambisol (soil depths very heterogeneous ranging between 15 and 230 cm). The content of clay and sand is about 15 % and 52 %, respectively. The humus content ranges between 1.3 % and 2.2 %. The location is composed of very light soils with a low water storage capacity, but these characteristics are representative for most parts of the Mur Valley between Graz and Bad Radkersburg.

The lysimeter station in Wagna is equipped with different types of lysimeters. In 1992, two refilled, non-weighable, gravity lysimeters were installed and in 2004, two of the test plots were equipped with high-precision lysimeters and soil hydrologic measuring profiles (SCIENCELYS; Figure 4.1). An additional grass-reference lysimeter (HYDROLYS; Figure 4.1) in combination with a weather station (weather station 1; Figure 4.1) was also installed at the southeast limit of the test site. Since 2003, further weather data has been acquired at a weather station of the national metrological service ZAMG (weather station 2; Figure 4.1), which is also situated at the test site (Klammler and Fank 2014).

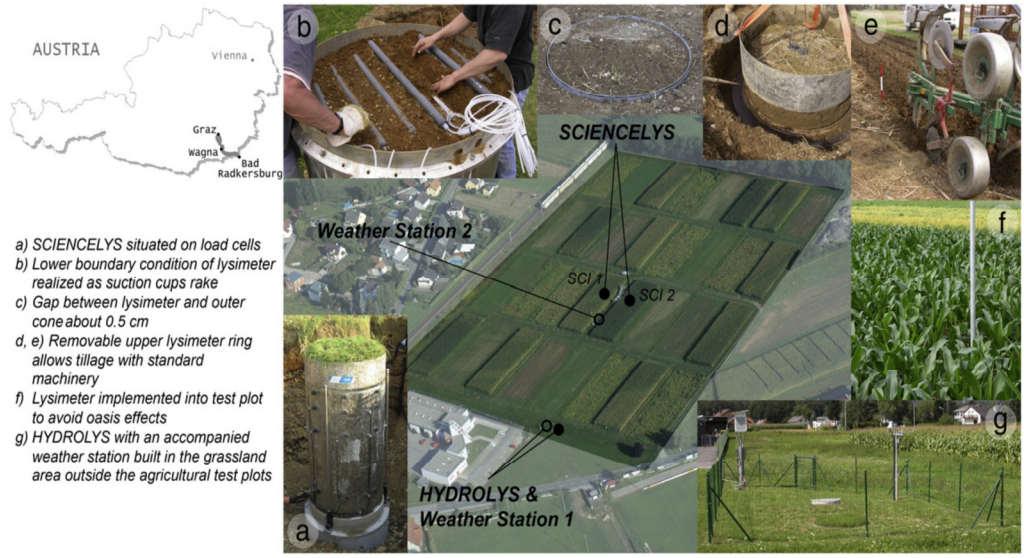

Figure 4.1: Location and overview of the test site Wagna, Austria (Klammler and Fank 2014)

4.1.1 SCIENCE-lysimeter (Publication II, III and IV)

The weighable, monolithic lysimeter (SCIENCELYS, dimensions 100×100×200 cm), installed directly in the agricultural test field (UMS 2013), contains undisturbed loamy to sandy soil over gravel and sand. The lysimeter is conventionally cultivated with agricultural machines (e.g. plough, harrow). A number of sensors and sampling devices allow describing

the soil water and solute transport situation stepwise down the lysimeter. The measurements inside the lysimeter monolith are supplemented by sensor readings in a soil hydrologic measurement profile in the undisturbed soil outside the lysimeter to investigate if there is an influence of the lysimeter construction on soil hydrologic properties inside the lysimeter. The bottom of the SCIENCELYS is designed as a potential boundary condition where a certain water potential can be applied at a suction cup rake to suck of leachate (controlled by a tensiometer installed in undisturbed soil outside the lysimeter).

Figure 4.2 shows the design and technical equipment of the SCIENCE-lysimeter described as follows (Klammler and Fank 2014):

- Dimensions: 2 m depth, 1 m^2 surface
- Cultivation ring: This is actually not a regular component of the SCIENCELYS by UMS, but at Wagna test site it was installed for machine operated tillage of the lysimeter. In other words, the upper 30 cm of the lysimeter cylinder can be removed to cultivate the top soil of the lysimeter with standard machinery (weighing cells have to be unloaded first).
- Soil water sampler: For analyzing soil water quality, suction cups are installed in 35, 60 and 90 cm depth.
- Soil moisture probes: TDR probes (Time Domain Reflectometry; TRIME-IT) are installed in 35, 60, 90 and 180 cm depth (temporarily also in 10 and 20 cm depth).
- Tensiometer: For measuring the hydraulic head, tensiometer with a measuring range between +100 hPa and −850 hPa are installed in 90 and 180 cm depth.
- Matrix sensors: For measuring the hydraulic head up to −2000 hPa, matrix sensors are installed in 35, 60 and 90 cm depth (temporarily also in 10 and 20 cm depth).
- Soil temperature probes: Installed in 35, 60, 90 and 180 cm depth (not illustrated in Fig. 2). Temporarily soil temperature was also measured in 10 and 20 cm depth.
- Silicon carbide porous suction cup rake: For a clearly defined lower boundary condition, the leachate is sucked off by a suction cup rake (surface 3600 cm^2) in 180 cm depth. Tension is applied by a vacuum pump which is controlled by a tensiometer in 180 cm depth of an undisturbed soil outside the lysimeter. This guaranties the same flow rates in the lysimeter as in the undisturbed field.
- Precision weighing system: For measuring mass changes of the lysimeter (e.g., due to ET, precipitation (P), leachate outflow) the lysimeter cylinder is situated on three high-precision weighing cells (resolution of 35 g or 0.035 mm water equivalent).
- Tipping bucket: For measuring the quantity of leachate, a tipping bucket with 0.1 mm resolution is installed (not illustrated in Figure 4.2, where leachate is quantified by a weighing gauge); water is sampled for chemical analysis.
- The following measurements are taken outside the lysimeter to detect possible influences of the lysimeter cylinder on the leachate dynamics:
    - Soil temperature probes in 35, 60, 90 and 180 cm depth (not illustrated in Figure 4.2)
    - Soil moisture probes in 35, 60, 90 and 180 cm depth (not illustrated in Figure 4.2)
    - Matrix sensors in 35, 60 and 90 cm depth
    - Tensiometer in 90 and 180 cm depth

The measuring interval of the probes is 10 seconds; average values are recorded every 10 minutes.

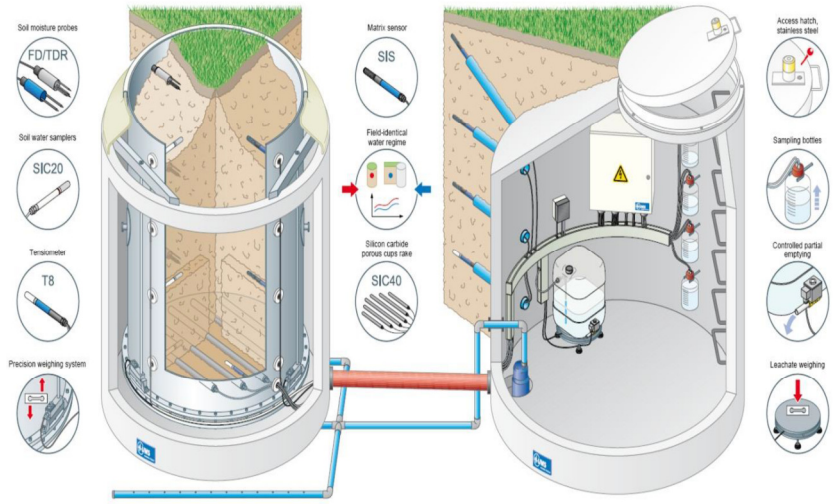

Figure 4.2: Design of a SCIENCE-lysimeter from UMS Munich (UMS 2013)

4.1.2 Gravity-lysimeter (Publication II)

The gravitation lysimeters (GRAVITYLYS) are two cube-shaped concrete containers encased in a steel sheet (dimensions 100×100×150 cm) which were manually filled with partly disturbed material from the surrounding area. Both containers are of the same dimensions and fillings. The filling comprises two soil horizons on a gravel drainage layer (100– 150 cm). The top soil Ap horizon (0–30 cm) and the B horizon (30–100 cm) consist of loamy sand, although the B horizon has a slightly higher silt content (Stumpp et al. 2009). The lysimeter tank is slightly raised compared to its surroundings, but is cultivated manually in the same manner as the field. Leachate outflow at the bottom of the lysimeter is only possible if saturation is reached. The leachate was monitored using a tipping bucket (±0.1 mm). The whole research site has been repeatedly described in detail (Fank 1999; Fank 2008; Von Unold and Fank 2008; Klammler and Fank 2014).

4.2 Experimental design

On April 21, 2010 Pyramin WG, containing 650 g kg^{-1} chloridazon, was applied pre-emergence as suspension onto both the soil surface of the field including the monolithic SCIENCELYS (2.5 x 4 m) and the soil surface of one GRAVITYLYS (1 x 1 m). Although the plant protection product Pyramin WG is normally not applied to maize, it was used in this study to assess transformation processes below the root zone to its main metabolites, desphenyl-chloridazon and methyl-desphenyl-chloridazon.

Figure 4.3: Application of Pyramin WG in April 2010 at the SCIENCELYS

On May 12, 2010 the commercial formulation Artett, containing bentazone and terbuthylazine, was applied post-emergence as suspension onto both the soil surface of the field including the monolithic SCIENCELYS (2.5 x 4 m) and the soil surface of the second GRAVITYLYS (1 x 1 m). An application rate of 2.73 kg ha^{-1} for each bentazone and terbuthylazine was used. The applications were performed by the company BASF (Vienna, Austria) using hand-held spraying apparatus following standard agronomic practices.

Figure 4.4: Application of Artett in May 2010 at the SCIENCELYS and GRAVITYLYS

On April 17, 2012 Poncho maize seeds (Bayer Austria GmbH) were sowed manually to a depth of 4 cm within the lysimeter surface of 1 m^2. Each seed carried 0.5 mg of clothianidin in its seed coating. At the vegetation stage of seedling the yield was reduced from eleven plants per row to six to better accommodating the lysimeter surface.

On May 24, 2012 Gardo Gold (Syngenta Agro GmbH), containing S-metolachlor and terbuthylazine, was applied by the company BASF (Vienna, Austria) using hand-held spraying apparatus (Figure 4.5). Application rates were equivalent to 3.47 kg ha^{-1} for S-metolachlor and 2.08 kg ha^{-1} for terbuthylazine. Dual Gold (Syngenta Agro GmbH), containing only S-metolachlor, was applied at a rate of 1.2 kg ha^{-1} on May 12, 2013 and 0.96 kg ha^{-1} on May 10, 2014 with typical agricultural machinery (e.g. field sprayer). The plant protection products were applied post-emergence as suspensions onto the soil surface of the field including the SCIENCELYS. The field area chosen for the application ensured an even distribution of the pesticide on the lysimeter surface and to minimize impacts from the surrounding area.

Figure 4.5: Maize six weeks after planting; Application of Gardo Gold in 2012 at the SCIENCELYS

The SCIENCELYS was cultivated by local farmers with crop rotation consisting of maize (2010, 2012, 2014), triticale (Triticosecale spp.) (2011 and 2015) and pumpkin (2013). Outside the crop vegetation periods, typical catch crops (ryegrass [Lolium multiflorum Lam.] or forage rye [Secale cereale L.]) were planted. The cultivation of the GRAVITYLYS were performed manually. In 2010 maize was grown on both surfaces and afterwards grass was planted during the remaining observation period.

4.3 Sampling and preparation

Leachate from the lysimeters was collected continuously at weekly intervals following the natural weather conditions and stored at -18°C prior to analysis. The observation period started in April 2010 and lasted until May 2015.

The soil sampling procedure at each lysimeter was equally in 2010 and 2012. Samples were collected directly before and after applications as well as 12, 30, 80 and 150 days after application at different soil depths (0-10, 10-20 and 20-30 cm). Sampling at 10-20 cm was initiated on day 12 and at 20-30 cm on day 30. The used stainless steel soil auger (30 cm x 10 mm) could take 20-30 g soil (Figure 4.6). The sampled point was marked and recorded after each withdrawal to avoid repetition of sampling at the same place (Figure 4.6). Samples were withdrawn each time from six different locations within the lysimeter surface. Samples taken randomly from two non-adjacent locations were pooled, giving a total of three replicate samples per horizon at each sampling time. The field-moist samples were well-homogenized, air-dreid and sieved to < 2 mm.

Figure 4.6: Soil sampling with a stainless steel auger; each borehole was marked

In 2010 maize plants were collected from both GRAVITYLYS and the SCIENCELYS while in 2012 only the SCIENCELYS were sampled. Maize samples were taken according to the growth stages seedling, tasseling, milk and dough, and physiological maturity for pesticide analysis. Samples from the SCIENCELYS were only collected from the lysimeter surface at the seedling and physiological maturity stage. In order to minimaze disturbance maize plants at tasselling and milk and dough were collected from the surrounded field area. At each stage, four plants (at seedling 6-7 plants were needed) were harvested and divided into leaves and stems, roots and maize kernels (only at grain maturity). Samples were freeze-dried, intensively homogenized and finely grounded prior to analysis.

Figure 4.7: Maize plants, roots and kernels at the maturity stage (October 2012)

5 RESULTS AND DISCUSSION

5.1 A long-term lysimeter experiment to investigate the environmental dispersion of the herbicide chloridazon and its metabolites - comparison of lysimeter types (Publication II)

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Clarification

The publication was written and prepared by Andrea Schuhmann. The simulations with PEARL were performed by Gernot Klammler. Comments of co-authors were included in the revised manuscript.

A long-term lysimeter experiment to investigate the environmental dispersion of the herbicide chloridazon and its metabolites - comparison of lysimeter types

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Abstract

Purpose In order to investigate the long-term fate of the herbicide chloridazon and its polar metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon, a lysimeter experiment was conducted.

Materials and methods The plant protection product *Pyramin WG*, which contains chloridazon, was applied to a weighable, monolithic lysimeter and a backfilled, gravitation lysimeter. Leachate, soil and maize samples were analysed and the data from the monolithic lysimeter was used to simulate the transport through the soil profile with the pesticide leaching model PEARL.

Results and discussion Chloridazon rapidly degraded to desphenyl-chloridazon and methyl-desphenyl-chloridazon. In leachate, chloridazon was therefore detected at concentrations up to 3.5 µg L^{-1}, whereas the metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon were present for more than two years at higher concentrations up to 24 and 6.1 µg L^{-1}. The concentrations of chloridazon in soil decreased significantly with depth and time, whereas both metabolites increased up to 370 and 16 µg kg^{-1}. The high concentrations, even 916 days after the application, clearly indicate a continuous downward migration and degradation in soil. In maize, chloridazon and purely the metabolite desphenyl-chloridazon were detected in leaves and stems. Desphenyl-chloridazon was additionally found in grains. It was noted that the design and setup of the two lysimeters lead to significant variations in terms of transformation rate, soil retention time and accumulation by plants. A comparison of the simulated results and the leaching rates measured from the monolithic lysimeter yielded adequate results for the metabolites, but rather poor correlation for chloridazon.

Conclusions The results obtained suggest persistence and high dispersion of chloridazon, and especially its metabolites, in leachate and soil. In maize, the migration in leaves and stems and the accumulation by grains might be relevant in terms of food security.

Keywords Chloridazon • Leachate • Lysimeter • Metabolites • Soil • PEARL-Model

1 Introduction

Transfer of pesticides into ground and surface water has become an important environmental concern following the widespread application of plant protection products in agriculture. Studies on groundwater contamination have reported increasing findings of either pesticides or their metabolites (Buttiglieri et al. 2009; Carabias-Martínez et al. 2003; Loos et al. 2010; Postigo and Barceló 2015). In Europe, groundwater - an important source of drinking water - is regularly monitored for the concentrations of a large array of pesticide and metabolites. The ground water directive (EC 2006) concerning pesticides supervened a drinking water directive (EC 1998), which outlined the general limits for pesticide concentrations of 0.1 $\mu g\ L^{-1}$ for single compounds and 0.5 $\mu g\ L^{-1}$ for the sum of all pesticides.

Metabolites are formed through processes affected by a pesticide's inherent properties as well as physical, chemical and biological soil characteristics, climatic conditions and cultivation practices. Metabolites are usually more polar, thermo-labile and less volatile than their parent compounds (Kuster et al. 2009) and may, therefore, depending on their dissipation behavior in soil, present an elevated risk of leaching. Although the physico-chemical properties (water solubility, octanol-water and organic carbon-water partitioning coefficients) of pesticides have been extensively studied in the past (Arias-Estévez et al. 2008, Dousset et al. 1994, Gevao et al. 2000), there is still a lack of environmental approaches in relation to treatment type and dose, soil type and climatic conditions. Problems deriving from the potential risk of contaminating soil and water are still an important issue.

Analytical methods to determine the extent of pesticides and their metabolites have improved, making it possible to detect low residue levels in complex matrices of environmental samples. With the introduction of liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS), high sensitivity and selectivity can be achieved, and compounds of medium to high polarity can be detected. Consequently, this technique is widely used for pesticide analysis, especially for the very polar metabolites (Reemtsma et al. 2013; Picó et al. 2006; Wille et al. 2011).

Lysimeters are an effective tool for studying the fate and transport of chemicals in the environment (Burauel and Führ 2000; Dousset et al. 1995; Francaviglia et al. 2000; Kasteel et al. 2007; Renaud et al. 2004; Schroll et al. 1999). Compared with laboratory studies, results are more realistic due to scale size and the natural rainfall situation. Thus, the use of lysimeters for agricultural research has increased (Lanthaler and Fank 2005). The technical design of lysimeters has improved from simply measuring water drainage to the precise computation of water and solute flux using high-resolution weighing systems (Von Unold and Fank 2008). The challenge is that variables such as the timing of weather conditions, e.g. the volume of rain after the application, cannot be influenced in situ.

Numerical models provide an efficient and low-cost method of analysing or predicting the long-term fate of pesticides in soil- and groundwater. Various models like MACRO (Jarvis 1995), PRZM (Suárez 2005) and PEARL (Leistra et al. 2001) have been developed to describe soil water movement and pesticide leaching. These models can not only simulate water flow and the transport of pesticides through the soil profile - they also consider transformation products to some degree. Baris et al. 2012 comprehensively review a number of models for pesticide leaching purposes. Several studies where pesticide leaching models were used are available in literature (Scorza and Boesten 2005; Leistra and Boesten 2010). Although such numerical simulation models require a number of sensitive input parameters,

the main advantage of their use is an increased understanding of these complex system processes. Furthermore, simulation models allow for a transfer of the application to other locations and the upscaling to applications of entire regional aquifers.

The present investigation focuses on the pesticide chloridazon and its polar metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon, which are some of the most frequently found pesticides in the ground and surface waters of Europe (Loos et al. 2010). Chloridazon (5-amino-4-chloro-2-phenyl-3-(2H)-pyridazinone) is a selective, systemic herbicide which inhibits photosynthesis. The herbicide is used, pre-emergence or early post-emergence, for weed control in sugar beet crops (EPA 2005). Chloridazon appears to be mobile in a variety of soil types and can thus be considered as a hazardous contaminant for groundwater (Céspedes et al. 2007). The predominant chloridazon metabolite is desphenyl-chloridazon, formed by aerobic degradation pathways in soil by splitting off the phenyl-group (EPA 2005; Tomlin 2006). The metabolite methyl-desphenyl-chloridazon was first detected by Weber et al. (2007) through a non-target screening of ground and surface water. According to European regulations (EU 2003) the European drinking water limit of 0.1 µg L^{-1} does not apply for "non-relevant" metabolites. Thus "non-relevant" metabolites of pesticides are either not specifically regulated or diverse threshold values are applied. In Austria and other European countries the metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon are classified as "non-relevant" metabolites and the threshold value for these metabolites in groundwater is 3 µg L^{-1}. Some studies about these metabolites in water (Buttiglieri et al. 2009; Loos et al. 2010; Reemtsma et al. 2013; Wode et al. 2012) and soil (Céspedes et al. 2007; Dechene et al. 2014) already exist.

In the present study two types of lysimeters a weighable, monolithic lysimeter and a backfilled, gravitation lysimeter to investigate the fate of chloridazon and its polar metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon in agricultural soil. The aims were to quantify the migration through soil, the leaching and the accumulation in maize. In addition, the PEARL model was used to simulate soil water dynamics and the pesticide fate using the field data from the weighable, monolithic lysimeter. The experimental setup of the weighable, monolithic lysimeter allowed in situ measurements of water contents and pesticide concentrations in different depths to yield adequate modelling results.

2 Materials and methods

2.1 Experimental site and chloridazon application

The experiment was conducted using two different types of lysimeters at an agricultural test site in Wagna (Styria, Austria). A weighable, monolithic lysimeter built in 2004 (UMS 2013) and a gravitation lysimeter filled in 1992 were utilized. Both had been used to cultivate maize which is a typical crop for this region. The soil is classified as loamy sandy Dystric Cambisol with 52% sand, 34% silt and 15% clay. Further characteristics are a pH of 6.6 ($CaCl_2$), an organic carbon content (OC) of 2.7% and a cation exchange capacity (CEC) of 11.53 cmolc kg^{-1} at a depth of 0-25 cm.

The weighable, monolithic lysimeter (SCIENCELYS, dimensions 100 x 100 x 200 cm) installed directly in the agricultural test field, contains undisturbed loamy to sandy soil over gravel and sand. The lysimeter is conventionally cultivated with agricultural machines (e.g. a plough). The load cells are lowered to the foundation and the upper ring of the lysimeter is

removed. After tillage, the ring is remounted and the lysimeter is lifted on to the load cells. A balance on the concrete foundation can measure the weight of the lysimeter with a resolution of 35 g (0.035 mm water equivalent). Inside the monolith, various measurements can be taken at 10 minute intervals - the water content using TDR-probes, the hydraulic potential using tensiometers and, SIS-sensors, and the soil temperature. A suction cup rake is implemented in the gravel filter layer as lower boundary condition at the bottom of the lysimeter. An automatic vacuum pump is used to transfer the soil water tension, measured at 180 cm below surface outside the lysimeter, to the suction cups at the lysimeter bottom. Thus, flow rates equivalent to those in the undisturbed field can be achieved. Leachate is sampled through the suction cup rake at the bottom of the lysimeter. The amount of leachate is detected using a tipping bucket with 0.1 mm resolution.

The gravitation lysimeter (GRAVITYLYS) is a cube-shaped concrete-container encased in a steel sheet (dimensions 100 x 100 x 150 cm) which was manually filled with partly disturbed material from the surrounding area. The filling comprises two soil horizons on a gravel drainage layer (100-150 cm). The top soil Ap-horizon (0-30 cm) and the B-horizon (30-100 cm) consist of loamy sand, although the B-horizon has a slightly higher silt content (Stumpp et al. 2009). The lysimeter tank is slightly raised compared to its surroundings but is cultivated manually in the same manner as the field. Leachate outflow at the bottom of the lysimeter is only possible if saturation is reached. The leachate was monitored using a tipping bucket (±0.1 mm). The whole research site has been repeatedly described in detail (Fank 1999; Fank 2008; Von Unold and Fank 2008; Klammler and Fank 2014).

The commercial product *Pyramin WG*, containing 650 g kg^{-1} pure chloridazon, was applied once pre-emergence in April 2010 as suspension onto both the soil surface of the field including the monolithic SCIENCELYS (2.5 x 4 m), and the soil surface of the GRAVITYLYS (1 x 1 m). The field area was chosen for the application to ensure an even distribution of the pesticide on the lysimeter surface and to minimize impacts from the surrounding area. The rate of application was 6.5 g equivalent to 5.9 kg chloridazon per hectare. The application was performed by the company BASF (Vienna, Austria) using hand-held spraying apparatus following standard agronomic practices. The experiment was performed under natural weather conditions without any additional irrigation. Precipitation, air pressure, relative humidity, air temperature, wind velocity, and global radiation were measured at a meteorological station at the test site.

2.2 Quantification of residues in leachate, soil and maize

Pesticides standards (chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The internal standard desphenyl-chloridazon-$^{15}N_2$ produced by Chiron (Trondheim, Norway) was obtained from CAMPRO (Berlin, Germany).

Leachate from the bottom of the lysimeters was collected weekly (when sufficient leachate/soil water flow) and stored at -18°C prior to analysis. Samples were analysed by direct injection after the addition of an internal standard (Desphenyl-chloridazon-$^{15}N_2$).

Soil samples at depths of 0-30 cm were taken from both lysimeters before the herbicide application. Immediately after the application, soil samples were taken at a depth of 0-10 cm. The sampling was repeated 12, 30, 80, 150, 497, 764 and 916 days after the application. Soil

samples were collected using a stainless steel soil auger (30 x 1 cm). The sampling points were carefully restocked with soil taken from the surrounding field area and marked to avoid sampling from the same place. Samples were taken randomly from six different locations within the lysimeter surface. On each occasion, two non-adjacent sampling points were mixed, giving a total of three replicate samples per depth for each sampling time. Samples were taken at soil depths 0-10 cm, 10-20 cm and 20-30 cm. The sampling at 10-20 cm depth was initiated on day 12 after the application and at 20-30 cm depths on day 30 after the application. Soil samples were air-dried, intensively homogenised and sieved (< 2 mm) before extraction. Soil samples were extracted and the resulting extracts were quantified according to the methods as described in Fuhrmann et al. (2014).

Maize plants were sampled at four vegetation stages: seedling (May 12, 2010), tasseling (July 12, 2010), milk and dough (August 2, 2010) and physiological maturity at the end of the maize growing season (September 27, 2010). At each stage, the plants were harvested and divided into leaves and stems, roots, and maize kernels (only at grain maturity). Samples were freeze dried and intensively homogenised. Aliquots of the homogenized samples were extracted and the resulting extracts were quantified according to the methods as described in Fuhrmann et al. (2014).

2.3 Liquid chromatography-mass spectrometry

The analytical method is described in detail elsewhere (Fuhrmann et al. 2014). It is based on an ultra-high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPLC-ESI-MS/MS) method. The MS was operated in positive ionization mode and quantitation performed by multiple reaction monitoring (MRM). A gradient of mobile phases constituted of 0.01 % formic acid and 2 mM ammonium acetate in water (A) and 2 mM ammonium acetate in methanol (B) for soil and maize samples was used. In analysing leachate samples, water modified with 0.01 % formic acid was used as eluent (A). Separation was achieved on a Kinetex column (C8, 2.6 µm particle size, 2.1×100 mm, Phenomenex, Aschaffenburg, Germany). The injection volume was 40 µl for soil and leachate and 3 µl for maize samples. The method detection limits for chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon are given in Table 1.

2.4 Numerical modelling

The one-dimensional model PEARL (FOCUSPEARL 4.4.4; Leistra et al. 2001) that describes the fate of pesticides and relevant transformation products in the soil-plant system was applied in this study using the data measured from the SCIENCELYS. Processes included in PEARL are pesticide application and deposition, diffusion through the gas and liquid phase, uptake of pesticides by plant roots, lateral discharge of pesticides with drainage water, and volatilization of pesticides at the soil surface. In addition, a convection-dispersion equation is used to describe the solute's transport. Instantaneous equilibrium or kinetic sorption is described by either a linear or a Freundlich equation, and degradation by first order kinetics, depending on soil water content, temperature and depth. The model does not allow the Freundlich exponent to vary with soil properties. PEARL uses exponential transformation, and changes the transformation rate according to temperature by using the Arrhenius equation and moisture content using a power law. PEARL does not limit the number of transformation products

or the transformation pathways that can be simulated. PEARL is linked with the SWAP model (Kroes et al. 2008), whose soil hydrology is described by Richard's equation.

The Van Genuchten-Mualem parameters for describing the hydraulic characteristic of the location investigated are derived from in situ measurements of water contents and matric potentials inside the lysimeters and are presented in Table 2. A dispersivity length of 0.05 m is assumed.

The potential evapotranspiration is the key variable affecting the uptake of water by plant roots and soil evaporation. SWAP provides the calculation according to a modified Penman-Monteith equation (Monteith 1965, Van Dam et al. 1997) or that from Makkink (1957). Furthermore, a user-defined reference evapotranspiration can be used. Since the grass reference evapotranspiration according to Allen et al. (1998) is also measured by a lysimeter at the Wagna test site, the reference evapotranspiration measurement has been used as input for the model approach presented here.

An antecedent sensitivity analysis indicates that the most sensitive input parameters concerning the simulated substances chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon are the sorption coefficients, the Freundlich exponents, the half-lives and the transformation factors from the parent compound to the metabolites. The Pesticide Properties DataBase (University of Hertfordshire 2013) has amassed an extensive compilation of pesticide properties which was utilised to parameterize the chemical model setup. In line with FOCUS (2009), a depth dependent degradation rate by multiplying the surface degradation rate with 0.5 between 30-60 cm and with 0.3 for 60-100 cm was applied. Below 100 cm soil depth no degradation is assumed.

Table 1 Limits of detection (LOD) and limits of quantitation (LOQ) of chloridazon and its metabolites

Compound	Leachate		Soil		Leaf/Stem		Maize kernels	
	LOD (µg L^{-1})	LOQ (µg L^{-1})	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	LOD (µg kg^{-1})	LOQ (µg kg^{-1})
Chloridazon	0.010	0.020	0.17	0.34	8.9	18	5.7	11
Desphenyl-chloridazon	0.040	0.080	1.5	3	58	11.5	7.4	15
Methyl-desphenyl-chloridazon	0.025	0.050	0.19	0.37	7.8	16	2.6	5.2

Table 2 Parameters of the Van Genuchten-Fitting to the soil physical data at the SCIENCELYS

Depth (cm)	θ_{sat} (-)	θ_{res} (-)	α (1/cm)	n (-)	k_{sat} (m/s)
0-30	0.39	0.16	0.05	1.33	4.6×10^{-6}
30-50	0.38	0.19	0.04	1.45	6.5×10^{-6}
50-80	0.44	0.11	0.065	1.2	6.7×10^{-6}
80-130	0.2	0.03	0.25	1.4	5×10^{-5}
>130	0.14	0.03	0.25	1.9	1.2×10^{-4}

θ_{sat} saturated water content, θ_{res} residual water content, α parameter related to the inverse of air entry suction (corresponds to the inflection point of the retention curve), n parameter related to pore-size distribution (corresponds to the slope of the retention curve), k_{sat} saturated hydraulic conductivity

The basis for a solute transport simulation is an accurate simulation of water movement in soil. Thus, this model calibration followed a stepwise approach starting with the calibration of water contents at depths 35, 60, 90 and 180 cm depth. Evapotranspiration and leachate amount were calibrated by adjusting soil physical parameters, evapotranspiration parameters and crop parameters. In a second step, the pesticide transport simulation was carried out by focusing on the objective function of leached pesticide mass. For the comparison of simulated and measured results of water contents, evapotranspiration, leachate amount and leached pesticide masses the Nash-Sutcliffe Efficiency (NSE; Nash and Sutcliffe 1970) is used. The dimensionless NSE ranges between 1 and -∞, where NSE = 1 denotes a perfect model fit and for NSE < 0 the average of the observations would be a better predictor than the model (Krause et al. 2005). In hydrological studies, it is common practice to assess the model performance on the basis of NSE, where NSE > 0.75 indicates a "good" performance and NSE < 0.36 indicates a "weak" similarity of model results with observations (Van Liew and Garbrecht, 2003). Furthermore, pesticide contents in the soil were also considered within the model calibration to ensure that dominant processes were simulated correctly with the model assumptions. The pesticide crop uptake is another important element of the pesticide mass balance, but as the ongoing degradation in the plant cannot be simulated by PEARL, this aspect was not considered in the present model calibration.

Table 3 Annual amounts of precipitation (P), grass reference evapotranspiration (ET_0) and leachate of the SCIENCELYS and GRAVITYLYS from 2010 to 2013

Year	P (mm a^{-1})	ET_0 (mm a^{-1})	SCIENCELYS (mm a^{-1})	GRAVITYLYS (mm a^{-1})
2010	1014	692	429	569
2011	730	767	63	142
2012	1000	780	325	445
2013	952	739	479	614
Mean	930	745	324	444

3 Results

3.1 Precipitation and leachate from the lysimeters

Table 3 shows the annual amounts of precipitation and leachate as regards each lysimeter. Annual precipitation at the test site in Wagna ranged between 730 mm (2011) and 1014 mm (2010), with a maximum precipitation event of 240 mm in August 2010. The annual amounts of leachate measured at the bottom outlet of the lysimeters varied mainly as a consequence of annual precipitation amounts and infiltration rates. No leachate was generated from the collection systems during certain periods, especially in July, August and September 2011 as well as in July 2012. In addition, an absence of leachate from November 2011 to March 2012 was observed solely in the SCIENCELYS. The effect of seasonal variations on leachate generation in terms of pesticide concentrations was noted. In general, the amount of leachate in the GRAVITYLYS was higher compared to the SCIENCELYS. The higher amount of leachate can be explained by the development of different hydraulic systems due to the manual filling of partly disturbed material.

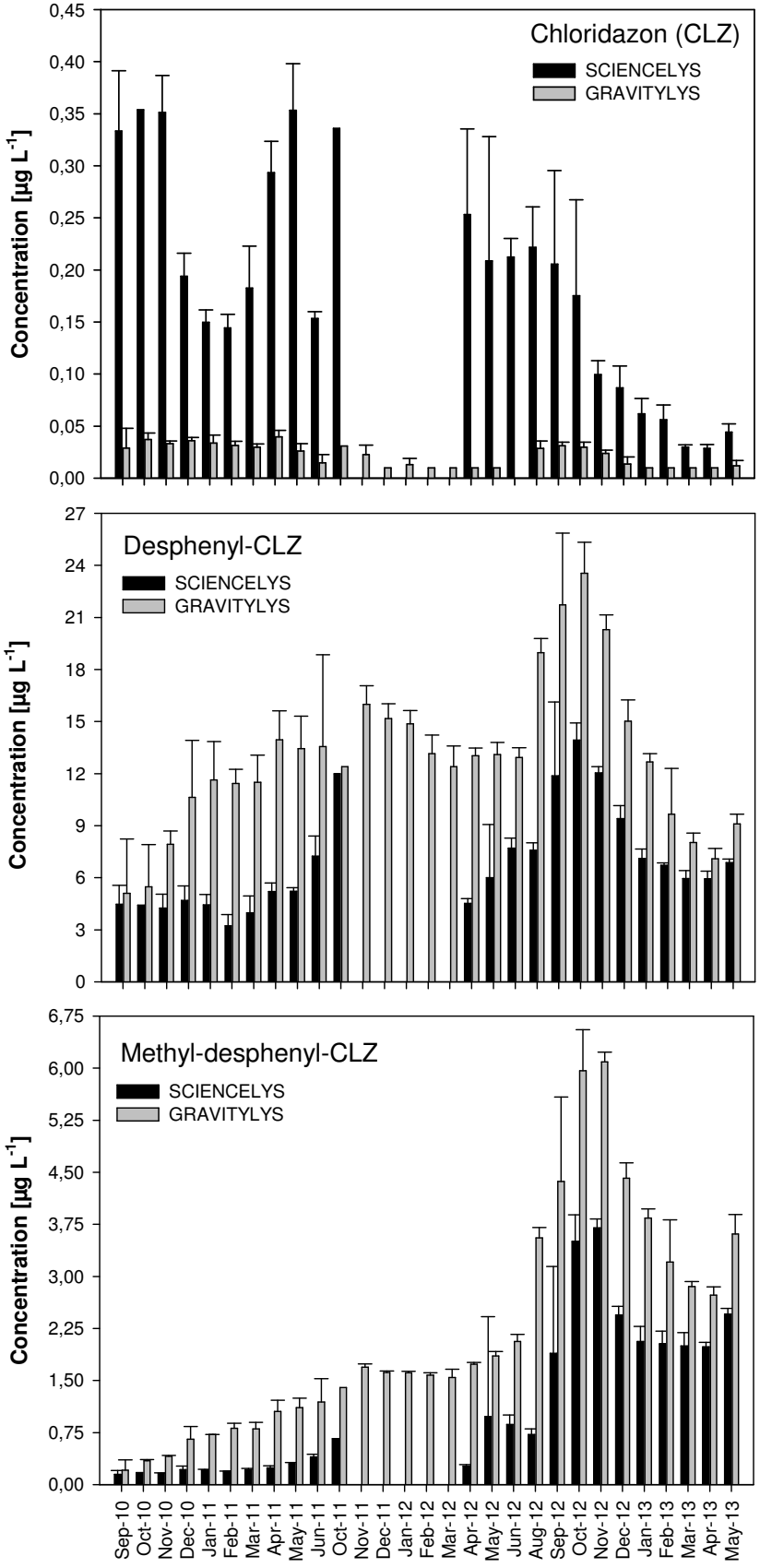

Fig. 1 Average concentrations per month of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon in leachate at the bottom of the SCIENCELYS (180 cm depth) and GRAVITYLYS (150 cm depth) from 2010 to 2013; No leachate occurred in July 2011, August 2011, September 2011 and July 2012

3.2 Loads in the leachate

Concentrations of chloridazon and its metabolites were measured in the leachate in order to quantify the amount of those substances leaching into shallow groundwater. A total of 111 and 137 leachate samples from the base outflows of the SCIENCELYS and GRAVITYLYS respectively were analysed for chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon. Fig. 1 shows the average concentrations per month of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon in the leachate of each lysimeter from 2010 to 2013. In September 2010 (five months after the application), chloridazon and the metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon were detected in the leachate of the SCIENCELYS and GRAVITYLYS.

Although the date of the initial appearance of the pesticides was the same, variability in leached pesticide loads from the different types of lysimeter was observed. From the SCIENCELYS, concentrations of 0.33, 4.5 and 0.15 µg L^{-1} for chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon respectively were detected five months after the application. The concentrations of chloridazon in the leachate exceeded the European threshold value for drinking water (0.1 µg L^{-1}). The concentrations of chloridazon ranged from 0.029 µg L^{-1} to 0.35 µg L^{-1}, with the highest concentrations occurring in October and November 2010, and May and October 2011. Concentrations higher than the EU drinking water limit were detected more than two years after the application. The concentrations of the metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon respectively in the leachate ranged from 3.2 to 14 µg L^{-1} and from 0.15 to 3.7 µg L^{-1}. The greatest concentrations of metabolites were detected from September to November 2012 as a result of the high monthly precipitation in July 2012 (207 mm). The metabolite desphenyl-chloridazon exceeded the Austrian threshold value of 3 µg L^{-1} in all the positive samples, whereas methyl-desphenyl-chloridazon reached this threshold value only in October and November 2012 (3.5 and 3.7 µg L^{-1}). The concentrations of desphenyl-chloridazon dropped rapidly to 9.4 µg L^{-1} in December 2012 and reached a mean concentration of 6.5 µg L^{-1} in the leachate at the end of the monitoring. Methyl-desphenyl-chloridazon declined to 2.5 µg L^{-1} in December 2012 and remained stable until the end of monitoring.

In the GRAVITYLYS, chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon appeared in the leachate at concentrations of 0.029, 5.1 and 0.21 µg L^{-1} on the same date as the SCIENCELYS. In the leachate of the GRAVITYLYS, only traces of the parent compound chloridazon were detected. High concentrations of both metabolites were detected in the leachate ranging from 5.1 to 24 µg L^{-1} and 0.21 to 6.1 µg L^{-1} for desphenyl-chloridazon and methyl-desphenyl-chloridazon respectively. The greatest concentrations of metabolites were also detected from September to November 2012. The metabolite desphenyl-chloridazon exceeded the Austrian threshold value of 3 µg L^{-1} in all the positive samples. The concentrations of desphenyl-chloridazon decreased gradually to 15 µg L^{-1} in December 2012 and reached a mean concentration of 9.3 µg L^{-1} in the leachate at the end of the monitoring. Methyl-desphenyl-chloridazon was found at concentration exceeding 3 µg L^{-1} in August 2012. The concentrations increased to 4.4 µg L^{-1} in December 2012 and it finally dropped to 3.6 µg L^{-1} in May 2013.

3.3 Residues in soil

Table 4 shows the soil concentrations of extractable chloridazon and its metabolites at different depths from the SCIENCELYS and GRAVITYLYS over a 916-day period. As expected, the concentrations of chloridazon where highest at the soil surface in both lysimeters. In the SCIENCELYS, the concentration of chloridazon in the topsoil layer (0-10 cm) was 1700 $\mu g\ kg^{-1}$ immediately after the application. On day 12, the chloridazon concentration increased to 2100 $\mu g\ kg^{-1}$ and then decayed from 30 days onwards. On day 916, the concentrations of chloridazon were considerably reduced, at 1.4 $\mu g\ kg^{-1}$. Residues of desphenyl-chloridazon in the topsoil peaked (370 $\mu g\ kg^{-1}$) in their formation on day 80 after the application and subsequently decayed to 4.1 $\mu g\ kg^{-1}$ on day 916. Methyl-desphenyl-chloridazon was detected in the topsoil of the SCIENCELYS on day 80 (2.4 $\mu g\ kg^{-1}$). The highest concentrations were found on day 150 (6.2 $\mu g\ kg^{-1}$) and finally decreased to 0.91 $\mu g\ kg^{-1}$ on day 916. Shortly after the application at the GRAVITYLYS, the chloridazon concentration was 3600 $\mu g\ kg^{-1}$ in the 0-10 cm soil layer. On day 12, the concentration of chloridazon had already decreased to 2700 $\mu g\ kg^{-1}$. On day 916, 8.7 $\mu g\ kg^{-1}$ of chloridazon remained in the top layer of the GRAVITYLYS. The highest concentration of desphenyl-chloridazon (250 $\mu g\ kg^{-1}$) in the topsoil was found on day 80 after the application – it subsequently decayed to 9.1 $\mu g\ kg^{-1}$ on day 916. Residues of methyl-desphenyl-chloridazon were extracted on day 12 (0.21 $\mu g\ kg^{-1}$) and had increased considerably by day 80 (5.6 $\mu g\ kg^{-1}$). The highest concentration was found on day 497 (8.9 $\mu g\ kg^{-1}$) and it finally decreased to 1.7 $\mu g\ kg^{-1}$ on day 916.

At 10-20 cm soil depth, pesticide concentrations showed a trend similar to that at 0-10 cm. However, peak concentrations were lower, and the occurrence of the peaks shifted within a few days of the application in both lysimeters. Sampling at 10-20 cm soil depth commenced 12 days after the pesticide application on both lysimeters. At the SCIENCELYS, the concentration of chloridazon was at 77 $\mu g\ kg^{-1}$ on day 12, increasing to 610 on day 30, decreasing to 59 $\mu g\ kg^{-1}$ on day 80, and ending up at 1.7 $\mu g\ kg^{-1}$ on day 916. Residues of desphenyl-chloridazon gradually increased, with the highest concentration recorded at 56 $\mu g\ kg^{-1}$ on day 150. On day 916, a desphenyl-chloridazon concentration of 9.0 $\mu g\ kg^{-1}$ remained in the soil of the SCIENCELYS. Methyl-desphenyl-chloridazon was detected on day 80 after the application (0.85 $\mu g\ kg^{-1}$) in the 10-20 cm soil layer of the SCIENCELYS. On day 497, a peak concentration of 6.5 $\mu g\ kg^{-1}$ was detected, which decreased to 2.4 $\mu g\ kg^{-1}$ on day 916. At the GRAVITYLYS, the concentration of chloridazon was at 160 $\mu g\ kg^{-1}$ on day 12 - it then increased to 670 on day 30, decreased to 21 $\mu g\ kg^{-1}$ on day 80 and ended up at 1.4 $\mu g\ kg^{-1}$ on day 916. High concentrations of desphenyl-chloridazon were detected at 10-20 cm soil depth. Peak concentrations at 81 $\mu g\ kg^{-1}$ on day 150 and 87 $\mu g\ kg^{-1}$ on day 497 were recorded. On day 916, a desphenyl-chloridazon concentration at 17 $\mu g\ kg^{-1}$ remained in the soil of the GRAVITYLYS. Residues of the methylated metabolite were found on day 30 (0.51 $\mu g\ kg^{-1}$),

Table 4 Concentrations (μg kg^{-1}) of extractable chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon on different sampling days and soil depths (0-10, 10-20 and 20-30 cm) from the SCIENCELYS and GRAVITYLYS

	SCIENCELYS								
Day of	Chloridazon (CLZ)			Desphenyl-CLZ			Methyl-desphenyl-CLZ		
Sampling	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm
0	1700	(-)	(-)	0.0	(-)	(-)	0.0	(-)	(-)
	2100		(-)			(-)			(-)
12		77		6.9	0.0		0.0	0.0	
30	1200	610	25	9.9	7.6	0.0	0.0	0.0	0.0
80	770	59	3.5	370	20	0.0	2.4	0.85	0.0
150	95	34	2.3	97	56	51	6.2	4.9	6.5
497	2.3	4.8	1.8	14	34	66	2.1	6.5	16
764	1.1	3.5	2.0	9.5	12	19	1.2	2.9	4.2
916	1.4	1.7	1.3	4.1	9.0	13	0.91	2.4	5.0
	GRAVITYLYS								
Day of	Chloridazon (CLZ)			Desphenyl-CLZ			Methyl-desphenyl-CLZ		
Sampling	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm	0-10 cm	10-20 cm	20-30 cm
0	3600	(-)	(-)	2.8	(-)	(-)	0.0	(-)	(-)
12	2700	160	(-)	15	1.1	(-)	0.21	0.0	(-)
30	2000	670	36	62	32	1.7	0.69	0.51	0.0
80	1000	21	3.9	250	20	1.9	5.6	1.6	0.79
150	54	37	4.5	92	81	55	4.5	5.9	3.6
497	16	8.0	1.2	75	87	73	8.9	13	15
764	10	5.1	2.0	32	43	55	3.4	7.2	12
916	8.7	1.4	0.90	9.1	17	22	1.7	5.3	8.8

(-) Samples were not taken at these days

n = 3

increasing to 13 µg kg^{-1} on day 497. On day 916, the concentration in the GRAVITYLYS decreased to 5.3 µg kg^{-1}.

At 20-30 cm soil depth, sampling initiated 30 days after the pesticide application. Concentrations of the parent compound declined markedly whereas the metabolites increased. At the SCIENCELYS, a chloridazon concentration of 25 µg kg^{-1} was detected 30 days after the application. The concentration rapidly decreased to 3.5 µg kg^{-1} on day 80, falling gradually from then on to 1.3 µg kg^{-1} on day 916. The metabolite desphenyl-chloridazon was detected 150 days after the application at a concentration of 51 µg kg^{-1}. By day 497, the concentration of desphenyl-chloridazon had increased to 66 µg kg^{-1}. The concentration of chloridazon-desphenyl declined to 13 µg kg^{-1} on day 916. Methyl-desphenyl-chloridazon was detected on day 150 (6.5 µg kg^{-1}) in the SCIENCELYS. The concentration of methyl-desphenyl-chloridazon reached its maximum of 16 µg kg^{-1} on day 497 and then declined to 5.0 µg kg^{-1} on day 916. At the GRAVITYLYS, a chloridazon concentration of 36 µg kg^{-1} was detected 30 days after the application. The concentration rapidly decreased to 3.9 µg kg^{-1} on day 80, falling gradually from then onwards to 0.90 µg kg^{-1} on day 916. On day 30 and 80, concentrations of the metabolite desphenyl-chloridazon (1.7 and 1.9 µg kg^{-1}) were quantified at 20 - 30 cm soil depths. On day 150 and 479, the concentration of desphenyl-chloridazon considerably increased to 55 µg kg^{-1} and 73 µg kg^{-1}. On day 916, desphenyl-chloridazon was still detected at 22 µg kg^{-1} in the GRAVITYLYS. The methylated metabolite was present on day 80 (0.79 µg kg^{-1}). A peak concentration of 15 µg kg^{-1} was recorded on day 497, falling to 8.8 µg kg^{-1} on day 916.

3.4 Accumulation by maize

Chloridazon and the metabolite desphenyl-chloridazon were accumulated by the leaves and stems of maize at different growth stages from both the SCIENCELYS and GRAVITYLYS (Fig. 2). The other metabolite, methyl-desphenyl-chloridazon, could not be detected in any maize material whatsoever. The concentration of the parent compound chloridazon decreased with the growth of maize, whereas the metabolite desphenyl-chloridazon increased. Only desphenyl-chloridazon was accumulated by maize kernels at grain maturity. As was found in the soil matrix, desphenyl-chloridazon was the major metabolite observed in maize.

For the SCIENCELYS, the extractable amount of chloridazon in maize was 500 µg kg^{-1} at the seedling stage. At the vegetative tasseling, the concentration was considerably reduced at 110 µg kg^{-1}, decreasing slightly to 66 µg kg^{-1} at the milk and dough stage. Chloridazon was not detected in the physiologically mature maize from the SCIENCELYS. The metabolite desphenyl-chloridazon was not accumulated by the leaves and stems of maize at the seedling stage. An amount of 190 µg kg^{-1} was preliminarily detected in the leaves and stems of maize at the vegetative tasseling of the SCIENCELYS. The amount of desphenyl-chloridazon at the milk and dough stage increased to 290 µg kg^{-1} and had decreased considerably to 50 µg kg^{-1} at maturity. In maize kernels from the SCIENCELYS, a concentration of 24 µg kg^{-1} desphenyl-chloridazon was detected at grain maturity.

For the GRAVITYLYS, the extractable amount of chloridazon in maize was 1400 µg kg^{-1} at the seedling stage. One possible explanation for the high chloridazon concentration might be that the uptake occurred more through the aerial pathway rather than from root to shoot translocation. Plant parts above the ground may become contaminated via pathways involving

direct contact between soil particles and plant surfaces. At the vegetative tasseling, the concentrations were considerably reduced at 120 µg kg^{-1}, decreasing slightly to 75 µg kg^{-1} at the milk and dough stage. At maturity, the parent compound chloridazon was no longer detectable. The metabolite desphenyl-chloridazon (55 µg kg^{-1}) was detected in the leaves and stems from the GRAVITYLYS at the seedling stage. At the vegetative tasseling, the amounts of the metabolite increased to 660 µg kg^{-1} and remained at 650 µg kg^{-1} in the leaves and stems at the milk and dough stage. At maturity, the concentrations of the metabolite desphenyl-chloridazon in leaves and stems of maize had decreased considerably to 140 µg kg^{-1}. Maize kernels accumulated 53 µg kg^{-1} of desphenyl-chloridazon at grain maturity.

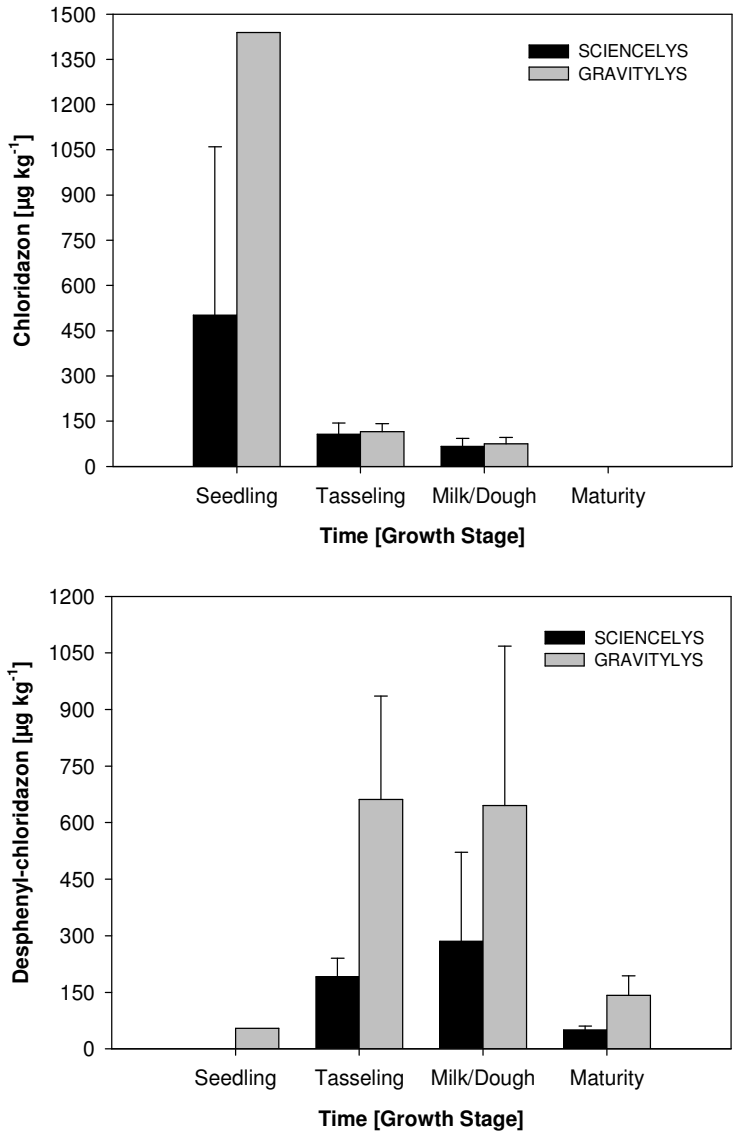

Fig. 2 Accumulation of extractable chloridazon and desphenyl-chloridazon in the leaves and stems of maize at different growth stages of the SCIENCELYS and GRAVITYLYS

3.5 Model simulations

The model period of the present simulation was from January 1, 2009 until December 31, 2013. Following good modelling practice, the period until the application of chloridazon on April 21, 2010 serves as warm-up period for the simulation.

The simulations of the SCIENCELYS water content were calibrated at depths of 35, 60, 90 and 180 cm and the results expressed by the Nash-Sutcliffe-Efficiency (NSE; Nash and Sutcliffe 1970) as given in Table 5.

Table 5 Goodness-of-fit of water contents for the SCIENCELYS at depths of 35, 60, 90 and 180 cm expressed by the Nash-Sutcliffe-Efficiency (NSE) based on daily results between 2010 and 2013

Depth [cm]	**NSE [-]**
35	0.94
60	0.93
90	0.82
180	0.87

The actual evapotranspiration can be simulated with an NSE of 0.94 for the period 2010 to 2013. The temporal interval for this statistical analysis concurs with the leachate sampling interval for pesticide analysis of approximately ten days on average, leading to 185 data pairs for consideration. Fig. 3 shows the cumulative leachate amount for crop periods representing an NSE of 0.31 (n = 185). It can be seen that the general level and the behavior of measured leachate can be represented by the simulation (especially in the years 2010, 2011 and 2012, including the period without any leachate in autumn 2011 and spring 2012). Nevertheless, there are still periods in which measured leachate rates which do not fit well to the measured curve (e.g. the peak in spring 2013 or catch crop in winter 2013/14). The mean annual leachate water rates between 2010 and 2013 are 324 mm and 321 mm for observed and simulated leachate, respectively.

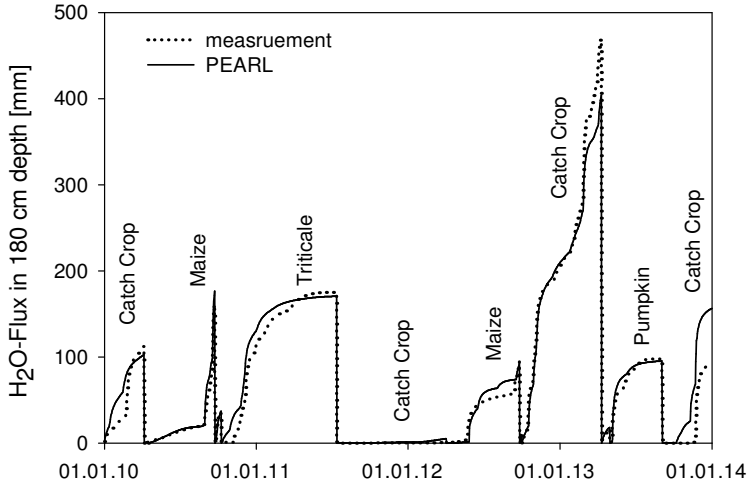

Fig. 3 Comparison of the measured (*dotted line*) and simulated (*continuous line*) cumulative leachate of the SCIENCELYS in 180 cm depth for crop periods

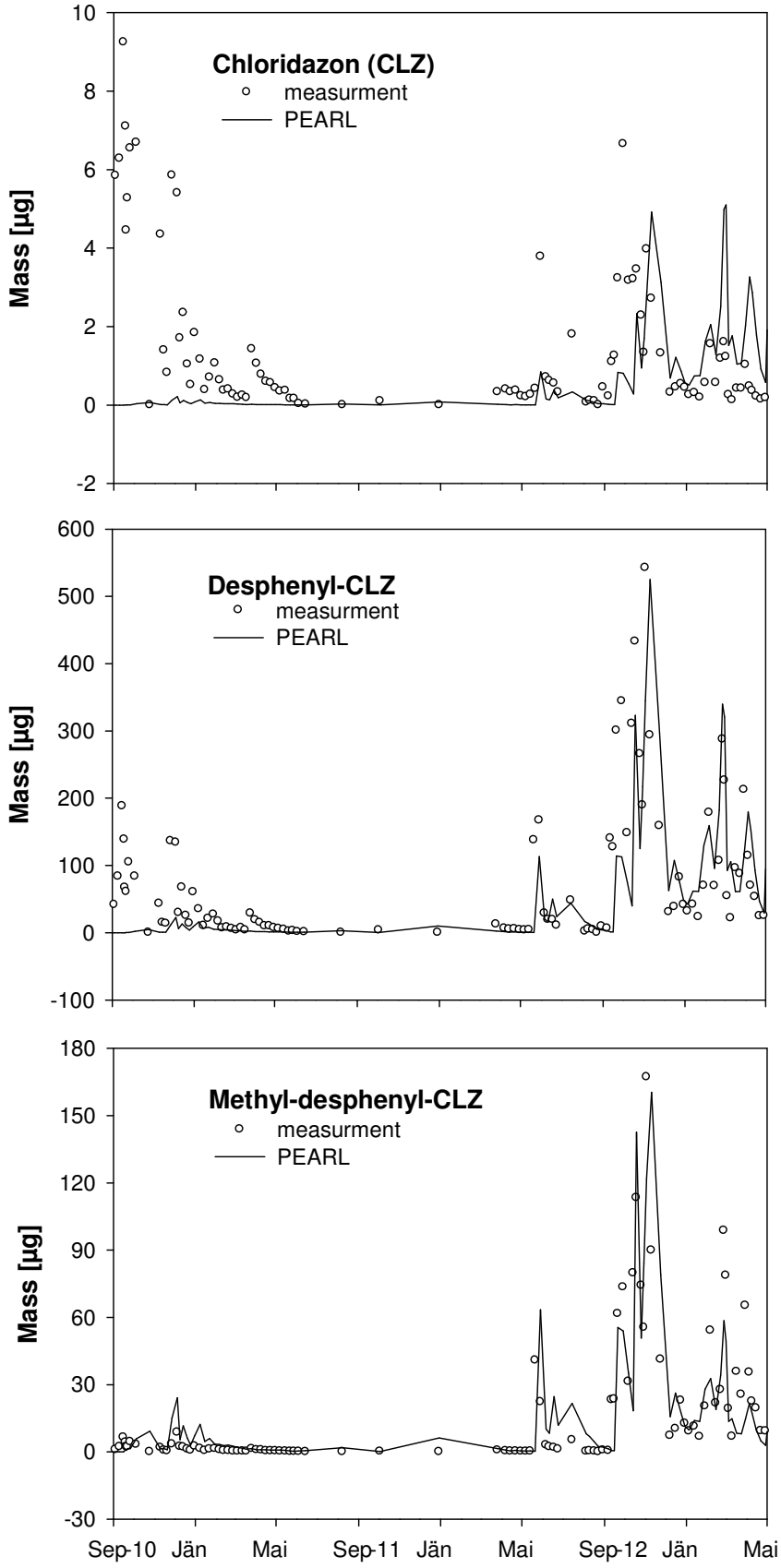

Fig. 4 Measured and simulated leached masses [µg] of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon of the SCIENCELYS for the period August 2010 to May 2013 using PEARL model: measured (*circle*) and fitted with PEARL (*continuous line*)

Table 6 Relevant chemical parameters (DT_{50}…half-live, K_{foc}…Freundlich sorption coefficient related to organic content, 1/n…Freundlich exponent) according to the Pesticide Properties DataBase (PPDB, University of Hertfordshire 2013) and parameters calibrated within the simulation for chloridazon (CLZ), desphenyl-chloridazon (desphenyl-CLZ) and methyl-desphenyl-chloridazon (methyldesphenyl-CLZ)

	CLZ		**Desphenyl-CLZ**		**Methyl-desphenyl-CLZ**	
	PPDB	**calibrated**	**PPDB**	**calibrated**	**PPDB**	**calibrated**
DT_{50} (d)	34.7 (3-93)	34.7	235.5 (130-360)	130	145 (118-170)	145
K_{foc} (mL/g)	199 (89-340)	300	50 (29-74)	74	92 (27-216)	92
1/n (-)	0.845 (0.568-1.03)	0.870	0.834 (0.804-0.868)	0.952	0.867 (0.794-0.915)	0.867
Coefficient of Transformation (-)	n.a.	n.a.	0.559*	0.3	n.s.	0.02

The numbers in brackets indicate the range of measured values as presented within the PPDB (University of Hertfordshire 2013)
*) Estimated maximum occurrence fraction
n.a. not available, n.s. not specified

The simulated results for leaching of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon are presented in Fig. 4 and are based on calibrated parameters as given in Table 6. The simulations of the leached masses resulted in NSEs of -0.58, 0.45 and 0.62 (based on the pesticide sampling interval between May 2010 and May 2013; n = 105) for chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon, respectively. It is obvious for chloridazon that the first peak (September 2010 to April 2011) cannot be represented by the simulation. Furthermore, some peaks for chloridazon in 2012 cannot be predicted and the simulation shows an overestimation in March and April 2013. Desphenyl-chloridazon-loads measured show the same behavior as chloridazon in the period September 2010 to April 2011, but this peak can also not be predicted by the simulation. The remaining period after May 2011 correlates smoothly as regards the temporal dynamic, as well as the magnitude of leached masses. The simulation of methyl-desphenyl-chloridazon - except for some overestimation at the beginning of the simulation and in June and July 2012, with some underestimation between February and April 2013 - strongly resembles the measured loads.

4 Discussion

The degradation of chloridazon in leachate, soil and maize after the application resulted in the formation of two known metabolites: desphenyl-chloridazon and methyl-desphenyl-chloridazon. These were also the main metabolic products of chloridazon in previous studies (Buttiglieri et al. 2009, EFSA 2007). While desphenyl-chloridazon, the predominant metabolite, was measured in all investigated matrices, the second metabolite methyl-desphenyl-chloridazon was detected at lower concentrations and only in leachate and soil. Desphenyl-chloridazon was one of the most abundant compounds monitored in the pan-European groundwater survey, at maximum concentrations of 13 and 52 µg L^{-1} (Loos et al. 2010), in line with our findings of slowly decreasing, long term stable or even increasing concentrations in leachate over multiple years (Fig. 1). It should be noted that both metabolites detected in this study are categorized as “non-relevant” metabolites for which the European drinking water limit (0.1 µg L^{-1}) is not applicable. In contrast to relevant metabolites, no general concentration limits are in force for so called “non-relevant” metabolites in the European Union. Desphenyl-chloridazon and methyl-desphenyl-chloridazon are classified in Austria as “non-relevant” metabolites for which a threshold value of 3 µg L^{-1} applies. It should be emphasized that both metabolites clearly exceeds this concentration limit and thus may be regarded as mobile and persistent in soil (EFSA, 2007).

The lysimeters used at the agricultural test site in Wagna have dissimilar designs and were installed with different technical equipment. The SCIENCELYS, built in 2004, houses modern lysimetric devices (i.e. a cultivation ring, suction cups, and a precision weighing system). Although the shortcomings of the relatively simple GRAVITYLYS lysimeter setup were known, this type of lysimeter was used to make a methodological comparison. The design of the lysimeter, as well as the environmental conditions, played a significant role in governing the variation of leachate. The first high-precipitation event (240 mm) in August 2010 generated a large amount of leachate (150 mm) in September 2010, five month after the application. On that occasion, chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon were detected for the first time in the leachate of both the SCIENCELYS and GRAVITYLYS. Seasonal variations of leachate generation, due to a lack of precipitation, were observed. Precipitation after a long drought period (i.e. hot summer) increased the

transport of chloridazon and its metabolites through the soil. This fact implies that an interruption to the water flow might induce sorption and degradation processes, and thus the concentrations of desphenyl-chloridazon and methyl-desphenyl-chloridazon detected in the leachate increased. In Fig. 5 the amounts of leachate and pesticide concentrations, respectively, show a clear seasonal pattern characterized by low load in dry seasons and high load in rainy seasons.

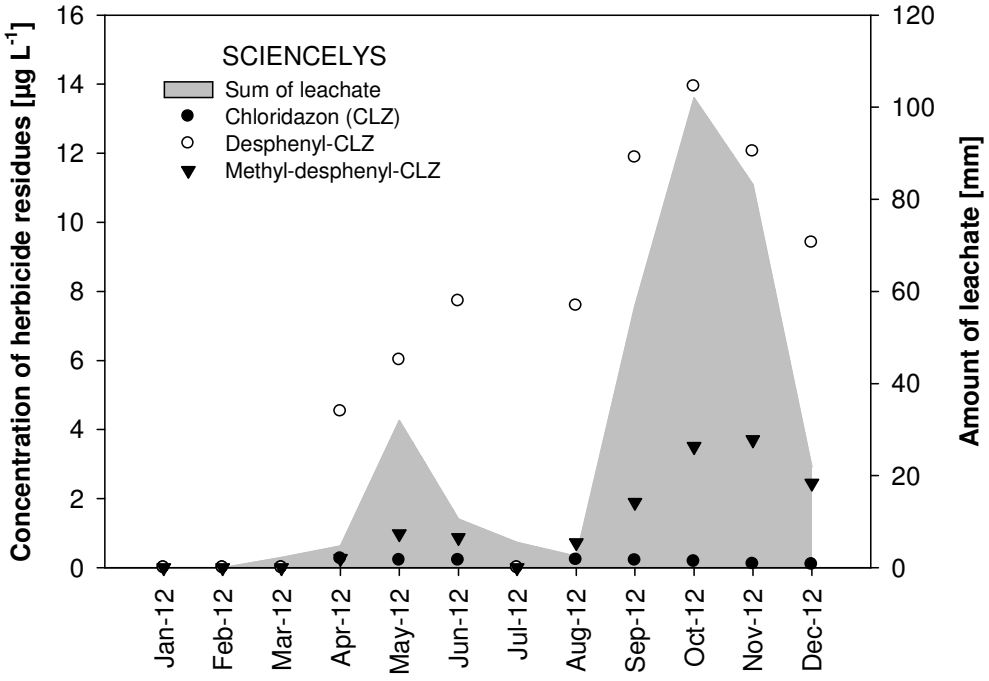

Fig. 5 Amount of leachate and concentration of chloridazon (*closed circle*), desphenyl-chloridazon (*open circle*) and methyl-desphenyl-chloridazon (*closed inverted triangle*) measured in leachate of the SCIENCELYS during the year 2012

Chloridazon was present in the SCIENCELYS leachate at levels above the EU threshold limit for drinking water (0.1 µg L^{-1}) during the two and a half years of monitoring. We suggest that the pesticide underwent slow degradation and strong adsorption. At the GRAVITYLYS, the parent compound was either more rapidly degraded or bound to soil particles, with very little leaching out (< 0.053 µg L^{-1}). Renaud et al. (2004) stated that the availability of pesticides for leaching over time is influenced by degradation measured as a decrease in total residues present. Although chloridazon was applied only once, desphenyl-chloridazon and methyl-desphenyl-chloridazon were detected at high concentrations in the leachate of both lysimeters after more than two years of monitoring. Higher concentrations of desphenyl-chloridazon were found in the leachate of the GRAVITYLYS compared to the SCIENCELYS. In comparison with the tension-controlled suction cups, which guarantee that the outflow of the SCIENCELYS occurs at the same time and magnitude as in the undisturbed field, the outflow of leachate at the GRAVITYLYS is only possible if saturation is reached. In the field, comparable local saturation does not occur. However, the more saturated conditions at the GRAVITYLYS might be an explanation for the higher degradation and concomitant detection of higher concentrations of desphenyl-chloridazon and methyl-desphenyl-chloridazon. In addition, the constant detection of the metabolites in both lysimeters verifies its persistence in

leachate. One rationale for the permanent presence and dispersion in leachate could be the retention of chloridazon in soil, which forms reservoirs for desphenyl-chloridazon and methyl-desphenyl-chloridazon precursors. The release of bound pesticide residues from soil depends on physic-chemical mechanisms or biochemical processes (Gevao et al. 2000).

Concentrations of chloridazon detected were highest at the soil surfaces of both lysimeters but decreased at lower depths, confirming the increase of degradation by time (Table 4). The rise in the SCIENCELYS chloridazon concentration on day 12 after the application might indicate a spatially heterogeneous application of the pesticide. Chloridazon was found to be completely degraded within 81 days of application of 2.6 kg ha^{-1} by Rouchaud et al. (1997). In the present study, chloridazon degraded over time to 1.4, 3.5 and 5.3 µg kg^{-1} in the SCIENCELYS and 8.7, 1.4 and 0.90 µg kg^{-1} in the GRAVITYLYS at 0-10, 10-20 and 20-30 cm soil depth respectively, 916 days after the application. Concentrations of chloridazon in the topsoil layer are higher in the GRAVITYLYS (8.7 µg kg^{-1}) compared to the SCIENCELYS (1.4 µg kg^{-1}) after 916 days which corresponds with the findings in leachate of the GRAVITYLYS, where only traces of chloridazon were found. Thus, the decline of chloridazon in soil due to degradation might be greater than that caused by leaching. Results show that under dry conditions the persistence of chloridazon in soil is higher than under wet conditions. Overall, low degradation rates resulted in the extended availability of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon for transport processes. Capri et al. (1995) reported that the degradation of chloridazon is affected by soil moisture and temperature. Since the GRAVITYLYS was aboveground, higher soil temperatures might have induced higher degradation rates, probably caused by the increase in soil microbial activity. An explanation for the disparities between the two lysimeters is that monolithic field lysimeters and backfilled lysimeters have different hydraulic systems. In addition, divergent cultivation methods could be a reason for the various soil hydraulic characteristics. The removable upper lysimeter ring at the SCIENCELYS enables mechanised soil tillage, whereas at the GRAVITYLYS, cultivation is performed manually. Thus, the two lysimeters might have different water retention characteristics. Fank (1999) reported soil compaction differing from the field conditions due to manual cultivation. Results show that investigations are more realistic using undisturbed lysimeters such as the monolithic SCIENCELYS.

Although no literature about the presence of chloridazon in maize could be found - the plant protection product *Pyramin WG* is normally used for sugar beet crops (Capri et al. 1995; Rouchaud et al. 1997) - our findings provide some insights into the accumulation of chloridazon and its metabolites in plants. Higher amounts of chloridazon and desphenyl-chloridazon were detected in the leaves and stems from the GRAVTIYLYS compared to the SCIENCELYS, conceivably because the amount of maize biomass recovered from the GRAVITYLYS was observed to be lower and this might have had an influence on the amount accumulated. The amount of chloridazon in the leaves and stems of maize at the seedling stage was strikingly higher than those at the two later stages (Fig. 2). The lack of detected residues of chloridazon at harvest might be due to a dilution effect through biomass increase of maize. Conversely, the amount of desphenyl-chloridazon in the leaves and stems of maize increased with time. The detection of the polar metabolite desphenyl-chloridazon in maize kernels proves the principal availability of such a compound for transfer into field-grown food, eventually threatening its security.

The experimental setup of the SCIENCELYS allowed in situ measurements of water contents and pesticide concentrations in different depths that were assumed to give additional information to simulate water transport. PEARL model simulations of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon concentrations at the SCIENCELYS showed similar patterns versus time at 0-30 cm depth. Model results for leached pesticide masses show the worst fit to measured data for chloridazon. The model performance for desphenyl-chloridazon and methyl-desphenyl-chloridazon can be described between a "weak" and "good" fit according to Van Liew and Garbrecht (2003). Leached masses of chloridazon and desphenyl-chloridazon showed a significant peak at the beginning of the simulation period which cannot be predicted by the simulation. One explanation for these unpredicted leaching events, which is also reflected in the NSEs of chloridazon and desphenyl-chloridazon, may be the existence of preferential flow in macropores. SWAP, in general, has been modified to account for preferential flow by introducing an adapted version of the FLOCR model (Hendriks et al. 1999), but this was not applied in our model approach.

Scorza and Boesten (2005), using the PEARL model, tried to simulate the movement of water, and the transport of bentazone and imidacloprid in cracked clay soil. Although the real and simulated values of water and imidacloprid were comparable, PEARL did not predict the bentazone concentrations well because of their preferential transport to soil macropores. Better correspondence between measured and simulated data - especially of the metabolite desphenyl-chloridazon - was achieved with the adjustment of certain parameters related to sorption and degradation. Parameters affecting sorption and degradation such as degradation rates, Freundlich exponent $1/n$ and sorption distribution coefficient K_{foc} were the most sensitive input parameters using the PEARL model. A sensitivity analysis of four mathematical models including PEARL also indicated the sensitivity of the related sorption and degradation parameters (Dubus et al. 2003).

PEARL did not calculate the degradation of compounds in the plants and thus the values measured in maize could not be considered in the parameterization. In order to provide an accurate mass balance for chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon, the degradation parameters in plants would be additionally required.

5 Conclusions

A lysimeter experiment using a weighable, monolithic lysimeter and a backfilled, gravitation lysimeter was carried out for chloridazon and its known polar metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon in leachate, soil and maize. Degradation of chloridazon to desphenyl-chloridazon and methyl-desphenyl-chloridazon was observed in both lysimeters. High concentrations, especially of the metabolite desphenyl-chloridazon were detected in leachate, soil and maize. Methyl-desphenyl-chloridazon was detected in leachate and soil at lower concentrations than desphenyl-chloridazon. Results obtained showed that precipitation and leachate volume might influence the concentrations observed in soil and leachate. Interestingly, chloridazon and desphenyl-chloridazon were detected in maize. Variations in terms of the transformation rate, soil retention time and accumulation by plants were found between the lysimeters. Due to the setup and design of the lysimeters, conditions in the monolithic, field lysimeter clearly differ from the backfilled, gravitation lysimeter. In addition, the monolithic lysimeter was designed to simulate the situation in the field, thus only the data from this lysimeter was used for the pesticide leaching model

PEARL. The PEARL simulation resulted in adequate correlation for mass transport of the metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon. Although the Pesticide Properties Data Base provides a good basis for the chemical setup, certain parameters (especially for desphenyl-chloridazon) have been adjusted within the model calibration. The supposed existence of preferential flow may be the cause of the discrepancy in the description of the chloridazon and desphenyl-chloridazon levels at the beginning of the simulation period between experiment and modeling.

Notwithstanding these issues, this study was performed over a number of years and offers a good insight into the migration of chloridazon and its main polar metabolites. The high concentrations in soil and leachate even years after a single application highlight the fact that chloridazon, and especially its known metabolites, persist in the long-term and thus, might represent a risk for groundwater contamination.

Acknowledgements

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5.1.1 Appendix A. Supplementary data

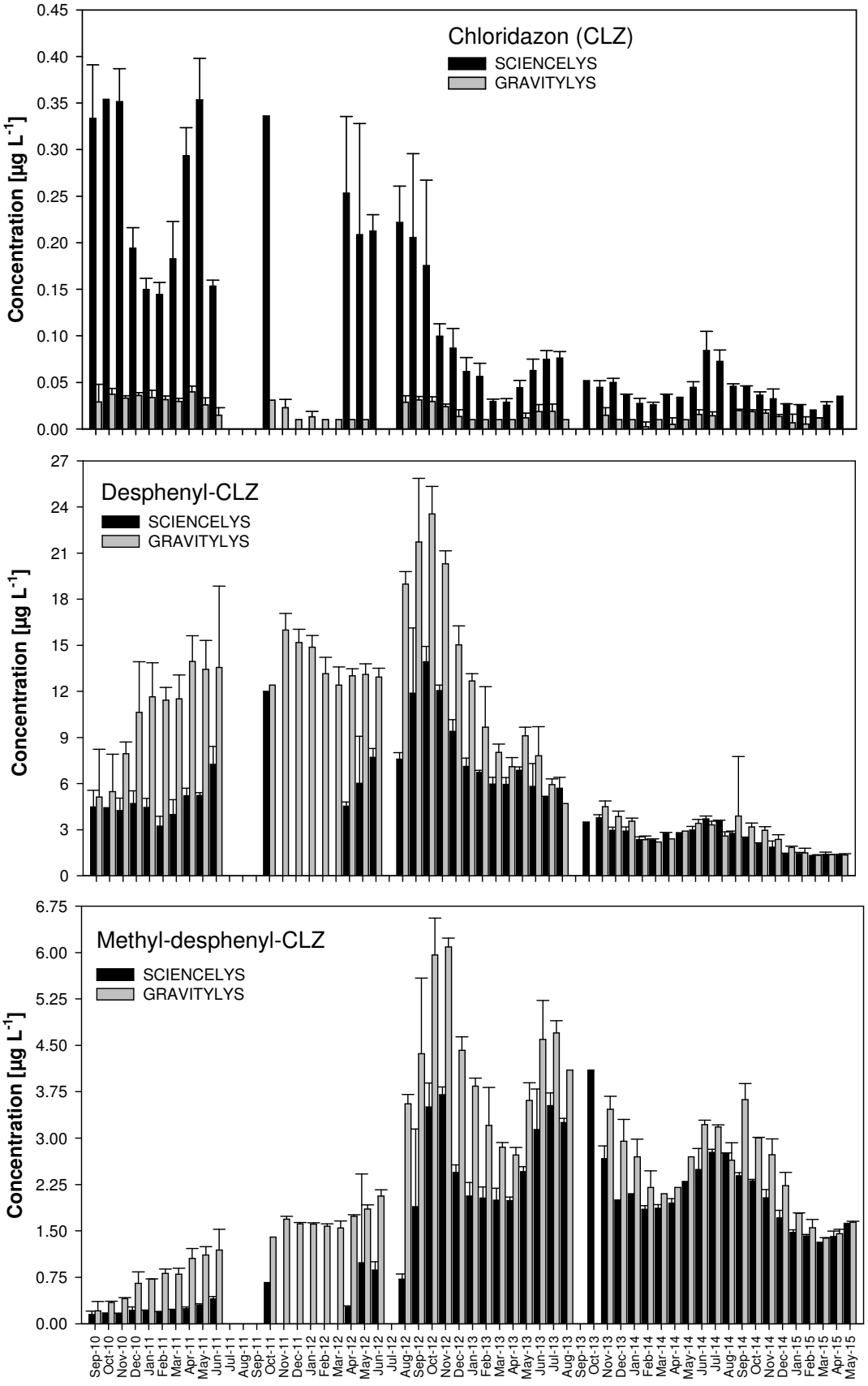

Figure 5.1: Average concentrations per month of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon in leachate at the bottom of the SCIENCELYS (180-cm depth) and GRAVITYLYS (150-cm depth) from 2010 to 2015

5.2 Modeling subsurface fate of s-metolachlor and metolachlor ethane sulfonic acid in the Westliches Leibnitzer Feld aquifer (Publication III)

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Clarification

The lysimeter data of the pesticides S-metolachlor and metolachlor-ESA from the thesis were used for this publication.

Modeling subsurface fate of s-metolachlor and metolachlor ethane sulfonic acid in the Westliches Leibnitzer Feld aquifer

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Core ideas

- Lysimeter experiments allow for site specific knowledge about the fate of pesticides.
- Lysimeter-based model calibration provides integrated parameter sets.
- Lysimeter scale-based models have been compiled to represent aquifer scale.
- Lysimeter scale-based models were coupled with a groundwater transport model.
- The groundwater model reproduced observed metabolite groundwater concentrations.

Pesticides and their metabolites have been increasingly detected in groundwater bodies in southeastern Austria in recent years. The main objective of this study was to model the fate of the herbicide *S*-metolachlor (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(1*S*)-2-methoxy-1-methylethyl] acetamide; SMET) and the main metabolite metolachlor ethane sulfonic acid (MESA) at the Westliches Leibnitzer Feld (WLF) aquifer. For this purpose, a modeling approach based on coupling the one-dimensional vadose zone model PEARL and the two-dimensional groundwater flow and solute transport model FEFLOW was developed. To calibrate the one-dimensional pesticide fate model, we used leachate concentrations of SMET and MESA from lysimeter experiments. Additionally, samples of representative soil types in the WLF aquifer were analyzed to infer SMET- and MESA-specific fate parameters (e.g., half-life DT_{50}, Freundlich sorption coefficient K_{foc}), which were used for the PEARL model. The results show that using SMET fate parameters derived from the lysimeter data considerably improved the fit of the simulation results with the field observations compared with the application of standard laboratory-derived fate parameters accounting for soil type differences. Although locally an overestimation of the monitoring data prevailed, the description of the subsurface fate of pesticides will improve the interpretation of concentration data and the design of mitigation measures.

Abbreviations: DT_{50}, half-life; MESA, metolachlor ethane sulfonic acid; NSE, Nash–Sutcliffe model efficiency; OC, organic carbon; PPDB, Pesticide Property Database; SMET, S-metolachlor; WLF, Westliches Leibnitzer Feld.

In Austria, almost all drinking water is supplied by untreated groundwater. Approximately half of it originates from springs out of karstified or fractured rocks, while the other half is provided by pumping wells from sand and gravel aquifers. Because of the Austrian topography, sediment-filled river valleys and basins are also intensively used by numerous human activities such as settlements, manufacturing, and in particular agriculture. Monitoring results show that the greatest threats to groundwater quality in Austria and at the European scale originate from the application of fertilizers and plant protection products as well as the emergence of corresponding metabolites in agriculture (e.g., Loos et al., 2010).

Among the vast number of plant protection products, in our present research we focused on the environmental fate of the herbicide SMET, which is often applied to maize (*Zea mays* L.) to combat the emergence of grass weeds. It transforms into the main metabolite MESA, which is classified as irrelevant in Austria. Thus, rather than the European drinking water limit of 0.1 mg L^{-1}, no general groundwater concentration limits for MESA apply (European Commission, 2003), although Austria has specified a threshold concentration in groundwater of 3 mg L^{-1}.

In the GeoPEARL-Austria study (Bundesministerium für Land und Forstwirtschaft, Umwelt und Wasserwirtschaft, 2013), MESA concentrations in the leachate between 1 and 10 mg L^{-1} were computed for southeastern Austria. These numbers are related to maize being the dominant crop (~50%), which is grown to feed pigs.

In recent years (2013–2015), groundwater quality data from the WLF aquifer has shown MESA concentrations in groundwater at numerous locations and different times varying between 0.074 and 1.834 mg L^{-1} (Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 2017). Although the values are below the threshold, the consistent detection of MESA concentrations in groundwater provides evidence of leaching of this substance from the soil into the groundwater.

For assessing the leaching potential of specific plant protection products into the groundwater, lysimeters are very valuable tools because they allow the detailed study of water movement as well as the environmental fate of fertilizers and plant protection products in the vadose zone under natural or controlled conditions.

Because the application of SMET is widespread within agricultural land, monitored groundwater concentrations of MESA can only be understood within a regional spatially distributed and temporally explicit framework. This means that the variable natural conditions in the aquifer (i.e., soil and weather) have to be considered in describing the movement and fate of SMET and its main metabolite in the vadose zone as well as the subsequent mixing into and advective transport with the lateral groundwater flow. This task can only be accomplished by using numerical models that address the most relevant processes along the subsurface pathway of the substances.

Several numeric models have been developed that describe the transfer of pesticides from the soil surface to leaching in the vadose zone. They are able to consider the major processes involved in the environmental fate of pesticides (e.g., sorption, degradation, leaching, volatilization, plant uptake, and wash-off) at varying degrees of complexity. Widely used models include PEARL (Leistra et al., 2001), MACRO (Larsbo and Jarvis, 2003), and PELMO (Klein, 1995), all of which are used for environmental risk assessment and registration purposes of plant protection products (FOCUS, 2009).

Baris et al. (2012) provided a comprehensive review of a number of pesticide leaching models. Marín-Benito et al. (2014) compared three pesticide fate models using equivalent parameterization with respect to the leaching of two herbicides under field conditions in an irrigated maize cropping system. They addressed the fact that some studies show an adequate description of the water and pesticide field data, whereas others show that models could not correctly simulate the monitored data. Because of the favorable performance of PEARL in these model assessments, and to be consistent with the GeoPEARL-Austria study, we used the model PEARL in our present research to simulate the monitored water movement and fate of SMET and MESA following herbicide applications on the Wagna lysimeter.

To match the monitored MESA concentrations in groundwater, the hydrodynamic transport of the metabolite with lateral groundwater flow needed to be examined. For this purpose, we sequentially coupled the pesticide fate model PEARL with the groundwater flow and contaminant transport model FEFLOW (Diersch, 2009).

Stenemo et al. (2005) pursued a similar approach by loosely linking the pesticide fate model MACRO to the three-dimensional discrete fracture–matrix diffusion model FRAC3DVS to describe the transport of the pesticide mecoprop [2-(4-chloro-2-methylphenoxy) propanoic acid] in a fractured moraine till and local sand aquifer. However, they restricted their application to field dimensions of 40 by 40 m. They concluded that the temporal resolution of the boundary conditions were much more important with respect to simulated concentrations leaching to the regional aquifer than the spatially variable pesticide input conditions.

Loague et al. (1998a, 1998b) combined the pesticide fate model PRZM-2 with the groundwater flow model MODFLOW and the solute transport model MT3D to simulate the regional distribution of the nematocide DBCP (1,2-dibromo-3-chloropropane) concentrations in groundwater in Fresno County, California. For the period between 1960 and 1994, they generated an annual DBCP water table loading map based on 1172 individual PRZM-2 runs at a 1-km^2 resolution of soil, land use, meteorology, irrigation, and groundwater table depth information. Loague et al. (1998b) inferred that nonpoint source application of DBCP was not responsible for the monitored hotspots in the study area. In both studies, the researchers stressed that it was not their intention to condition simulations to an individual site, but rather they used the best available models in an uncalibrated mode and then asked what-if questions at a regional scale, which were subsequently interpreted in terms of limiting assumptions.

Christiansen et al. (2004) added a macropore description to the coupled MIKE SHE/Daisy code and applied the model to a small catchment in Denmark. They concluded that although macropore processes have no dominating effect on groundwater recharge at the catchment scale, they will have significant effects on pesticide leaching to groundwater because some of the pesticides are transported rapidly downward in the soil to zones with less sorption and degradation. Bergvall et al. (2011) coupled the vadose zone model HYDRUS-1D to the groundwater flow model MODFLOW and the solute transport model MT3D to describe significant processes that govern the subsurface transport of the pesticide metabolite 2,6-dichlorobenzoamide in a glaciofluvial esker aquifer. They reproduced the observed concentrations at the regional scale, attributed half of the model uncertainty to hydraulic conductivity in the aquifer and infiltration rate, and applied the model to optimize the location of extraction wells for remediation. Herbst et al. (2005) linked the models TRACE and 3DLEWASTE to reveal the behavior of the pesticide isoproturon (N,N-dimethyl-N¢-[4-(1-methylethyl)phenyl]urea) at a 20-km^2 test area in the lower Rhine embayment. The highest

concentrations of isoproturon in groundwater were estimated for areas with a thin and permeable soil layer, whereby the researchers stressed the importance of the processes in the unsaturated zone.

It was the objective of our study to apply the sequentially coupled PEARL and FEFLOW models at the WLF aquifer. Additionally, we tested the use of lysimeter-derived pesticide fate data for regional modeling of MESA groundwater concentrations and compared the results against the use of standard laboratory-derived fate parameters for SMET and MESA considering soil type variability.

Materials and Methods

Investigation area

The investigation area, the WLF (see Fig. 1), is located ~30 km south of Graz, Austria, and has a size of 44 km^2. The hydrogeological characteristics of the area have already been depicted in Klammler et al. (2013) and Händel et al. (2013). The WLF aquifer comprises quaternary gravel with an average thickness of 8 m. Groundwater recharge in the investigation area is mainly provided by infiltrating precipitation without any predominant periods for groundwater recharge. The average groundwater depth is ~3.5 m. Aquifer hydraulic conductivities vary between 1 x 10^{-4} and 7 x 10^{-3} m s^{-1}. The mean annual precipitation is 938 mm, with a maximum in August (125 mm) and a minimum in January (28 mm), while the average annual air temperature is 10.5°C (monitoring period 1991–2016).

Spatial soil information is available only for arable land according to the Austrian Soil Mapping (Bundesministerium für Land und Forstwirtschaft, 1974; maximum exploration depth of 1 m). Thirty percent of this area is covered with sandy-clayey Dystric Cambisols and in the floodplains along the rivers mainly Dystric Fluvisols can be found. Neighboring soil types were allocated to residential areas and forests for the simulations.

The WLF is intensively used for agriculture. At present, ~54% of the investigation area is arable land used for cultivating maize (50%), oil pumpkin (*Cucurbita maxima* Duchesne) (13%), grassland (17%), and some other crops with minor portions (average percentages 1996–2014, based on the integrated administration and control system of the EU). Approximately 28% of the investigation area is used for residential purposes and 14% is forest.

Lysimeter Setup in Wagna

In the WLF aquifer, we have operated several lysimeters within an agricultural test field located in Wagna for >20 yr to investigate the impact of conventional and organic farming schemes on yield and leachate characteristics. The lysimeter setup has been repeatedly described in detail (von Unold and Fank, 2008; Klammler and Fank, 2014; Schuhmann et al., 2016). The weighable, monolithic lysimeters (1-m^2 surface, 2-m depth) are installed at the center of the agricultural test field at Wagna and contain undisturbed loamy to sandy soil over gravel and sand. The soil is classified as a loamy sandy Dystric Cambisol with 52% sand, 34% silt, and 15% clay. Further characteristics are a pH of 6.6 (in $CaCl_2$) and an organic C (OC) content of 2.7%.

The lysimeters were cultivated by local farmers with typical agricultural machinery (e.g., a plow) during which the upper ring of the lysimeter was removed. The lysimeter scale has a resolution of 35g (0.035 mm water equivalent). Inside the monolith, various measurements can be taken at 10-min intervals at depths of 35, 60, 90, and 180 cm: (i) the water content using time-domain reflectometry probes, (ii) the hydraulic potential using tensiometers, and (iii) the soil temperature. A layer of suction cups is installed in the gravel filter layer to control the lower boundary condition of the lysimeter. An automatic vacuum pump is used to transfer the soil water tension - measured at 180 cm below the surface outside the lysimeter - to the suction cups at the lysimeter bottom. Thus, flow rates equivalent to those in the undisturbed field can be achieved. Leachate is sampled through the suction cup layer, whereby the amount of leachate is detected using a tipping bucket with 0.1-mm resolution.

Application of S-Metolachlor on the Wagna Lysimeter

The conventional lysimeter was cultivated with a crop rotation consisting of maize (2010, 2012, 2014), triticale (*Triticosecale* spp.) (2011 and 2015) and pumpkin (2013). Outside the crop vegetation periods, typical catch crops (ryegrass [*Lolium multiflorum* Lam.] or forage rye [*Secale cereale* L.]) were planted. Gardo Gold (Syngenta Agro GmbH), containing SMET and terbuthylazine [*6-chloro-N*-(1,1-dimethylethyl)-*N'-ethyl-1,3,5- triazine-2,4-diamine*], was applied on the lysimeter on 24 May 2012. Application rates were equivalent to 3.47 kg ha^{-1} for SMET and 2.08 kg ha^{-1} for terbuthylazine. Dual Gold (Syngenta Agro GmbH), containing only SMET, was applied at a rate of 1.2 kg ha^{-1} on 12 May 2013 and 0.96 kg ha^{-1} on 10 May 2014. The plant protection products were applied postemergence as suspensions onto the soil surface of the field including the lysimeter without any additional irrigation. The field area was chosen for the application to ensure an even distribution of the pesticide on the lysimeter surface and minimize impacts from the surrounding area.

Leachate from the lysimeter was collected at an average sampling interval of 10 d (when sufficient leachate had been produced) and stored at −18°C prior to analysis. Samples were analyzed by liquid chromatography–mass spectrometry and direct injection after the addition of an internal standard. Based on the Pesticide Property Database (PPDB; University of Hertfordshire, 2017), SMET is moderately soluble in water (480 mg L^{-1}), nonpersistent with a half-life (DT_{50}) value in the field of 21 d, and moderately mobile with a Freundlich K_{foc} of 226.1 mL g^{-1}. It transforms into the key metabolites metolachlor oxanilic acid and MESA with estimated maximum occurrence fractions in soil of 0.109 and 0.124, respectively. Metolachlor ethane sulfonic acid is described as having a high solubility in water (212,461 mg L^{-1}), persistent with a typical DT_{50} value of 132 d, and very mobile with a linear K_{foc} of 9 mL g^{-1}.

Regional Variation of Environmental Fate Parameters of S-Metolachlor and Metolachlor Ethane Sulfonic Acid

The degradation of SMET was determined for seven representative soils (samples taken not deeper than 20 cm) and one deeper sample (between 40 and 60 cm) within the investigated area. The locations of the soil samples, which were taken at the beginning of May 2016, are shown in Fig. 1. One soil sample was taken at each of the two agriculture-operated lysimeters in Wagna, each having a different soil type.

The soil samples varied with respect to OC content (3.2–19.4 g kg^{-1}), clay (12–19%), cation exchange capacity (6.3–13.2 $cmol_c$ kg^{-1}), and pH (6.0–6.4). Degradation half-life was measured according to the Organization for Economic Cooperation and Development (2002) and at 18°C and 22 and 32% gravimetric water content, respectively. The DT_{50} varied between 28.1 and 38.8 d for the surface soils and was much higher (DT_{50} = 123.2 d) in the subsurface sample.

Freundlich adsorption coefficients of SMET were determined using a standard batch equilibrium method following the Organization for Economic Cooperation and Development (2000). The K_f values ranged from 1.39 to 1.9 mL g^{-1} (K_{foc} = 93.7– 121.9 mL g^{-1}, n^{-1} = 0.71–0.77) in four surface soils and was 0.19 mL g^{-1} (K_{foc} = 60.8 mL g^{-1}, n^{-1} = 0.8) in the subsurface soil. The most important soil property influencing the sorption was the OC content of the soil. The linear distribution coefficient (KD) for MESA varied between 0.05 and 0.19 mL g^{-1} (KOC = 6.4–14.6 mL g^{-1}).

Calibration of PEARL on Lysimeter Data

For modeling the monitored SMET and MESA concentrations in the leachate resulting from the three applications of plant protection products, we used the environmental fate model PEARL (FOCUSPEARL 4.4.4; Leistra et al., 2001). This one-dimensional model uses a convection–dispersion equation to simulate the transport of solutes in the vadose zone. Instantaneous equilibrium or kinetic sorption is described by either a linear or a Freundlich equation and degradation by first-order kinetics depending on soil water content, temperature, and depth. PEARL is linked with the SWAP (Soil, Water, Atmosphere, and Plant) model (Kroes et al., 2008), whose soil hydrology is described by the Richards equation. The SWAP model calculates the evapotranspiration according to a modified Penman–Monteith equation (Monteith, 1965; Van Dam et al., 1997) or according to the Makkink equation (Makkink, 1957).

The van Genuchten–Mualem parameters for describing the hydraulic characteristics of the lysimeter were derived from in situ measurements of water contents and matrix potentials inside the lysimeters and are presented in Table 1. A dispersivity length of 0.1 m was assumed.

Table 1. Parameters of the van Genuchten model used for the PEARL simulation, including saturated and residual water content (θ_{sat} and θ_{res}, respectively), shape fitting parameters α and n, and saturated hydraulic conductivity (K_{sat}).

Depth	**θ_{sat}**	**θ_{res}**	**α**	**n**	**k_{sat}**
cm	m^3	m^{-3}	1 cm^{-1}		m s^{-1}
0-30	0.39	0.16	0.05	1.33	5 x 10^{-6}
30-50	0.38	0.19	0.04	1.45	6.5 x 10^{-6}
50-80	0.44	0.11	0.065	1.2	6.7 x 10^{-6}
80-130	0.,2	0.03	0.25	1.4	5 x 10^{-5}
>130	0.14	0.03	0.25	1.9	1.2 x 10^{-4}

During the calibration, we used values from the PPDB and from the lysimeter-specific soil analysis in the laboratory to match the time series of SMET and MESA fluxes between April

2012 and June 2015. Based on the results of an upfront sensitivity analysis, we focused on DT_{50} and n^{-1} values for the fitting of SMET observations and DT_{50} and K_{foc} values as well as the coefficient of transformation for the matching of monitored MESA concentrations in the leachate in a manual trial-and-error procedure. We chose this method over an automated calibration procedure because of the higher flexibility to handle options to steer the numerous hydraulic and environmental fate parameters given the characteristics of the monitored time series.

Application of PEARL to the Agricultural Part of the Aquifer

To take the nonpoint-source nature of the application of plant protection products into account, the parameterization specified at the Wagna lysimeter needed to be transferred to the aquifer scale. For this purpose, the WLF aquifer was divided into homogeneous subunits that showed uniform soil, weather, and land-use (e.g., forests, settlements, farmland) conditions. In the area of the WLF aquifer, data from one meteorological station were available to drive the PEARL simulations.

The van Genuchten parameters required for the simulation of the 23 different soil types within the investigation area were derived by curve fitting to existing retention curves. For the definition of these curves, Murer (1998) used values for pore volumes and field capacities derived from the Austrian Soil Mapping (after AG Boden [1994] and Eisenhut [1990]). He also provided hydraulic conductivity curves, which were the basis for defining the required saturated hydraulic conductivities for PEARL.

The actual set of crops grown each year was recorded based on cadastral municipalities in Austria. The percentage of maize grown can vary among different municipalities in the same year and change between years in the same municipality. In the PEARL simulations, only cultivation of maize with the application of SMET as the sole herbicide was considered per subunit. The overlay between the spatial distribution of different soil types and the cadastral municipalities yielded a total of 79 homogeneous subunits in the vadose zone. During the PEARL simulations, different environmental parameter sets were used in the subunits, which are described below.

Because maize represents only a fraction of the crops grown, the computed time series of MESA fluxes needed to be reduced by multiplication with the corresponding maize percentage for this particular cadastral municipality and year. Moreover, we assumed that each farmer used the maximum dose of SMET (i.e., 1.25 L ha^{-1}) with no reduction for wind drift, surface runoff, or interception. Additionally, because of the effective absence of organic material, no decay in the vadose zone underneath the soil layers or in the saturated groundwater body was taken into account. Consequently, below the soil layers and in the saturated part of the groundwater body, only conservative transport of MESA along with groundwater flow was considered. This approach is consistent with the application practice of pesticide fate models (Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 2013).

Sequential Coupling between PEARL and FEFLOW

To match the monitored MESA concentrations in the groundwater, the pesticide fate model PEARL was sequentially coupled with the groundwater flow and contaminant transport model FEFLOW (Diersch, 2009). Because PEARL is a one-dimensional vertical model, this procedure is suitable only if lateral flow in the vadose zone can be neglected, which is the case in most porous media aquifers in river valleys and basin fills. The sequential coupling approach, which was developed to simulate the impact of diffuse N application as fertilizer, was discussed in detail by Klammler et al. (2013).

Within the sequential coupling between PEARL and FEFLOW, the results of unsaturated water and MESA fluxes were provided as an upper boundary condition time series to FEFLOW (i.e., as a source term for flow or mass). By adding a gravel horizon underneath the soil layers that was sufficiently deep to cover the varying thickness of the vadose zone as a result of the fluctuating groundwater level, PEARL results were stored at a depth interval of 10 cm for daily time steps, generating a corresponding depth–time matrix. A specific add-in module for FEFLOW was developed to use these depth–time matrices of unsaturated water flow and MESA mass as look-up tables, that is, FEFLOW picked up water and MESA fluxes from the look-up tables for the depth of the groundwater table at the corresponding time step.

Groundwater Flow and Conservative Transport Model for the Aquifer

Groundwater transport of MESA driven by lateral groundwater flow was simulated with the groundwater flow and transport model of the WLF aquifer described by Klammler et al. (2013). It is a two-dimensional groundwater model implemented in FEFLOW covering the time period between 1993 and 2009 and it has recently been extended to 2015. Because NO3 and MESA can both be treated as conservative substances in the saturated domain, we assumed that the saturated transport model derived by Klammler et al. (2013) could also be used to simulated groundwater transport of MESA. In this context, we followed a practical approach by not aiming to exactly match a monitored time series of metabolite concentrations in the groundwater but rather attempting to reproduce the essential patterns of the concentration time series behavior.

The dynamic groundwater recharge input distribution into the groundwater under agriculture was computed at a daily time interval using the soil water and N transformation model SIMWASER–STORASIM (Feichtinger, 1998). Groundwater recharge for nonagricultural areas in the WLF aquifer (e.g., forests) was calculated by using the FAO Penman–Monteith method (Allen et al., 1998) to estimate evapotranspiration. Groundwater recharge from residential areas was determined following a combined approach, whereby precipitation falling on paved areas was collected and infiltrated directly into the aquifer by drainage shafts. Groundwater recharge from grassland was simulated by running the one-dimensional vertical soil water movement and plant growth model SIMWASER (Stenitzer, 1988).

Groundwater recharge for the cultivation of maize was computed with the SWAP model within the coupled PEARL–FEFLOW approach. For representative subunits, these groundwater recharge time series were compared with those resulting from the approach described by Klammler et al. (2013) for agricultural land to ensure that the two groundwater recharge patterns were compatible and could be used in the same model domain. The comparisons showed a favorable match, whereby implementing the groundwater recharge

time series computed with PEARL into the existing groundwater flow and transport model did not introduce a systematic error in the further computations.

Modeling of Metolachlor Ethane Sulfonic Acid Groundwater Concentrations in the Aquifer

Aquifer-wide MESA groundwater concentrations were simulated for four different versions of fate parameter distributions (shown in Table 2) and application doses. The simulated MESA groundwater concentrations were assessed at six monitoring wells (for locations, see Fig. 4) representing areas of different MESA groundwater concentration levels:

1. In Version 1, the most likely SMET and MESA fate parameters from the PPDB (University of Hertfordshire, 2017) were uniformly applied to simulate the MESA groundwater concentrations in the WLF aquifer to illustrate the situation whereby no site-specific fate information is available (i.e., no knowledge from calibration on lysimeter data or soil-specific physicochemical analysis). For plant uptake, values according to Briggs et al. (1982) were used since there are no values specified in the PPDB.
2. In Version 2, the SMET and MESA fate parameters as derived from the calibration of the Wagna lysimeter concentration data were used uniformly distributed across the 23 different soil types in the model domain.

Table 2. Relevant chemical parameters according to the Pesticide Properties DataBase (PPDB; University of Hertfordshire, 2017) given in Version 1 and parameters calibrated at the Wagna lysimeter (Version 2). Values for Version 4 apply to the soil type covering 50% of the Westliches Leibnitzer Feld aquifer.

	S-metolachlor			**Metolachlor ethane sulfonic acid**		
Parameter†	**Version 1**	**Version 2**	**Version 4**	**Version 1**	**Version 2**	**Version 4**
DT_{50}, d	21 (11-31)‡	33	41	132 (94-169)	94	94
K_{foc}, mL g^{-1}	226 (110-369)	226	131	9 (3-22)	18.3	7.8
n^{-1}	1.06 (1.053-1.071)	0.95	0.79	1.0	1.0	1.0
FacUpt§	0.27	0.27	0.27	0.003	0.003	0.003
Coefficient of transformation	na¶	na		0.124#	0.06	0.07

† DT_{50}, half-life; K_{foc}, Freundlich sorption coefficient related to organic content; n^{-1}, Freundlich exponent; FacUpt, coefficient for plant uptake.
‡ The range of measured values as presented within the PPDB provided in parentheses.
§ Values according to Briggs et al. (1982); not specified in PPDB.
¶ na, not applicable.
# Estimated maximum occurrence fraction.

3. In Version 3, the effect of reducing the overall applied volume of SMET by 30% on the resulting MESA groundwater concentrations was evaluated in combination with the fate parameters from Version 2 to consider the fact that not all farmers might have used the SMET compound as a herbicide. The selected percentage is only an assumption to quantify the sensitivity of the input dosage uncertainty.
4. In Version 4, soil-specific fate parameters were applied to ~50% of the agricultural area in the WLF aquifer following the results of the laboratory analysis for the investigated surface soil types. In this version, the 30% dose reduction from Version 3 was still used.

Results

Modeling of S-Metolachlor and Metolachlor Ethane Sulfonic Acid Application at the Wagna Lysimeter

Figure 2 shows the cumulative leachate amount for crop periods with a Nash–Sutcliffe model efficiency coefficient (NSE; Nash and Sutcliffe, 1970) value of 0.56 (n = 195). It can be seen that the general level and the behavior of the measured leachate is well represented by the simulation (especially in 2010, 2011, and 2012, including the period without any leachate between autumn 2011 and spring 2012), which was based on daily values. Nevertheless, there are still periods in which measured leachate rates do not fit well to the modeled curve (e.g., during the peak in spring 2013, the catch crop in winter 2013–2014, or the fallow period in autumn and winter 2015). The mean annual leachate water rates between 2010 and 2014 were 371 and 367 mm for monitored and simulated leachate, respectively.

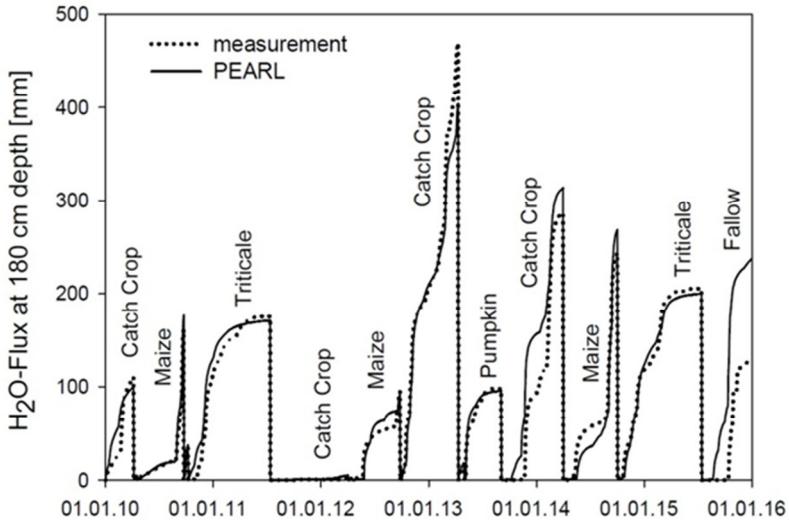

Fig. 2. Comparison of the measured (dotted line) and simulated (continuous line) cumulative leachate of the lysimeter at the 180-cm depth for crop periods. After each vegetation period, cumulative water flux was reset to 0 mm.

The simulation results for leaching of SMET and MESA are presented in Fig. 3 and were based on the calibrated parameters (Version 2) given in Table 2. It can be seen that the first occurrences of SMET in the leachate in the summer of 2012 could not be reproduced with PEARL (top image of Fig. 3). However, the nature of the monitored SMET concentrations between August 2014 and June 2015 were well represented by PEARL, although there was a

slight delay in the simulated results. Because the analysis of leachate SMET concentrations was terminated in June 2015, the predicted peak in SMET concentrations in autumn 2015 could not be compared against data. The NSE for SMET computed between May 2012 and June 2015 is 0.19.

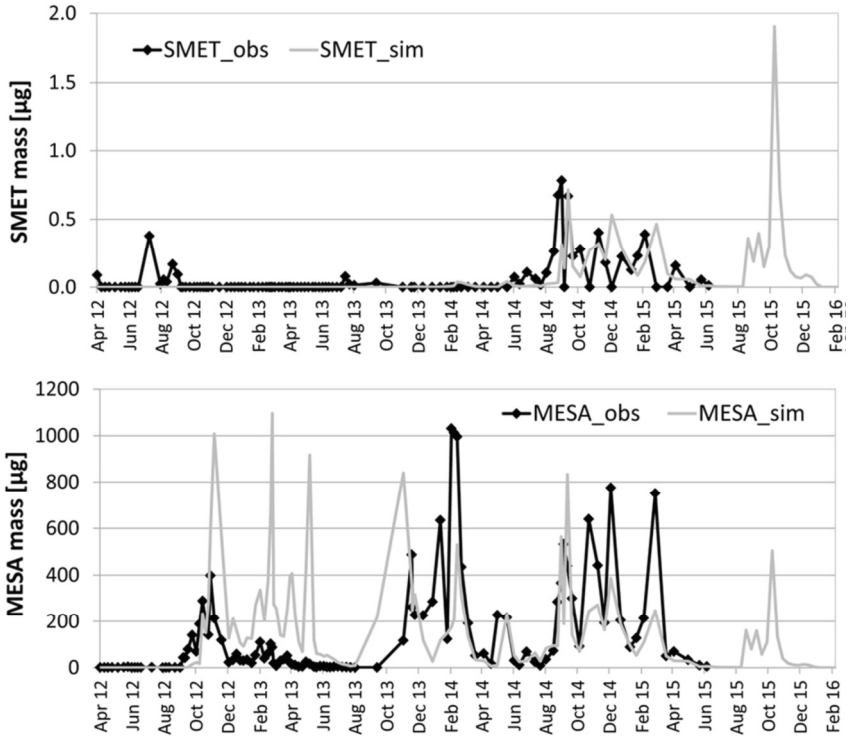

Fig. 3. Measured (obs; April 2012–June 2015) and simulated (sim; April 2012–February 2016) leached masses of S-metolachlor (SMET) (top) and metolachlor ethane sulfonic acid (MESA) (bottom) in the lysimeter at the 180-cm depth.

For MESA, the comparison between monitored and simulated leachate concentrations (bottom image of Fig. 3) looks different. While the monitored first arrival of MESA in the leachate (in October 2012) could be matched with PEARL, the simulated values thereafter clearly overestimated the monitored concentrations until November 2013. The second and third coherent appearances of MESA in the leachate were better reproduced with PEARL, yet absolute single peak values were noticeably underestimated by the model. Therefore, corresponding NSE values are low and vary between −0.19 for the period between May 2012 and June 2015 and 0.16 for the time span between February 2014 and June 2015.

Comparison between Computed Time Series of Metolachlor Ethane Sulfonic Acid Groundwater Concentrations and Observations

The location of the selected monitoring wells (observation wells) is shown in Fig. 4 against the background of the simulated MESA groundwater concentrations averaged between 2011 and 2015. The overall distribution pattern of the simulated MESA groundwater concentrations (based on an average node distance of 27 m in the computational mesh) is dominated by areas with low predicted values gradually changing into adjacent regions with medium range MESA groundwater concentrations. Two distinct areas showing high averaged MESA

groundwater concentrations emerge in the eastern and the far southwestern part of the WLF aquifer.

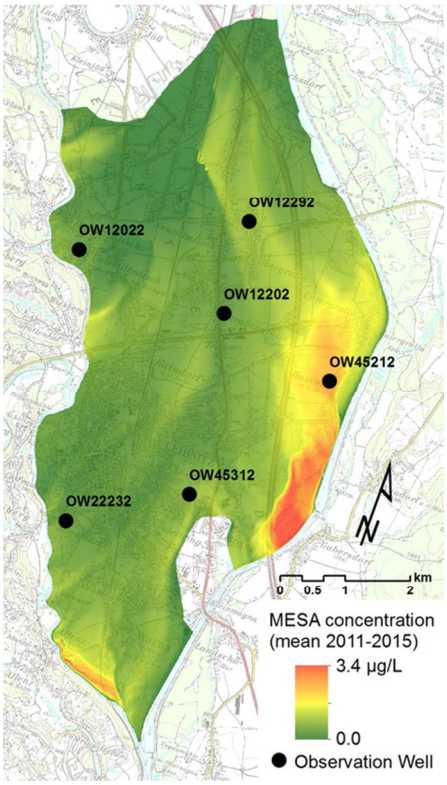

Fig. 4. Distribution of the simulated metolachlor ethane sulfonic acid (MESA) groundwater concentrations averaged between 2011 and 2015 along with the groundwater quality monitoring wells in the Westliches Leibnitzer Feld aquifer.

Figure 5 shows the comparison between the MESA groundwater concentration time series for the four simulation versions and the available observations at selected monitoring wells. At OW45212 (a region of high simulated MESA groundwater concentrations; top left image in Fig. 5), the fluctuation range of the MESA groundwater time series based on the four different versions reduced from between ~4 and 10 mg L^{-1} for Version 1 to between roughly 1 and 3 mg L^{-1} for Version 2. The time series at OW45212 tended to have an annual concentration maximum in the summer followed by a 4- to 5-mo decline. The uniform application of the fate parameter set derived at the Wagna lysimeter within the entire WLF aquifer (Version 2) significantly shifted the simulated MESA groundwater concentrations toward the observations. Reducing the application dose of SMET by 30% (Version 3) further improved the simulated MESA groundwater concentrations through lowering them on average by 0.5 mg L^{-1}. Assigning the soil-specific fate parameters to the dominant (~50%) soil type in the WLF aquifer (Version 4) yielded roughly the same MESA groundwater concentrations as uniformly using the lysimeter-derived fate parameters.

At OW12292 (center left image in Fig. 5), using the fate parameter set of Version 2 and the reduced SMET application (Version 3) led to a decrease in the simulated MESA groundwater concentrations by a factor of approximately five compared with the simulated values using Version 1. The two MESA groundwater observations could be well described by either Version 2 or 3, which was not the case for Version 1. For OW22232 (bottom left image in Fig. 5), a pattern of annual MESA groundwater concentration maxima in autumn can be observed throughout the time series, whereas the MESA groundwater concentration minima do not show a clear distribution. The order of magnitude and the trend of the MESA groundwater concentration measurements were well captured by the corresponding time series applying parameters from Versions 2 and 3. The MESA groundwater concentrations from Version 4 were slightly higher than those from Version 2, which was also true for OW12292.

Fig. 5. Metolachlor ethane sulfonic acid (MESA) groundwater concentration time series as the available discrete measurements at monitoring wells OW45212, OW12292, OW22232, OW12022, OW12202, and OW45312 and simulated using no site-specific information (Version 1), uniformly distributed parameters derived from site-specific calibration (Version 2), a 30% reduction in applied volume of S-metolachlor to quantify input dosage uncertainty (Version 3), and soil-specific fate parameters applied to ?50% of the agricultural area following the results of the laboratory analysis for the investigated surface soil types and the 30% dose reduction from Version 3 (Version 4).

The same basic features can also be found by comparing the simulated time series of MESA groundwater concentrations at further monitoring wells OW12022 (top right image of Fig. 5), OW12202 (center right image of Fig. 5), and OW45312 (lower right image of Fig. 5). However, at OW12202 and OW45312, the time series using the input sets from Versions 2 and 3 consistently overestimated the monitored MESA groundwater concentrations, albeit these are small absolute differences only. At OW12202, the trend of the monitored MESA groundwater concentrations was properly met by the simulated values. Using the laboratory-derived fate parameters for the dominant soil type (Version 4) led to a worse reproduction (i.e., higher absolute values) of the observed MESA groundwater concentrations than Version 2 for wells OW12202 and OW45312, whereas it yielded about the same results for OW12022.

Discussion

With PEARL, the major characteristics of the monitored time series of SMET mass leached in the lysimeter at the 180-cm depth could be captured well. However, this was not the case for MESA leachate concentrations where, particularly after the first SMET application in 2012,

the simulation results clearly overestimated the monitored MESA leachate concentrations. The discrepancies between the monitored and the modeled time series can also be seen in the low (i.e., close to zero) NSE values, which mainly were due to the mismatch of monitored and modeled spikes. Thus, the predictive power of the fitted model is limited given that an NSE value of 1 indicates a perfect match.

It was not possible to find a single fate parameter set with which the observed MESA leachate concentrations from the first SMET application and the two consecutive applications could be reproduced. This also may be related to the fact that experimental conditions during the 2012 SMET application were different (i.e., individual maize plants were covered during the application, a handheld spray gun was used, and some of the maize plants were removed from the lysimeter), which could not be implemented in the PEARL model, probably leading to an overestimation of the simulated MESA concentrations. Hence, during the calibration process, the focus was placed on matching the second and third groups of MESA occurrences in the leachate with PEARL. Water contents at different depths were calibrated separately and yielded NSE values between 0.48 and 0.62.

For modeling the fate of SMET and MESA, it was a great benefit to have site-specific fate parameters available instead of using only parameter values given in the PPDB. Only the Freundlich sorption coefficient for SMET and the Freundlich exponent for MESA were used as the most likely values from the PPDB, whereas the majority of the parameter values were modified sometimes even beyond the parameter range indicated in the PPDB (e.g., DT_{50} and n^{-1} for SMET) because of the physicochemical characterization of the lysimeter soil type. This implies that if no lysimeter data from a compound application and no physicochemical analysis of local soils are available, the use of the most likely values from the PPDB for environmental fate parameters might lead to only a coarse description of the leaching features for a given compound or unrealistic fitted parameters that will not be appropriate for predictions. Thus, it can be deduced that the use of environmental fate parameters derived from calibrating leachate concentration time series in a lysimeter represents a central element within our regional model approach because the concentration time series in lysimeters also include the impacts of flow processes on the leaching characteristics of the compounds.

The transfer of the resulting groundwater recharge and MESA fluxes to the saturated domain from PEARL was implemented considering the actual depth of the groundwater table. Thus, the temporal and spatial aspects of groundwater recharge and MESA fluxes reaching the groundwater body were taken into account. This is an important advancement over using typical results from environmental fate model studies for groundwater model applications, where leaching conditions are assessed at a standard depth of 1 m below ground.

Aquifer-wide MESA groundwater concentrations were simulated for four different versions of fate parameters and application doses. At some monitoring wells, a tendency of overestimation of the observed MESA groundwater concentrations by the simulated wells can be noted (OW45312, OW12202, and OW45212 in part) even for the best-performing version. The overestimation cannot be explained only by the moderate success in calibrating the MESA leachate concentration time series at the Wagna lysimeter because in 2013 and 2014, the simulated mass flux was lower than the monitored fluxes. The extent to which these findings are compound specific and dependent on the prevalent vertical profile (i.e., coarse sand and gravel underlying soil layers) and weather conditions must be further investigated.

As a surprising outcome, the simulated MESA groundwater concentrations in Version 4 increased, leading to a clear overestimation of the observations whereby the overall simulation results deteriorated. Nonetheless, making use of the laboratory-derived fate parameters improved the calibration of the lysimeter leachate data. Thus, it can be inferred that the use of spatially distributed, laboratory-derived fate parameters without placing them in a hydraulic context (as with the lysimeter experiment) does not necessarily improve regional groundwater concentration simulation results.

From a technical perspective, the implementation of the sequential coupling between PEARL and FEFLOW to simulate the continuous subsurface pathway of SMET and MESA as well as the related fate processes appears to be a promising approach. The variability of soil types and agricultural practices (i.e., percentages of maize cultivated per cadastral municipality) led to a total of 79 PEARL simulation models to be set up. This number is still small compared with gridded nationwide studies about the leaching potential of numerous plant protection products, which can include several hundred thousand simulation runs (e.g., Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 2013).

The one-dimensional vertical representation in PEARL implies that horizontal flow processes in the vadose zone hold minor significance and can be neglected, which is the case for the WLF aquifer and most river valleys and basins where agricultural production is conducted. Moreover, there is no process-driven need for feedback between the saturated and the vadose model domain in the WLF aquifer (i.e., the groundwater table is below the reach of plant roots), whereby the sequential coupling approach can be followed without further limits of applicability.

If horizontal flow exists in the vadose zone, a three-dimensional model needs to be applied to simulate flow and environmental fate processes. The resulting recharge and leaching mass time series can subsequently be coupled with a groundwater flow and pollutant transport model. Alternatively, a three-dimensional model that describes water flow and solute movement in variably saturated media could be applied (e.g., as an extension to the approach described by Bergvall et al., 2011). In the case of shallow groundwater tables, where plant roots reach into the groundwater, the coupling of models between the vadose and the saturated zone has to be implemented at an appropriate time interval (e.g., 1 d, depending on the temporal dynamics of the dominating processes); current results from the groundwater model (i.e., groundwater level and compound concentration) are then used as the lower boundary conditions for the following time step in the vadose zone model. Subsequently, the results from the vadose zone model (i.e., water flux and leachate mass) are back-transferred to the groundwater model as the upper boundary conditions for solution of the actual time step.

We highly recommend using lysimeter experiments to understand groundwater concentrations of a given compound because the derived environmental fate parameters from lysimeter data reflect the interplay between hydraulic and physicochemical processes in the vadose zone. Soil-specific environmental fate parameters determined in the laboratory narrow the range of parameter values indicated in the PPDB and assist in the modeling of leachate data from lysimeter experiments. If no lysimeter experiments for the targeted compound in a given aquifer exist, it is challenging to specify a general modeling protocol. For aquifer-scale modeling, our findings indicate that difficulties in modeling the observed groundwater concentrations may arise. Nonetheless, we still suggest using a groundwater transport model

to run scenarios whereby vadose zone processes can be related to groundwater concentration data.

In general, the overall modeling approach (i.e., sequential coupling between PEARL and FEFLOW) is transferable to other aquifers and for different compounds and allows a transparent methodology to study the impact of different agricultural practices on groundwater concentrations of plant protection products and their metabolites. Additionally, it denotes a reasonable modeling procedure to link the risk assessment of plant protection products to groundwater protection goals for drinking water use based on which groundwater quality monitoring schemes can be developed. Because of lower detection limits and lower analysis costs per sample, it can be expected that in the near future the frequency of compound concentrations in the groundwater exceeding the corresponding detection limit will increase and thus the available database for matching simulation results will quickly grow.

Conclusions

We have implemented the sequential coupling between PEARL and FEFLOW models to describe the subsurface fate of MESA at the aquifer scale. The parameterization of PEARL was supported by an experimental application of SMET on a well-established lysimeter in the WLF aquifer and laboratory analysis of soil samples to delineate location-specific fate parameters. Nonetheless, matching the monitored MESA leachate concentration time series with PEARL proved a challenging task and did not lead to satisfactory results for high application doses of SMET.

The lysimeter-derived fate parameter set was used to simulate the leaching of MESA into the groundwater body at the aquifer scale considering the distribution of soil types and maize cultivation percentages. For the transfer of groundwater and MESA fluxes to groundwater model inputs, the actual groundwater table was taken into account and therefore the complete vadose zone passage is represented. Monitored MESA groundwater concentrations were simulated by running the coupled models with four different combinations of fate parameter sets and application dosages. For the given compounds and prevailing natural conditions, the application of the fate parameter set derived during the lysimeter data calibration at the entire WLF aquifer notably drives the simulation results toward the magnitude of monitored MESA groundwater concentrations. An assumed 30% reduction of the SMET application (Version 3) improves the simulated MESA groundwater concentrations at some locations. We also tested the additional use of specific fate parameters for the dominant soil type in the WLF aquifer, which surprisingly resulted in a deterioration of the simulated MESA groundwater concentrations. Thus, it can be inferred that lysimeter experiments and the subsequent modeling of leachate dynamics yield integrated parameter sets that are better suited for regional groundwater quality simulations than the use of spatially distributed laboratory-derived fate parameters.

Overall, the developed model system is well suited and robust for describing the entire subsurface pathway and fate of plant protection products including groundwater transport at the aquifer scale. Although a lot of site-specific information was available and has been implemented to the best of our understanding, the simulated MESA groundwater concentrations at some locations tended to overestimate the observations, which may be

associated with uncertainties about the real application dose of SMET throughout the WLF aquifer.

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5.3 Degradation and leaching of bentazone, terbuthylazine and S-metolachlor and some of their metabolites: A long-term lysimeter experiment (Publication IV)

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Degradation and leaching of bentazone, terbuthylazine and S-metolachlor and some of their metabolites: A long-term lysimeter experiment

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ABSTRACT

The degradation and leaching of bentazone, terbuthylazine and S-metolachlor and their metabolites N-methyl-bentazone, desethyl-terbuthylazine, 2-hydroxy-terbuthylazine, metolachlor ethane sulfonic acid (ESA) and metolachlor oxanilic acid (OA) were investigated using the plant protection products Artett (bentazone/terbuthylazine), Gardo Gold (S-metolachlor/terbuthylazine) and Dual Gold (S-metolachlor) applied to a weighable, monolithic, high precision lysimeter with a loamy sandy soil. Artett and Gardo Gold were applied at higher doses than recommended according to good agricultural practice. In leachate, S-metolachlor was detected at concentrations of up to 0.15 µg/L, whereas metolachlor-ESA and metolachlor-OA were present at higher concentrations of up to 37 and 8.4 µg/L respectively. In a second terbuthylazine application, concentrations of desethyl-terbuthylazine of up to 0.1 µg/L were detected. In soil, bentazone degraded faster than terbuthylazine and S-metolachlor, whereas the metabolization of terbuthylazine after the second application resulted in an enhanced formation of desethyl-terbuthylazine and a highly increased hydroxylation of terbuthylazine. The importance of analysing both parent compounds and metabolites on a long-term scale was demonstrated to better understand the environmental fate and transport.

Keywords: agriculture; herbicide; contamination; metabolism

INTRODUCTION

The use of pesticides to increase agricultural production continues to be an important topic for environmental research. Once applied to the field, pesticides can be degraded by the influence of physical, chemical and biological factors, volatilized, adsorbed by soil colloids and transported through surface runoff and leaching. The transport of pesticides is influenced by

the water movement (e.g. infiltration, plant uptake, drainage) and the interaction with the soil matrix (e.g. organic matter, clay content, iron oxides) (Arias-Estévez et al. 2008).

Bentazone, terbuthylazine and S-metolachlor are important herbicides applied to maize and other crops to control pre-emergence or early post-emergence broadleaf and grass weeds. These herbicides are often used in combination to enhance the herbicidal effect. They undergo transformation in the environment to form corresponding metabolites such as N-methyl-bentazone, desethyl-terbuthylazine, 2-hydroxy-terbuthylazine, metolachlor ethane sulfonic acid (ESA) and -oxanilic acid (OA). Due to their solubility and mobility, residues of these parent compounds and of their metabolites have been detected in surface and groundwater, in some cases at concentrations higher than the European drinking water limit of 0.1 µg/L (Guzzella et al. 2003, Loos et al. 2010, BMLFUW 2013).

Bentazone, terbuthylazine and S-metolachlor and thus their metabolites have different chemical properties (Table 1) linked to different soil sorption and degradation processes. Bentazone poses an environmental risk because of its high mobility and susceptibility to leaching from soil to groundwater (Boesten and Van der Pas 2000, Li et al. 2003). The most stable metabolite of bentazone in soil is N-methyl-bentazone, which is very prone to microbially-mediated degradation (Wagner et al. 1996). Terbuthylazine became a commonly used triazine herbicide in Austria. Microbial degradation of triazines proceeds mainly via dealkylation, hydroxylation and ring cleavage of the parent compound. Dealkylated products of terbuthylazine can be considered hazardous contaminants for groundwater pollution because they are generally more persistent and water soluble than the parent compound (Gerstl et al. 1997). On the other hand, hydroxylated compounds are characterized by low water solubility and are therefore considered less important potential contaminants for groundwater (Guzzella et al. 2003). The chloroacetanilide herbicide S-metolachlor transforms into the prominent metabolites metolachlor-ESA and metolachlor-OA which are well known groundwater contaminates. In Austria (BMG 2014) and Germany (Umweltbundesamt 2015), both metabolites are classified as ‘non-relevant’ metabolites with a threshold value of 3 µg/L in groundwater. ‘Non-relevant’ metabolites (EC 2003) are either not specifically regulated or diverse threshold values are applied among EU member states.

The pesticides used in this study are among those used extensively in Austrian agriculture. Due to the lack of long-term degradation experiments, a field-based lysimeter study of three to five years of local agricultural practices was conducted. The fate of bentazone, terbuthylazine and S-metolachlor and the occurrence of their metabolites in leachate and soil at different depths were investigated. The aim was to identify and quantify degradation and migration.

Table 1. Physico-chemical properties based on the pesticide property database (Lewis et al. 2016)

Compound	**Bentazone**	**Bentazone-methyl**	**Terbuthylazine**	**Terbuthylazine-desethyl**	**Terbuthylazine-2-hydroxy**	**S-Metolachlor**	**Metolachlor-ESA**	**Metolachlor-OA**
CAS number	25057-89-0	61592-45-8	5915-41-3	30125-63-4	66753-07-9	87392-12-9	171118-09-5	152019-73-3
Molar mass (g/mol)	240.28	254.31	229.71	201.68	211.33	283.79	329.41	279.33
Chemical formula	$C_{10}H_{12}N_2O_3S$	$C_{11}H_{14}N_2O_3S$	$C_9H_{16}ClN_5$	$C_7H_{12}ClN_5$	$C_9H_{17}N_5O$	$C_{15}H_{22}ClNO_2$	$C_{15}H_{23}NO_5S$	$C_{15}H_{21}NO_4$
Solubility in water at 20 °C (mg/L)	570	na	6.6	327	7.2	480	212,461	238
Dissociation constant (pK_a)	3.5	na	1.9	na	na	3.1	na	na
Octanol-water partition coefficient (log K_{ow})	0.77	na	3.4	2.3	1.5[a]	na	-1.9	na
Freundlich K_{foc}	60	258	231	78	187	226	na	7.3
DT_{50} in the field (days)	8.0		22	29		21	70[b]	128

na -not available; [a]Kaune et al. (1998); [b]Bayless et al. (2008); ESA – ethane sulfonic acid; OA – oxanilic acid

MATERIAL AND METHODS

Experimental site and pesticide application

The experiment was conducted using a weighable, monolithic lysimeter (1 m² surface area, 2 m depth) built in 2004 at the agricultural test site in Wagna (Styria, Austria). The lysimeter could be tilled with agricultural machines (e.g. a plough) during which the upper ring of the lysimeter was removed. Leachate was collected at a depth of 180 cm by seven suction cups (total surface 3600 cm²), which were controlled by a vacuum pump operated to mimic matrix potentials measured next to the lysimeter at a depth of 180 cm. The lysimeter setup has been repeatedly described in detail (Klammler and Fank 2014, Schuhmann et al. 2016, Kupfersberger et al. 2018). The lysimeter was located within a test plot of 1000 m² area, which was cultivated with a crop rotation consisting of maize (2010, 2012, 2014), triticale (2011, 2015) and pumpkin (2013). After the crop vegetation periods, typical catch crops were cultivated (ryegrass or forage rye). Soil tillage was done by plough and harrow in early spring and early autumn. Since 2014 the plough has been replaced by ripper to reduce the soil surface treatment. Details about the soil characteristics are given in Table 2.

Table 2. Soil characteristics of the lysimeter

Depth (cm)	Clay	Silt	Sand	Gravel	Carbon content	Dry bulk density (kg/m³)	pH
			(%)				
0-30	20	33	45	2	1.1	1510	6.3
30-50	20	27	53	0	0.52	1550	6.5
50-80	14	24	62	0	0.35	1550	6.6
80-130	0	1.0	33	66	< 0.08	na	6.8
>130	0	1.0	25	74	< 0.08	na	7.1

na - not available (1500 kg/m³ assumed)

Since the initial scientific question of the experiment intended to avoid direct pesticide uptake via the leaves in order to measure the uptake from soil into maize (data not available), the maize plants were covered with plastic bags during the pesticide applications in 2010 and 2012. The plastic bags were removed immediately after the application without a wash-off (artificially or by precipitation) of pesticide residues from the plastic bags. Details about the applications are given in Table 3. All plant protection products were applied as suspensions onto the field including the monolithic lysimeter. The applications in 2010 and 2012 were performed using a hand-held spraying apparatus, whereas mechanical sprayers were used in 2013 and 2014. In addition, a higher pesticide doses than normal in agriculture were used for the applications in 2010 and 2012. The field area chosen for the application ensured an even distribution of the pesticide on the lysimeter surface. All experiments were performed under natural weather conditions without an additional irrigation. Precipitation was measured by a meteorological station located at the test site. Table 4 shows the annual amounts of precipitation, evapotranspiration and leachate from the lysimeter.

Table 3. Details of the pesticide applications at the lysimeter surface

Date	Crop	Formulation	Active ingredients (a.i.)	Application form	Growth stage at time of application	Application dose (kg a.i./ha)
April 28, 2009 *	pumpkin	Dual Gold	S-metolachlor (960 g/L)	pre-emergence	no crop cover	2.4
May 12, 2010	maize	Artett	bentazone (150 g/L)/ terbuthylazine 150 g/L)	post-emergence	3- to 4-leaf stage	2.7 / 2.7
May 24, 2012	maize	Gardo Gold	S-metolachlor (312.5 g/L)/ terbuthylazine (187.5 g/L)	post-emergence	6- to 7-leaf stage	3.5 /2.1
May 12, 2013	pumpkin	Dual Gold	S-metolachlor (960 g/L)	pre-emergence	no crop cover	1.2
May 10, 2014	maize	Dual Gold	S-metolachlor (960 g/L)	post-emergence	7-leaf stage	0.96

* The first application of Dual Gold in 2009 was done by a local farmer before the start of the project.

Table 4. Annual amounts of precipitation (P), real evapotranspiration (ET_r) and leachate (L) together with the annual mean concentrations of S-metolachlor, metolachlor-ESA and metolachlor-OA from April 2012 to June 2015

Year	Crop	P	ET_r	L	S-Metolachlor	Metolachlor-ESA	Metolachlor-OA
		(mm)			(µg/L)		
2010	maize	1014	599	429			
2011	triticale	730	736	63			
2012	maize	1000	685	325	0.0070	6.4	0.19
2013	pumpkin	952	529	479	0.0019	4.5	0.54
2014	maize	1171	598	561	0.0085	17	1.7
2015	triticale	864	721	88*	0.012	16	0.041

* until June 2015; ESA – ethane sulfonic acid; OA – oxanilid acid

Quantification of residues in leachate and soil

Leachate from the lysimeter at depths of 35, 90 and 180 cm was collected by suction cups at an average sampling interval of 10 days. The samples were stored at -18°C and analysed by direct injection-liquid chromatography-electrospray ionization-tandem mass spectrometry after the addition of an internal standard (bentazone-d_6, terbuthylazine-d_5, desethyl-terbuthylazine-d_9, metolachlor-d_6) described in Fuhrmann et al. (2014).

Soil samples at depths of 0-30 cm were taken from the lysimeter surface before the herbicide applications in 2010 and 2012. Immediately after the applications, soil samples were taken at a depth of 0-10 cm. The sampling was repeated 12, 30, 80, 150 (in 2010), 476 (in 2011) and 743 (in 2012) days after the application of bentazone and terbuthylazine on May 12, 2010 and 12, 30, 80 and 150 days after the application of S-metolachlor and terbuthylazine on May 24, 2012. Details about the soil sampling procedure are given in Schuhmann et al. (2016). Soil samples were extracted with a modified QuEChERS method specified in Fuhrmann et al. (2014) and the resulting extracts were quantified according to the liquid chromatography mass spectrometry methods described in Fuhrmann et al. (2014) and Schuhmann et al. (2016).

Estimation of half-life from measured soil residues

The half-life of pesticides belongs together with the sorption coefficient and the Freundlich exponent to the parameters which mainly control degradation and leaching. The DT_{50} values of bentazone, S-metolachlor and terbutylazine were estimated from the total mass (measured pesticide concentrations multiplied by soil bulk density) in the soil profile between 0 - 30 cm depth, assuming first order degradation according to FOCUS (2006). The initial mass of terbuthylazine and S-metolachlor on day 0 (directly after the application) was calculated from the application amount, because the measured data was lower on day 0 compared to day 12. Losses due to leaching or plant uptake were not considered in these simplified estimations.

RESULTS AND DISCUSSION

Leachate

The annual leachate volume varied between 63 mm in 2011 to 561 mm in 2014 (Table 4), which was mainly a consequence of annual precipitation because no additional irrigation was applied. Recently, Klaus et al. (2014) and Meite et al. (2018) have shown that precipitation characteristics have an important role on pesticide leaching. The experimental duration of 5-years comprised both leaching and non-leaching periods. Almost no leachate was observed during certain periods, especially from June 2011 to mid-May 2012 (in total 10 mm) and from August to October 2013 (in total 0.5 mm) (Figure 1).

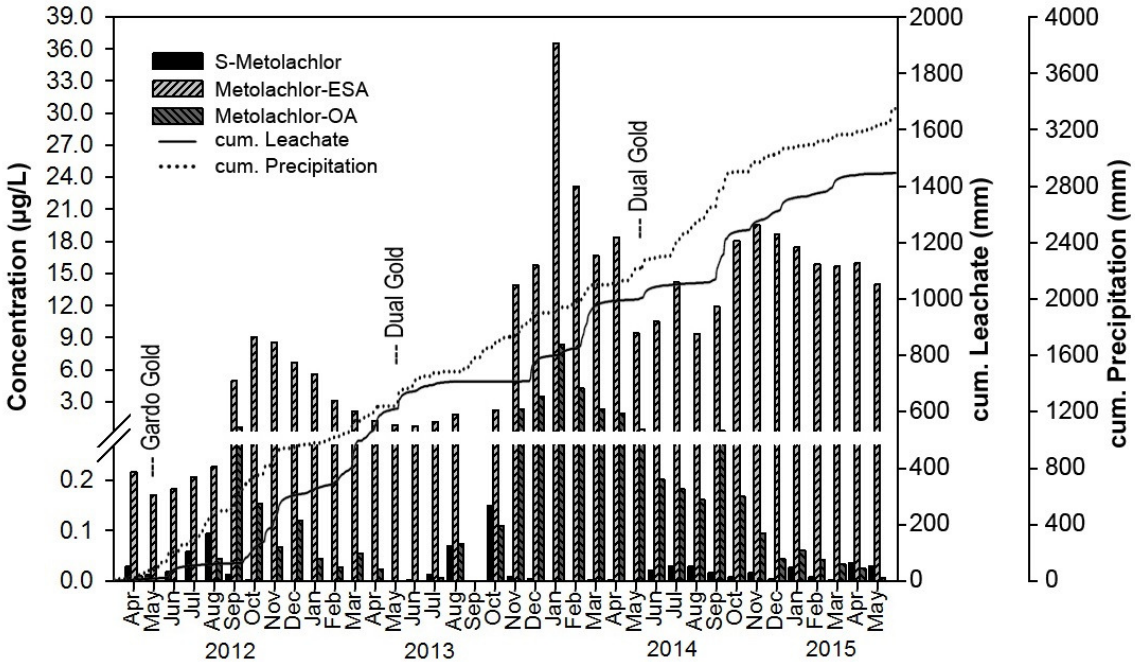

Figure 1. Average concentrations per month of S-metolachlor, metolachlor-ESA (ethane sulfonic acid) and metolachlor-OA (oxanilic acid) in leachate at the bottom of the lysimeter (180 cm depth) after repeated annual applications together wtih the cumulative precipitation and the cumulative leachate; no leachate was produced in September 2013. The scale on left y-axis has been disrupted for better visibility of low concentrations

Bentazone and terbuthylazine, as well as their metabolites N-methyl-bentazone and 2-hydroxy-terbuthylazine, were not detected, and only traces of the metabolite desethyl-

terbuthylazine were found. This might be due to the fact that low leachate volumes in June (5.4 mm), July (4.4 mm) and August (17 mm) were observed after the application in 2010 and 2-hydroxy-terbuthylazine is a lypophilic compound that is rarely detectable in water samples (Guzzella et al. 2003). After the second application of terbuthylazine in May 2012, the metabolite desethyl-terbuthylazine was detected more frequently at concentrations ranging from 0.055 to 0.1 µg/L at 180 cm of depth, with the highest concentrations occurring in October and December 2012. It has been reported that repeated application reduces the formation of bound residues and accelerates the rate of metabolite formation (Gevao et al. 2000).

At depths of 35 cm and 90 cm, S-metolachlor was not detected from May 2012 to May 2015. Due to the fact that S-metolachlor was already detected at a depth of 180 cm in April 2012 (0.029 µg/L), which was before the application of S-metolachlor in 2012, the detected concentrations of S-metolachlor in 2012, 2013 and 2014 (Figure 1) seem to originate mainly from the S-metolachlor application in 2009. High precipitation events in 2009 (1360 mm) which led to the rapid translocation of S-metolachlor and dry periods in 2010 and 2011 which decreased the effective degradation rate in the subsoil might be responsible. Kahl et al. (2014) also stated that substances located in the subsoil can remain there for a very long time and being degraded or leached a long time later. Mean monthly S-metolachlor concentrations were – with one exception in October 2013 – generally lower than 0.1 µg/L (Figure 1). Over the entire investigation period from May 2012 to May 2015, the cumulative leached load of S-metolachlor (8.0 µg) at 180 cm depth was very little in comparison to loads of metolachlor-ESA (15 360 µg) and metolachlor-OA (1300 µg).

Metolachlor-ESA had also already been detected with a background concentration of 0.22 µg/L in the leachate before the S-metolachlor application in May 2012 (Figure 1). Within six months after this application, metolachlor-ESA concentrations in the leachate increased up to 9.1 µg/L in October 2012, followed by a decline to 0.76 µg/L by June 2013. After the S-metolachlor application in 2013, the metolachlor-ESA concentration rose to 37 µg/L in January 2014, which was the maximum value measured during the investigation period. Afterward, the concentrations of metolachlor-ESA dropped to 9.4 µg/L in May 2014 and increased again after the third application to 20 µg/L in November 2014. In May 2015 a mean concentration of 14 µg/L metolachlor-ESA was still detected in the leachate. Traces of metolachlor-OA initially appeared in August 2012 and reached a relative maximum of 0.64 µg/L in September 2012 (Figure 1). After that peak, the metolachlor-OA concentrations decreased until no metolachlor-OA could be detected between May and June 2013. The highest metolachlor-OA concentration (8.4 µg/L) was detected in January 2014 – eight months after the S-metolachlor application in May 2013. Metolachlor-OA declined to 0.16 µg/L in August 2014 and increased again to 0.29 µg/L in September 2014, after which only traces were detected in leachate.

At a depth of 35 cm, the detected concentrations of both metabolites rapidly increased after each application. The highest concentrations of metolachlor-ESA were detected in September 2014 (52 µg/L), whereas metolachlor-OA showed the greatest measured concentrations in July 2012 (24 µg/L). At a depth of 90 cm, metolachlor-ESA showed a behavior similar to that seen at 35 cm, but concentrations were in general approximately 50% lower with the highest peak in November 2014 (27 µg/L). The concentrations of metolachlor-OA declined between 35 cm and 90 cm of depth. Because only traces of metolachlor-OA were detected at a depth of

90 cm, it can be assumed that the degradation of metolachlor-OA after the applications in 2012 and 2014 occurred between depths of 35 cm and 90 cm.

Soil

The applied pesticides were not detected in the soil samples taken before the applications in 2010 and 2012. As expected, after the applications the concentrations of bentazone, terbuthylazine and S-metolachlor and their metabolites were highest in the soil surface layer of the lysimeter (Tables 5 and 6) and decreased until 30 cm, confirming the increase of degradation with time. The application in 2010 and 2012 were performed post-emergence with a hand-held spraying apparatus and the maize plants were covered with plastic bags. The low concentrations on day 0 and the higher findings on day 12 can be explained by interception of the applied pesticides on the plastic bags. Because the plastic bags also covered parts of the lysimeter surface ($1m^2$), it can be assumed that non-representative samples were collected on day 0. The plastic bags were removed immediately after application before soil samples were taken randomly from six different locations within the lysimeter surface. Thus, the applied pesticide mass on the plastic bags that might have reached to soil after a wash-off was lost in 2010 and 2012.

Table 5. Mean and standard deviation values (µg/kg) of extractable bentazone, N-methyl-bentazone, terbuthylazine, desethyl-terbuthylazine and 2-hydroxy-terbuthylazine on different sampling days at varying soil depths (0-10, 10-20 and 20-30 cm) from 2010 to 2012. The sampling at 10-20 cm depth was initiated on day 12 after the application and at depths of 20-30 cm on day 30 after the application

Day of sampling/Date	**Bentazone**			**N-methyl-bentazone**					
	0-10	10-20	20-30	0-10	10-20	20-30			
0 (May 12, 2010)*	230 ± 72	(-)	(-)	0.65 ± 0.13	(-)	(-)			
12 (May 24, 2010)	61 ± 23	31 ± 17	(-)	3.7 ± 1.0	2.5 ± 1.4	(-)			
30 (Jun 14, 2010)	8.3 ± 7.7	1.2 ± 0.38	0.90 ± 0.50	3.1 ± 2.3	0.32 ± 0.14	0.60 ± 0.29			
80 (Aug 8, 2010)	1.7 ± 0.39	0.55 ± 0.065	0.19 ± 0.028	3.0 ± 0.95	0.30 ± 0.13	0.16 ± 0.074			
150 (Oct 11, 2010)	0.12 ± 0.013	0.21 ± 0.029	0.40 ± 0.16	nd	nd	0.26 ± 0.11			
476 (Aug 31,2011)	0.079 ± 0.042	0.11 ± 0.021	(-)	nd	nd	(-)			
743 (May 24, 2012)	nd	nd	nd	nd	nd	nd			
Day of sampling/Date	**Terbuthylazine (TA)**			**Desethyl-TA**			**2-hydroxy-TA**		
	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
0 (May 12, 2010)*	38 ± 12	-	-	0.28 ± 0.062	-	-	98 ± 26	-	-
12 (May 24, 2010)	240 ± 88	100 ± 34	-	8.7 ± 2.6	4.2 ± 1.2	-	130 ± 39	58 ± 21	-
30 (Jun 14, 2010)	76 ± 2.9	5.2 ± 4.9	23 ± 3.4	7.4 ± 4.4	0.61 ± 0.32	1.5 ± 0.38	180 ± 160	6.8 ± 5.6	23 ± 3.3
80 (Aug 8, 2010)	26 ± 11	3.1 ± 2.5	0.56 ± 0.45	5.4 ± 1.2	1.1 ± 0.38	0.34 ± 0.071	120 ± 44	13 ± 10	3.3 ± 2.0
150 (Oct 11, 2010)	0.67 ± 0.17	1.8 ± 0.52	3.5 ± 0.20	0.41 ± 0.052	1.3 ± 0.13	1.6 ± 0.12	3.9 ± 0.58	9.3 ± 1.8	20 ± 1.7
476 (Aug 31,2011)	0.45 ± 0.17	1.3 ± 0.33	-	0.26 ± 0.0049	0.53 ± 0.12	-	2.3 ± 0.67	4.9 ± 0.34	-
743 (May 24, 2012)	0.47 ± 0.35	0.51 ± 0.22	0.50 ± 0.23	0.30 ± 0.097	0.27 ± 0.018	nd	1.6 ± 0.46	2.7 ± 1.6	2.8 ± 0.41

*The sampling on day 0 was immediately after application; - samples were not taken at these days; $n = 3$; nd – not detected

Table 6. Mean and standard deviation values (µg/kg) of extractable S-metolachlor, metolachlor-ESA (ethane sulfonic acid) and metolachlor-OA (oxanilic acid) on different sampling days at varying soil depths (0-10, 10-20 and 20-30 cm) from 2012. The sampling at a depth of 10-20 cm was initiated on day 12 after the application and at depths of 20-30 cm on day 30 after the application

Day of sampling/Date	**S-Metolachlor (MET)**			**MET-ESA**			**MET-OA**		
	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
0 (May 24, 2012)*	510 ± 180	-	-	5.5 ± 0.81	-	-	6.2 ± 1.6	-	-
12 (Jun 5, 2012)	600 ± 110	11 ± 2.4	-	26 ± 7.8	0.91 ± 0.19	-	33 ± 4.6	0.8 ± 0.27	-
30 (Jun 22, 2012)	270 ± 110	120 ± 94	12 ± 6.0	21 ± 11	15 ± 12	3.4 ± 0.89	27 ± 17	17 ± 11	2.2 ± 0.36
80 (Aug 13, 2012)	63 ± 33	4.6 ± 3.7	1.9 ± 0.56	76 ± 14	62 ± 13	41 ± 15	69 ± 20	16 ± 5.4	12 ± 6.9
150 (Oct 23, 2012)	12 ± 4.2	3.3 ± 1.3	1.5 ± 0.46	6.0 ± 0.77	5.1 ± 0.78	5.1 ± 1.2	4.9 ± 0.89	2.1 ± 0.37	1.2 ± 0.34
Day of sampling/Date	**Terbuthylazine (TA)**			**Desethyl-TA**			**2-hydroxy-TA**		
	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30
0 (May 24, 2012)*	150 ± 50	-	-	1.0 ± 0.29	-	-	190 ± 69	-	-
12 (Jun 5, 2012)	300 ± 45	3.7 ± 0.58	-	30 ± 6.8	1.0 ± 0.45	-	230 ± 54	6.2 ± 0.88	-
30 (Jun 22, 2012)	170 ± 34	68 ± 36	5.7 ± 1.5	20 ± 4.8	9.0 ± 3.8	1.3 ± 0.61	93 ± 25	46 ± 30	6.4 ± 1.7
80 (Aug 13, 2012)	77 ± 27	8.8 ± 8.5	2.9 ± 1.4	18 ± 4.6	3.0 ± 1.7	1.1 ± 0.58	31 ± 9.3	7.1 ± 3.7	5.3 ± 0.27
150 (Oct 23, 2012)	16 ± 1.2	6.5 ± 1.6	1.8 ± 0.36	4.7 ± 0.74	3.2 ± 0.32	1.3 ± 0.30	12 ± 1.6	6.6 ± 1.2	4.5 ± 0.45

*The sampling on day 0 was immediately after application; - samples were not taken at these days; $n = 3$

The concentrations of bentazone (230 µg/kg) steadily decreased following the application in 2010 until two years later on day 743 no residues were detected. The metabolite N-methyl-bentazone was detected immediately following the application in the top soil (0.65 µg/kg). No residues of N-methyl-bentazone were left at soil depths of 0-10 cm and 10-20 cm after day 80. At 20-30 cm, residues of bentazone and its metabolite N-methyl-bentazone were only detected on days 30, 80 and 150 (i.e. in the year of the application). Within the topsoil, there can be significant within-field spatial variability in pesticide degradation rates, associated with variation in soil properties controlling degradation processes or the localization of specific pesticide-degrading microbial populations in the topsoil (Walker et al. 2001). Although the application rate of bentazone and terbuthylazine in 2010 was the same, a rise of the soil residue concentration of terbuthylazine (240 µg/kg) was observed on day 12. 2-hydroxy-terbuthylazine (180 µg/kg) was detected at concentrations much higher than those of desethyl-terbuthylazine (8.7 µg/kg). The concentrations of terbuthylazine, desethyl-terbuthylazine and 2-hydroxy-terbuthylazine were still detectable at 0.47, 0.30 and 1.6 µg/kg, respectively, on day 743. At 10-20 cm, the high concentration of terbuthylazine (100 µg/kg) on day 12 rapidly decreased to 5.2 µg/kg by day 30. On day 743, 0.51 µg/kg of terbuthylazine remained in the soil. The highest concentrations of desethyl-terbuthylazine and 2-hydroxy-terbuthylazine were detected on day 12 (4.2 µg/kg and 58 µg/kg). At 20-30 cm, the concentration of terbuthylazine (23 µg/kg) detected on day 30 finally decreased to 0.50 µg/kg by day 743. After a decline until day 80, the concentrations of both metabolites increased again to 1.6 µg/kg and 20 µg/kg by day 150. On day 743, only 2-hydroxy-terbuthylazine remained in the soil.

Day 743 (May 24, 2012) was the same day as the application (day 0) of S-metolachlor and terbuthylazine. The highest concentration of terbuthylazine (310 µg/kg) was again detected on day 12. One explanation could be interception, since the herbicides were applied post-emergence and the maize was covered with plastic bags during the application in 2010 and 2012, so that the chosen sampling points on day 0 might have been affected by the covering of maize. On day 150, 16 µg/kg of terbuthylazine remained in the topsoil. High concentrations of 2-hydroxy-terbuthylazine were already found on day 0 (190 µg/kg). At 10-20 cm, the highest concentration of terbuthylazine (68 µg/kg) was detected on day 30. Peak concentrations of desethyl-terbuthylazine and 2-hydroxy-terbuthylazine were also detected on day 30. At 20-30 cm, a terbuthylazine concentration of 5.7 µg/kg was detected on day 30, with levels falling gradually from then on to 1.8 µg/kg by day 150. On day 30, 80 and 150, similar concentrations of the metabolite desethyl-terbuthylazine were measured. Residues of 2-hydroxy-terbuthylazine slightly decreased from 6.4 µg/kg on day 30 to 4.5 µg/kg on day 150.

After the application on May 24, 2012, the concentrations of S-metolachlor peaked on day 12 and then decreased gradually. On day 150, 12 µg/kg of S-metolachlor remained in the topsoil. The metabolites metolachlor-ESA and metolachlor-OA were detected immediately after the application. The concentrations of both have reached their maximum (76 µg/kg and 69 µg/kg respectively) on day 80. At 10-20 cm, the high concentration of S-metolachlor (120 µg/kg) on day 30 rapidly decreased to 4.6 µg/kg by day 80. Except metolachlor-OA at a depth of 10-20 cm (17 µg/kg on day 30), both metabolites peaked on day 80 in each soil depth. In comparison, higher concentrations of metolachlor-ESA were detected. At 20-30 cm, an S-metolachlor concentration of 12 µg/kg was detected 30 days after the application. Similar concentrations (1.9 µg/kg and 1.5 µg/kg) of S-metolachlor were recorded on days 80 and 150,

respectively. Peak concentrations of metolachlor-ESA (41 µg/kg) and metolachlor-OA (12 µg/kg) were detected on day 80.

Bentazone, terbuthylazine and S-metoalchlor showed varying degradation rates. The concentrations of bentazone detected in the soil were generally lower than the concentrations of terbuthylazine. Fitting sum-up residues from 0-30 cm soil depth to the first order decay model resulted in estimated DT_{50} values of 17 and 25 days for bentazone and terbuthylazine, respectively. According to the estimated DT_{50} values, 26 and 37 days for S-metolachlor and terbuthylazine in 2012, S-metolachlor was mineralized faster than terbuthylazine. These calculated half-lives are apart from bentazone within the range of values in the literature (Table 1; Lewis et al. 2016). Whereas, most of the reported DT_{50} values in literature were normalized to standard conditions.

In conclusion, bentazone, terbuthylazine, N-methyl-bentazone, and 2-hydroxy-terbuthylazine were not detected in leachate. Desethyl-terbuthylazine was found more frequently and at higher concentrations in leachate after the repeated application of terbuthylazine. In contrast to the leachate, where only desethyl-terbuthylazine was detected, 2-hydroxy-terbuthylazine was the predominant metabolite found in soil. While metolachlor-ESA was transported to a depth of 180 cm, metolachlor-OA was mainly degraded at depths of between 35 cm and 90 cm. S-metolachlor residues remained at depths of 0-35 cm after applications in 2012, 2013 and 2014 and were not translocated into deeper soil layers. The S-metolachlor concentrations detected between 2012 and 2014 originated from a previous S-metolachlor application in 2009, which can be explained by a high precipitation rate followed by dry periods in 2010 and 2011.

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5.3.1 Appendix A. Supplementary data: clothianidin

Neonicotinoid insecticides represent some of the most popular and widely used class of insecticides in the world controlling many pests and associated diseases in crop production. The active ingredients imidacloprid, thiamethoxam, clothianidin, acetamiprid and nitenpyram, are the most commonly used systemic insecticides for treatment of seeds (Jeschke and Nauen, 2008). A key characteristic distinguishing neonicotinoids from other currently popular insecticide classes is their systemic nature. Neonicotinoids are relatively small molecules and are highly water soluble. Seed coating with neonicotinoids is an application method that has been regarded as more ecologically friendly than spraying because the insecticides are put into soil. Concerns regarding the environmental fate and effects of neonicotinoids increased since the damage of honey bees associated with the use of seed-coating insecticides have been identified (Reetz et al. 2011). An intensive debate about the potential risk for honey bees and other pollinators posed by neonicotinoids in seed treatments across Europe was provoked. Apart from the risk of bees, it is important to investigate the impact of these pesticides in the environment especially in water and soil. In this work, commercially available clothianidin-coated maize seeds were planted on the surface of the weighable, monolithic lysimeter to quantiy the transport of clothianidin in leachate and soil.

Poncho maize seeds (Bayer Austria GmbH) were sowed manually to a depth of 4 cm within the lysimeter surface of 1 m^2 on April 17, 2012. Each seed carried 0.5 mg of clothianidin in its seed coating. Leachate from the bottom of the lysimeter was measured before, during, and after the maize growing season in 2012 and finally until May 2015. Soil from different depths was only collected over the growing season in 2012 in a similar fashion at the lysimeter as for S-metolachlor and its metabolites. Details about the sampling procedure, extraction and quantification methods are given above in Schuhmann et al. (2019).

In leachate, clothianidin was present during all sampling events regardless the presents of maize (Figure 5.2). Concentrations of clothianidin (> 0.1 µg L^{-1}) were already detected before the seedling of clothianidin-coated maize in April 2012. The contamination might be from dust emissions during drilling of neighbouring fields or accumulation from former applications (Nuyttens et al. 2013). Clothiandin residues can also be detected due to former applications of thiamethoxam on neighbouring fields. Clothianidin is the main metabolite of thiamethoxam (EFSA 2013) and the metabolism of thiamethoxam to clothianidn occurs very rapid (Nauen et al. 2003). After the seedling, the concentrations of clothianidin increased gradually over the growing season. This is likely due to the close proximity of the coated seed to the soil pore water and the relatively high water solubility of clothianidin (340 mg L^{-1} at 20 °C). The highest concentration (0.3 µg L^{-1}) was recorded in October 2012 at the physiological maturity of the maize plants. Clothianidin was present in the leachate during all sampling events regardless of the presence from dry periods (Figure 5.2). Whiting et al. (2014) also found clothianidin in water samples with concentrations up to 0.3 µg L^{-1} at the middle vegetative stage of maize. In 2013, a steady increase in clothianidin concentrations similar to 2012 was observed, although oil pumpkin was planted. A peak concentration of 0.25 µg L^{-1} was detected in July 2013, which was during pollination. As a result of dry conditions in summer periods no leachate could be collected in September 2013. In response to the following rainfall event an increase of clothianidin to 0.15 µg L^{-1} in November 2013 was observed. In 2014, the detected concentrations of clothianidin (0.14 to 0.095 µg L^{-1}) were considerably lower over the growing season. However, measurable concentrations of clothianidin were still detected in 2015 (Figure 5.2) indicating movement and dispersal within

the lysimeter. These observations are in line with others who have reported a long persistence of neonicotinoids in the environment (Bonmatin et al. 2015).

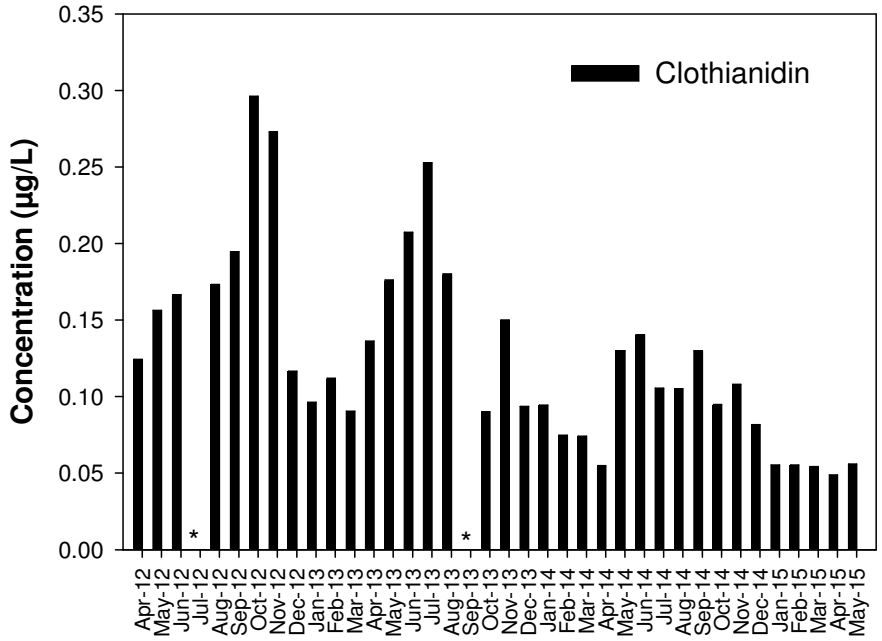

Figure 5.2: Average concentrations per month of clothianidin concentrations measured in leachate at the bottom of the lysimeter (180 cm depth). An asterisk indicates no leachate was produced in July 2012 and September 2013 due to drought conditions

In soil, residual clothianidin concentrations were measured in different depths during the maize growing season in 2012 (Figure 5.3). On Day 37 after the maize planting the application (day 0) of S-metolachlor and terbuthylazine as suspension on the lysimeter surface was performed. The concentrations of clothianidin (15 µg kg^{-1}) in the top soil peaked on day 49, then decreased to 9.1 µg kg^{-1} on day 118 and slightly increased to 10 µg kg^{-1} on day 189. At 10-20 cm clothianidin concentrations showed a trend similar to that at 0-10 cm, but concentrations were lower. At 20-30 cm, the concentration of clothianidin (9.6 µg kg^{-1}) detected on day 70 finally decreased to 5.8 µg kg^{-1} by day 189. Whiting et al. (2014) and Li et al. (2012) reported that the residual clothianidin concentrations decreased throughout the growing season. Plant uptake processes together with degradation of clothianidin believed to cause soil concentrations to decrease over time. However, the amount of clothianidin taken up by the maize or lost due to degradation can only be assumed in this study.

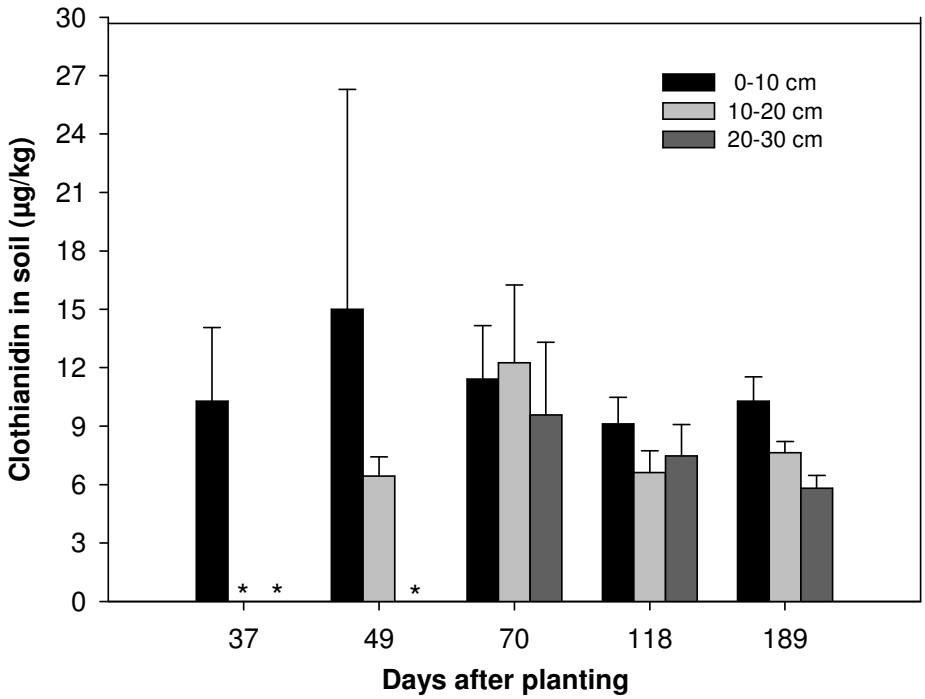

Figure 5.3: Clothianidin concentrations in soil measured over the growing season at five time points in 2012. Values indicate the mean ± standard deviation. An asterisk indicates no samples were collected at this depth

Independently from uptake by plants or microbial breakdown the neonicotinoid pesticide clothianidin could be detected in 100 % of the soil samples seeded with treated seeds during the same year and remained in leachate for extended periods of time well beyond the maize growing season. When treated seeds are planted clothianidin residues might be transported in soil through different processes. Further experiments with labelled compounds may further elucidate these findings and explain the ongoing processes. Nevertheless results proof that clothianidin is mobile in the soil and might represent a potential contamination threat to surface and groundwater. In this context, the European Union (EU) has recently included five neonicotinoid pesticides including clothianidin in the Watch List (Decision 2018/840) as potential priority pollutants with the aim of monitoring their concentration in EU water basins and assessing their associated environmental risks.

5.3.3 Appendix B. A modeling approach (Unpublished Manuscript)

ABSTRACT

The PEARL model was parameterised with lysimeter, laboratory and literature data to predict the degradation and leaching of S-metolachlor and its metabolites metolachlor ethane sulfonic acid (ESA) and metolachlor oxanilic acid (OA). The data used was obtained from herbicide concentrations measured in leachate and soil at different depths on a weighable, monolithic lysimeter after repeated S-metolachlor applications. In addition, input parameters such as half-life DT_{50} and sorption coefficient for S-metolachlor, metolachlor-ESA and metolachlor-OA were determined using the loamy-sandy lysimeter soil in laboratory experiments. The results of the simulation with PEARL show a good agreement with the measured water flow. While the simulated metolachlor-ESA leaching is quite close to the measured data, the metolachlor-OA simulation results significantly overestimate the measured data. The dynamic of measured S-metolachlor leaching is not simulated very well. Nevertheless, the general processes of S-metolachlor degradation and sorption over three to five years between application and leaching can be described with the model.

Keywords: pesticide; soil; leachate; lysimeter; PEARL model

INTRODUCTION

The use of pesticides for weed and pest control has also resulted in their widespread occurrence in the environment. The chloroacetanilide herbicide metolachlor has been used extensively in agriculture and subsequently, its ionic metabolites metolachlor-ESA and metolachlor-OA are the most frequently detected agricultural compounds in surface and groundwater, whereas metolachlor-ESA is detected more frequently than metolachlor-OA (Kalkhoff et al. 1998, Kolpin et al. 1998).

Lysimeter studies can be used to identify and quantify pesticides and metabolites that have the potential to leach to groundwater. Thus, the interest in lysimeter experiments to generate data for risk assessment (Dousset et al. 1995, Renaud et al. 2004) or modeling purposes has increased (Francaviglia et al. 2000, Kasteel et al. 2007). Numerical models like MACRO (Jarvis 1995), PRZM (Suárez 2005) and PEARL (Leistra et al. 2001) have been developed to describe the leaching of pesticides into deeper soil layers while taking into account important processes like degradation, sorption, plant uptake and the formation of transformation products. Models provide an effective tool to predict the fate of pesticides, in addition to field and laboratory data (Marín-Benito et al. 2014, Kahl et al. 2014).

The aim of this study is to simulate the environmental fate of S-metolachlor and its metabolites metolachlor-ESA and metolachlor-OA in the lysimeter at Wagna test site using the PEARL model. This location was already used for a previous PEARL application simulating chloridazon and its metabolites (Schuhmann et al. 2016), which provides the hydraulic basis for this model approach. Although Kupfersberger et al. (2018) already published simulation results of metolachlor and its metabolites for the location of the Wagna lysimeter, the present study focuses on the optimisation of the previous work. Simulation

results are compared to the measured herbicide leaching observed in a long-term lysimeter experiment, which is described in Schuhmann et al. (2019).

MATERIAL AND METHODS

Lysimeter experiment

The details of the experimental site, pesticide applications and quantification of the residues in leachate and soil are described in Schuhmann et al. 2019. The experimental data were collected during a three years period with repeated applications of S-metolachlor on a weighable, monolithic lysimeter at the agricultural test site in Wagna (Styria, Austria). The study was primarily set-up to investigate the potential risk of groundwater contamination from herbicides commonly used in Austria. The application dates, used formulations for which crop and application amounts are given in Table 1.

Table 1. Application doses (kg/ha) of S-metolachlor at the different application dates

Date	Crop	Formulation	Application doses (kg/ha)
April 28, 2009	Pumpkin	Dual Gold	2.4
May 24, 2012	Maize	Gardo Gold	3.47
May 12, 2013	Pumpkin	Dual Gold	1.2
May 10, 2014	Maize	Dual Gold	0.96

40% of the applied rate was assumed to be intercepted through the covering of maize with plastic bags in 2012 (expert judgement). No interception of S-metolachlor is assumed for pumpkin in 2013 (almost no crop cover) and by maize in 2014 (heavy rain the following day).

Model set-up

The one-dimensional model PEARL (FOCUSPEARL 4.4.4, Leistra et al. 2001) that describes the transport (convection/dispersion equation) and degradation of solutes in the soil-plant system was applied for S-metolachlor, metolachlor-ESA and metolachlor-OA. The water flow was calculated by the SWAP model (Kroes et al. 2008), which is integrated into PEARL by default. Soil water movement is described by Richard's equation and the reference evapotranspiration in the presented simulation was defined according to Penman-Monteith (Allen et al. 1998).

The parameterisation of soil hydraulic properties in PEARL follows the van Genuchten-Mualem approach (van Genuchten 1980, Mualem 1976) and is derived from in situ measurements of water contents and matrix potentials in different depths inside the lysimeter (Table 2). A dispersivity length of 0.1 m was assumed, which is based on an evaluation of a conservative tracer test described in Klammler and Fank (2014).

The calibration of water flow followed a manual approach by simultaneously using data of water contents at depths of 35, 60, 90 and 180 cm and of evapotranspiration and leachate amount. While soil hydraulic parameters were kept unchanged within this calibration step, crop parameters like leaf area indices, crop factors, rooting depths and crop heights have been modified. In a second step, pesticide transport was simulated by focusing on minimizing the

difference between simulated and observed leached substance mass as well as substance concentrations in soil water at depths of 35 and 90 cm and substance content in soil between 0-30 cm.

According to Scorza Júnior and Da Silva (2011) the most sensitive input parameters concerning the fate of pesticides in soil are sorption (Freundlich coefficient Kfoc and exponent 1/n), degradation (half-life DT_{50}) and organic carbon content. Sorption (Kfoc, 1/n) and degradation (DT_{50} at reference temperature) parameters of S-metolachlor, metolachlor-ESA and metolachlor-OA were taken from laboratory experiments carried out with the soil of the lysimeter (Brückner et al. 2017). Freundlich adsorption coefficients of S-metolachlor, metolachlor-ESA and metolachlor-OA were determined using a standard batch equilibrium method following OECD Guideline 106 (OECD 2000). Degradation half-life was determined according to OECD-Guideline 307 (OECD 2002). 250 g of soil from the lysimeter test site (triplicate) were spiked with 10 mg/kg of pesticide solution (equates to an application rate of 1.2 kg/ha) and incubated in darkness, at 18°C and at 22 % water content. Soil samples (10 g) were taken after 0, 1, 3, 6, 11, 19, 36 and 51 days and were analysed for contents of S-metolachlor and its metabolites metolachlor-ESA and metolachlor-OA. Degradation followed a first-order kinetic. The transformation coefficients of the metabolites were calculated from the molar fraction of formed metabolite and degraded parent compound (degradation of the metabolites was taken into account). Table 3 shows the parameter and corresponding references used for the environmental fate simulations.

The organic carbon content as well as other soil properties were derived from soil chemical and physical analysis. In line with FOCUS (2009), a depth-dependent degradation rate was applied by multiplying the surface degradation rate by 0.5 between 30-60 cm and 0.3 for 60-100 cm. Below 100 cm soil depth no further degradation was assumed. The model period of the present simulation was from January 1, 2005 until June 30, 2015. Following good modeling practice, the period until the first pesticide application on April 21, 2010 served as a warm-up period for the simulations.

Table 2. Soil characteristics and van Genuchten-parameters used for PEARL-simulation

Depth (cm)	Clay (m%)	Silt (m%)	Sand (m%)	Gravel (m%)	Humus (m%)	Dry bulk density (kg/m³)	pH (-)	θ_{sat} (-)	θ_{res} (-)	α (1/cm)	n (-)	k_{sat} (m/s)
0-30	20	33	45	2	1.9	1,510	6.3	0.39	0.16	0.050	1.3	$5.0*10^{-6}$
30-50	20	27	53	0	0.90	1,550	6.5	0.38	0.19	0.040	1.5	$6.5*10^{-6}$
50-80*)	14	24	62	0	0.60	1,550	6.6	0.44	0.11	0.065	1.2	$6.7*10^{-6}$
80-130	0	1.0	33	66	< 0.13	n.a.	6.8	0.20	0.030	0.25	1.4	$5.0*10^{-5}$
>130	0	1.0	25	74	< 0.13	n.a.	7.1	0.14	0.030	0.25	1.9	$1.2*10^{-4}$

θ_{sat}… saturated water content

θ_{res}… residual water content

α… parameter related to the inverse of the air entry suction (corresponding to the inflection point of the retention curve)

n… parameter related to pore-size distribution (corresponding to the slope of the retention curve)

k_{sat}… saturated hydraulic conductivity

*) In Klammler and Fank (2014) this depth is defined as 50-60 cm referring to a soil profile of approximately 3 meters next to the lysimeter. Soil texture conditions at the Wagna test site are known to be very heterogeneous (fluctuations between 30 and 200 cm). Due to the water content measured at depths of 60 and 90 cm inside the lysimeter, fine textured soil can be assumed to be deeper than 60 cm but above 90 cm. The calibration of the water contents in soil obtained the best results assuming this horizon between 50-80 cm depth.

n.a. not available (1,500 kg/m³ assumed)

Table 3. Selected chemical parameters (DT_{50}…half-life at reference temperature, K_{foc}…Freundlich-sorption-coefficient related to organic content, 1/n…Freundlich exponent, FacUpt…coefficient for plant uptake) used within the simulation for metolachlor, metolachlor-ESA and metolachlor-OA

	S-metolachlor		**Metolachlor-ESA**		**Metolachlor-OA**	
		Reference		**Reference**		**Reference**
DT_{50} (days)	29	Brückner et al. (2017), modified	94	Lewis et al. (2016)	127.5 / 50	Lewis et al. (2016) / Webb et al. (2008)
K_{foc} (mL/g)	121.9	Brückner et al. (2017)	10.1	Brückner et al. (2017)	7.9	Brückner et al. (2017)
1/n (-)	0.74	Brückner et al. (2017), modified	1.0	Brückner et al. (2017)	0.58	Brückner et al. (2017)
FacUpt (-)	0.4	Briggs et al. (1982)*	0.5	FOCUS (2014)	0.5	FOCUS (2014)
Coefficient of transformation (-)	-	-	0.12	estimated from data according to Brückner et al. (2017)	0.18	estimated from data according to Brückner et al. (2017)

*) log K_{ow} = 3.05 (Pesticide Properties Database, Lewis et al. 2016)

RESULTS AND DISCUSSION

The simulations of water contents were calibrated at depths of 35, 60, 90 and 180 cm against daily measurements and the results expressed by the Nash-Sutcliffe-Efficiency (NSE; Nash and Sutcliffe 1970) as given in Table 4.

Table 4. Goodness-of-fit for water content at depths of 35, 60, 90 and 180 cm expressed by Nash-Sutcliffe - Efficiency (NSE) based on daily results between 2010 and 2015

Depth (cm)	NSE (-)
35	0.93
60	0.93
90	0.82
180	0.86

The dimensionless NSE ranges between 1 and -∞, where a NSE of 1 denotes a perfect model fit and for NSE < 0 the average of the observations would be a better predictor than the model (Krause et al. 2005). The actual evapotranspiration was simulated with a NSE of 0.92 for the period of 2010 to July 2015. The temporal interval for this statistical analysis concurs with the leachate sampling interval for pesticide analysis of approximately ten days on average, leading to 195 data pairs for consideration. Figure 1 shows the cumulative leachate amount for crop periods. Compared to Schuhmann et al. (2016) and Kupfersberger et al. (2018), the simulation of the leachate in the presented paper was improved by modifying plant parameters. It can be seen that the general level and the behavior of measured leachate is well represented over the entire simulation period. Nevertheless, there are still periods where the simulation does not exactly fit the measured leachate (e.g. in May and June 2010, in January and February 2011, in April 2013 or in January 2015). Furthermore, there are a few periods – e.g., 21.11.-25.11.2013 and 02.09.-21.09.2014 – where the total leachate amount is simulated correctly, but time-based shifts between observed and simulated leachate occurred. Thus, the resulting NSE = 0.45 (n = 195) is rather low. However, neglecting these two periods of time-based shifts for the statistical determination would increase the NSE for simulated leachate to 0.68. The mean annual leachate water rates between 2010 and 2014 were 371 mm and 362 mm for observed and simulated leachate, respectively.

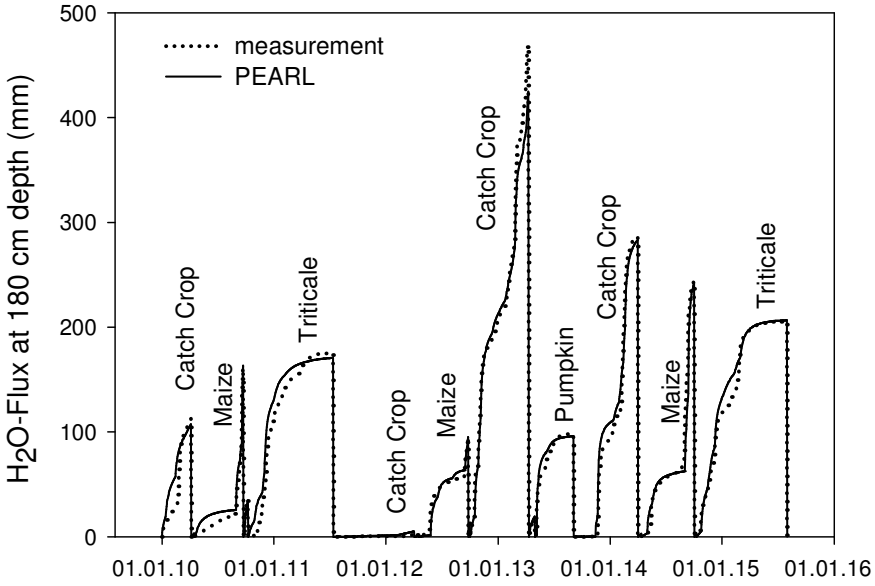

Figure 1. Comparison of the measured (dotted line) and simulated (continuous line) cumulative leachate of the lysimeter at a depth of 180 cm for crop periods. After each vegetation period, cumulative water flux was reset to 0 mm.

The model simulations of S-metolachlor, metolachlor-ESA and metolachlor-OA are based on parameters given in Table 3. Although Brückner et al. (2017) specified a DT_{50} of 32.1 days for S-metolachlor, a DT_{50} of 29 days is assumed in the present paper due to the fact that bioactivity in the soil samples reduced between the sampling of the soil and the end of the experiment. Furthermore, 1/n has been modified from 0.72 to 0.74 in order to better fit measured metolachlor leaching. Since no location-specific determinations of DT_{50} for metolachlor-ESA and metolachlor-OA were performed from Brückner et al. (2017), parameters from literature were used. While a mean DT_{50} of 132 days for metolachlor-ESA is given by the PPDB (Lewis et al. 2016), we decided to use a DT_{50} of 94 days (which represents the lower bound of the given range in the PPDB) to better fit the measured loads. Other literature shows a DT_{50} of 70 days (Webb et al. 2008), which confirms the choice of using the minimum rather than the maximum value of the given range in Lewis et al. (2016). Literature values of DT_{50} for metolachlor-OA are rather rare. For metolachlor-OA, DT_{50} values according to Lewis et al. (2016) of 127.5 days and according to Webb et al. (2008) of 50 days have been used.

Simulated and measured loads of S-metolachlor, metolachlor-ESA und metolachlor-OA at depths of 180 cm are presented in Figure 2. The simulation results for S-metolachlor show only a similar behavior to the measured loads in the periods May to September 2012 and September 2013 to January 2014. In the other periods, simulated S-metolachlor loads were either overestimated (October 2012 to May 2013) or underestimated (July 2014 to May 2015). Although the temporal dynamic of S-metolachlor cannot be simulated very well, the general level of simulated S-metolachlor is in the order of the measured load. According to Schuhmann et al. (2019) metolachlor was detected at times in the leachate at a depth of 180 cm, while it was not detected at depths of 35 and 90 cm. Due to the fact that metolachlor was already detected at a depth of 180 cm in April 2012, which was before the application of S-

metolachlor in 2012, the detected concentrations seem to originate from a previous S-metolachlor application in 2009. Thus, further simulation runs were performed to identify the depth translocation of each single S-metolachlor application. These simulations confirmed the assumption and, furthermore, showed that the total S-metolachlor measured in leachate between May 2012 and May 2015 derived from the application in 2009. Although S-metolachlor is described as non-persistent, this is possible because of high precipitation events in 2009 (1,360 mm) which led to the rapid translocation of S-metolachlor and dry periods in 2010 and 2011 which decreased the effective degradation rate in the subsoil. Kahl et al. (2014) has also stated that pesticides located in the subsoil can remain there for a very long time without being degraded or leached into the groundwater. The S-metolachlor applications from the years 2012, 2013 and 2014 are not translocated to the lysimeter outlet at a depth of 180 cm. According to the simulation results, metolachlor concentrations which originated from applications in 2012, 2013 and 2014 are not translocated deeper than 75 cm. Moreover – at least for the location of the suction cup installed in the lysimeter at a depth of 35 cm – no S-metalochlor concentrations higher than the limit of quantification (0.030 µg/L) were measured over the entire investigation period. Thus, the entire S-metolachlor load from the applications in 2012, 2013 and 2014 was adsorbed and degraded in the upper soil at depths of between 0 and 35 cm. A further simulation run where the actual application rate of 2.4 kg/ha in 2009 was reduced to only 1.2 kg/ha resulted in a maximum S-metolachlor translocation depth of 90 cm. This result indicates that applying only half of the rate of S-metolachlor in 2009 would have led to no S-metolachlor leaching.

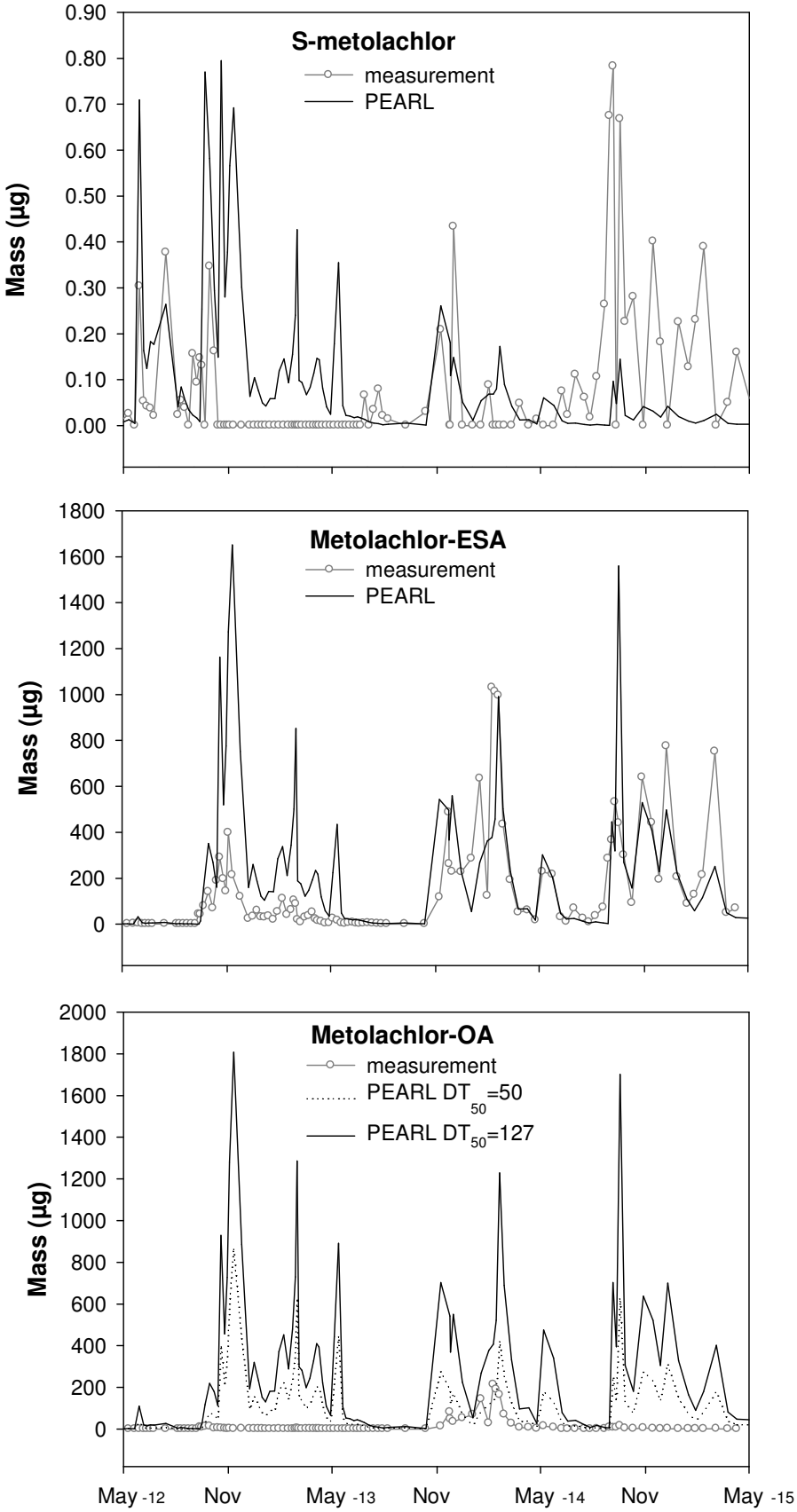

Figure 2. Measured and simulated (PEARL) leached masses of S-metolachlor, metolachlor-ESA and metolachlor-OA at 180 cm depth for the period from May 2012 to May 2015

The results of simulated and measured metolachlor-ESA loads generally show an acceptable agreement for the years 2014 and 2015 (Figure 2), while simulated loads in autumn 2012 and spring 2013 are clearly overestimated. However, the temporal dynamic of simulated metolachlor-ESA is quite similar to the measurements from May 2013 on. The metolachlor-OA simulation generally shows leached loads higher than those actually measured, regardless of which DT_{50} value (127.5 days of Lewis et al. (2016) or 50 days of Webb et al. (2008)) is used. The only period where measured metolachlor-OA can be roughly simulated – although it is still overestimated – is between November 2013 and April 2014, using a DT_{50} of 50 days. With the exception of this period, no significant loads of metolachlor-OA were measured, which cannot be reproduced by the model. Based on metolachlor-OA concentrations measured at different depths, it can be stated that the greatest differences between simulation and measurement occur at depths between 35 cm and 90 cm, where measured metolachlor-OA concentrations strongly decrease while the simulated concentrations do not.

The simulation with PEARL was intended to focus not only on the pesticide loads in the leachate, but also on the measured residues of S-metolachlor and its metabolites in the topsoil (Figure 3). The measured substance masses are derived from measured soil concentrations after the application in 2012 (Schuhmann et al. 2019) by multiplication with the measured density of the solid soil matter of 2,720 kg/m and are summarized for the soil depth between 0 and 30 cm. For S-metolachlor it can be seen that – except for the measured substance mass detected on May 24, 2012 (day 0) – the simulated masses are generally close to the measured means. Due to interception losses resulting from covering the maize with plastic bags during the application in 2012, the simulation did not focus on the metolachlor concentrations measured in the top soil on day 0. The simulation results of S-metolachlor on days 12, 30 and 80 are within the standard deviations; the simulation on day 150 is slightly overestimated. On days 0, 12 and 30, high standard deviations of soil concentration measurements for S-metolachlor were observed.

The measured masses of metolachlor-ESA and metolachlor-OA can only partially be reproduced by the model simulations. While on days 0, 12 and 30 after the S-metolachlor application the simulated masses of both metabolites are similar to those measured (even if there is some underestimation), the measured masses on day 80 were much higher than the simulation results. The significant overestimation on day 80 for metolachlor-ESA and metolachlor-OA may be due to a different transport behavior between field and simulation. A faster simulated translocation of metolachlor-ESA and metolachlor-OA from the topsoil (0-30 cm) to lower depths would explain the underestimation of soil concentrations. However, further simulation runs assuming depth-dependent parameter modifications of DT_{50} and K_{foc} (within a plausible range) did not improve the results. On day 150 the simulated mass of metolachlor-OA is slightly overestimated, whereas the metolachlor-ESA simulation underestimates the measured mass.

The transformation rate has a significant impact on the leaching rates of metabolites. If more mass of metabolites is produced, the risk of higher leaching rates rises, especially for very mobile metabolites like metolachlor-ESA and metolachlor-OA. Based on data derived from Brückner et al. (2017), we estimated transformation coefficients of 0.12 and 0.18 for metolachlor-ESA and metolachlor-OA, respectively. Compared to the maximum estimated occurrence fractions of 0.124 and 0.109 for metolachlor-ESA and metolachlor-OA given in Lewis et al. (2016), our values derived from laboratory results are rather high. However, the

values used appeared plausible since using lower transformation coefficients would have worsened the simulated concentrations in the topsoil. Furthermore, the plant uptake factor also influences the fate of the pesticides. Since this parameter was not measured in our study, but assumed, a potential uncertainty remains.

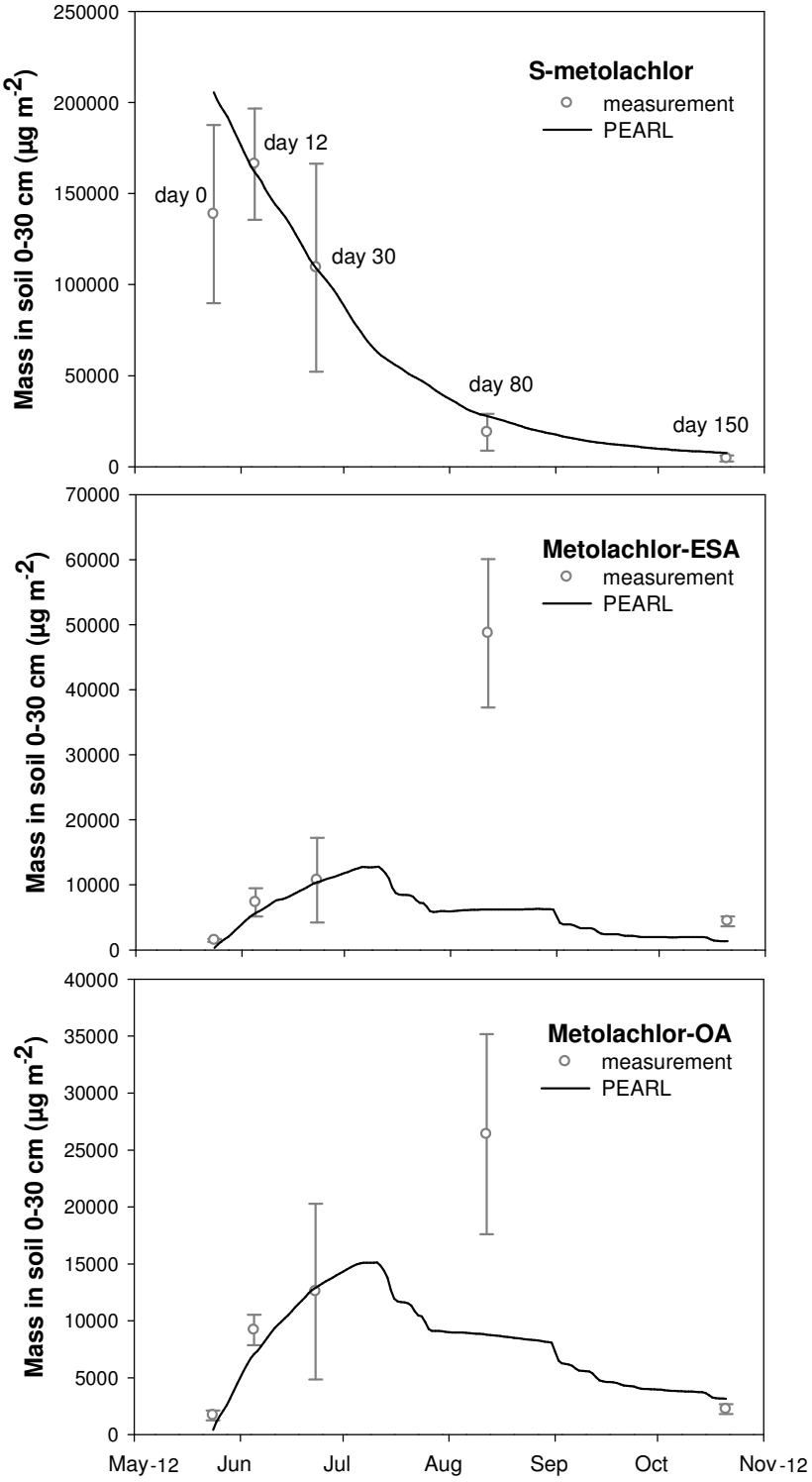

Figure 3. Measured and simulated (PEARL) masses of S-metolachlor, metolachlor-ESA and metolachlor-OA in soil (0-30 cm depth) after the application in 2012. Vertical bars represent standard error of the mean of three measurements

By using an appropriate soil and plant parameterization, the pesticide transport model PEARL was able to properly simulate the hydraulic conditions in soil. In combination with lysimeter, laboratory and literature data, general processes of metolachlor degradation and sorption over a period from three to five years between application and leaching can be described with the model. The results obtained illustrate the complexity of parameterizing the PEARL model due to sorption and degradation processes. This study clearly demonstrates the importance of measuring and modeling both parent compounds and metabolites to better understand the transport of pesticides and thereby quantify the potential risk of groundwater contamination.

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6 CONCLUSIONS

The analytical methods are valid, accurate and rugged procedures to determine bentazone, chloridazon, clothianidin, terbuthylazine, S-metolachlor, and their corresponding metabolites N-methyl-bentazone, desphenyl-chloridazon, methyl-desphenyl-chloridazon, desethyl-terbutyhlazine, 2-hydroxy-terbuthylazine, metolachlor-ESA and metolachor-OA in leachate, soil and maize.

The long-term lysimeter experiments with chloridazon and its metabolites clearly showed that the degradation pocess took place over years as evidenced by the detection of chloridazon, desphenyl-chloridazon and methyl-desphenyl-chloridazon at high concentrations in leachate and soil. In addition, chloridazon and desphenyl-chloridazon were also detected in maize. The use of different lysimeter types resulted in different transformation rates, soil retention times and accumulation by plants for chloridazon and its metabolites. Due to the setup and design of the lysimeters, conditions in the monolithic, field lysimeter clearly differ from the backfilled, gravitation lysimeter. Based on this lysimeter study, the design of the weighabale, monolithic lysimeter proved to be more suitable and thus was further used for investigations with bentazone, clothianidin, S-metolachlor and terbuthylazine.

Bentazone and terbuthylazine and their metabolites N-methyl-bentazone and 2-hydroxy-terbuthylazine were not detected in the leachate. An explanation might be the low leachate volumes (June 5.4 mm, July 4.4 mm and August 17 mm) observed after the application of bentazone and terbuthylazine in 2010. In contrast to the leachate, 2-hydroxy-terbuthylazine was the predominant metabolite of terbuthylazine found in soil. High residues of 2-hydroxy-terbuthylazine at day 0 of sampling in 2010 and 2012 indicate almost immediate decay of terbuthylazine shortly after application. Results on residues in soil were checked to ensure unintentional degradation of terbuthylazine. Clothianidin was transported from maize seed coatings through the soil profile and were detected in leachate over years.

The plant protection products Pyramin WG, Artett and Gardo Gold were applied with a hand-held apparatus at higher doses than recommended according to good agricultural practice. The application of Artett and Gardo Gold was carried out post emergence at the seedling stage of maize. Plastic bags were used to cover the plants only during the application to subsequently measure the pesticide uptake from soil into maize. Unfortunately, the usage of the plastic bags significantly affected the initial soil concentrations because in most cases the pesticide concentration on day 12 after the application is higher than on day 0 (directly after the application).

S-metolachlor was applied several times during the long-term lysimeter experiment at different rates. As a surprising outcome, S-metolachlor residues remained at depths of 0-35 cm after applications in 2012, 2013 and 2014 and were not translocated into deeper soil layers. Results of the soil water at 35 and 90 cm depth show that no S-metolachlor was detected within the period from May 2012 to May 2015. This implies that all residues measured in the leachate at the bottom of the lysimeter are related to a previous S-metolachlor application in 2009 which can be explained by a high precipitation rate followed by dry periods in 2010 and 2011. In addition, the simulation with PEARL for each single year confirmed this observation.

Results showed that the monolithic lysimeter provide excellent data for the modeling approach with PEARL. However, some model parameters like the degradation half-life or the

sorption coefficient were not measured on-site. Thus, laboratory and literature data were additionally used to predict the degradation of S-metolachlor and its metabolites. The PEARL simulation resulted in adequate correlation for mass transport of the metabolites, but the temporal dynamics of S-metolachlor, especially the first occurrence in 2012, cannot be represented well by the simulation. Similar discrepancies between the predicted and measured first peak of the parent compound chloridazon in 2010 were observed. The results obtained illustrate the complexity of parameterizing the PEARL model due to the existence of preferential flow or sorption and degradation processes.

Notwithstanding these issues, this study was performed over a number of years and provided valuable data for the commonly used pesticides and metabolites. Under the given lysimeter conditions and the fact that the applied dose was higher than used in agriculture, the presence of the parent compound chloridazon and its metabolites in leachate and soil is remarkable. In addition, hydrological conditions were found to play a significant role in governing the leachate. It is also important to mention the high leaching potential of metolachlor-ESA and metolachlor-OA after repeated applications of S-metolachlor. Clothianidin remained in leachate for extended periods of time well beyond the maize growing season.

Despite the limitations of lysimeter experiments our results nonetheless still indicates the persistence of chloridazon, S-metolachlor, terbuthylazine and their metabolites as well as clothianidin in the environment and thus, might represent a risk for groundwater contamination. This study may motivate for more extensive pesticide monitoring compaigns in order to provide data to compare the environmental fate within different soil types and thus improve the quality of model simulations.

7 LIMITATIONS, FURTHER RESEARCH CHALLENGES AND OUTLOOK

In the past few decades, farmers worldwide have tried to shift from using highly toxic pesticides to less toxic alternatives. A famous example is the ban of atrazine which led to the extended use of terbuthylazine and S-metolachlor for maize. However, the use of plant protection products always has an indirect effect on the ecosystem and biological diversity. When registering pesticides for use, a thorough review is conducted to determine if, under good agricultural practice, compounds can reach environmental compartements such as soil, water, sediment and biota. Although the European Union maintains a strict authorization procedure before pesticides are introduced, long time effects are often not investigated and information on all possible transformation products is not known. For instance, sowings with neonicotinoid-dressed seeds have caused damage on bees despite the needed risk assessment. Authorities regularly monitor the water quality of ground and surfacewater and thus prominent metabolites of atrazine, terbuthylazine and S-metolachlor are still detected (Karlsson et al. 2020; Casado et al. 2019).

Serious measures should be taken on global scale to handle environmental pollution due to pesticides. The problem is that different plant protection products are available in different EU member states which is in contrast to the EU harmonized market approach. Agrochemical companies such as BASF, Bayer CropScience, DuPont or Syngenta govern the European market for plant protection products and are preliminary responsible for the registration of new products.

Studies generally focusing on water contamination are more common than the analysis of pesticides in soil. Although soils act as sink or even reservoirs for pesticides and might pose a greater environmental threat. However, the analysis of soils is by far much more time consuming and complicated compared to the analysis of water samples. The focus on analysing water samples might be motivated by public or political concern. Monitoring or screening studies about a broad range of compounds to poof the water quality were performed due to the the European Council Directive (EC 1998) and the Austian Ordinance on the Monitoring of the Quality of Water Bodies (GZÜV 2006). Low detection limits and analysis cost per samples additionally foster monitoring studies which provide data for further risk assessment and modeling purpuses.

In the last years, metabolites have gained increasing attention due to their higher mobility and persistence. Their detection frequency in surface and groundwater is often high, with concentrations exceeding those of their parent compounds. In our study the metabolites of chloridazon, S-metolachlor and terbuthylazine resulted in a higher mobility than their parent compounds. However, metabolites are still neglected because within regulatory schemes only known and relevant metabolites are considered and need to be assessed (EC 2009). It is necessary to implement metabolites more consequently into the existing regulations to prevent the occurrence and effects of metabolites in water.

The improvement of analytical methods contributed significantly to the detection frequency of metabolites at low concentrations. Since new metabolites had been identified and confirmed each year, the analytical developments have to be adapted constantly. In general, LC-MS/MS is the most widely used method for analysing pesticides. Nowadays liquid or gas chromatography coupled to high-resolution mass spectrometry, such as LC-Orbitrap MS and

LC-time-of-flight (TOF) MS, operating in full-scan mode have been successfully applied for screening. Advantages of non-targed acquisition over targeted MS/MS are the measurement of a high number of analyts in one run and the ability to use comprehensive databases to identify analytes that were not considered at the time the sample was analysed (Mol et al. 2016; Cotton et al. 2016). Without the recent advances in the sensitivity, resolution and mass accuracy of mass spectrometers, the detection of complex mixtures of unknown components would not have been possible. Thus, environmental samples can now be screened for a range of contaminants at extremely low concentrations.

Our approach with concentration measurements and systematic long-term lysimeter experiments holds promise to quanitfy the degradation of the parent compound and its metabolites even at low concentration ranges. However, concentrations alone make it difficult to inform how much of the metabolite has been transformed. For instance, the continuous input of chloridazon-desphenyl from chloridazon makes it challenging to evaluate its transformation from concentration data. Thus further laboratory experiments studies to gain insight to possible additional transformation pathways will be needed. Another possibility to identify degradation processes might be the combination of concentration based methods with compound specific stable isotope analysis (CSIA) as described in Melsbach et al. (2020).

Lysimeters are effective tools in assessing and predicting water and solute transport in soil. A wide range of lysimeter types exist, which are equipped with different measuring devices. Using a high precison weighing lysimeter, like we used in this thesis, excellent data for precipitation measurements or modeling purposes can be provided. The actual evapotranspiration can be calculated with a high accuracy from the weight (mass) change. However, drawbacks of these lysimeter type are the high construction costs and the effort for maintenance which makes it difficult for future long-term studies. The research done in this thesis clearly demonstrated the need for long-term studies evaluating the occurrence of degradation products in soil and leachate. Further research activities are necessary regarding a better connection of lysimeter studies with laboratory and field experiments. In addition, technical developments are needed in the field of lysimeter data management, especially the measuring frequency of parameters and the resulting data volume. The combination of lysimeter studies with field experiments opens new possibilities for modeling the dynamics of pesticides in soil. Consequently, preventive strategies can be adopted and assessed to avoid pesticide contamination.

In addition, results from lysimeter experiments under natural conditions become more and more important for simulation scenarios of current and future climatic and hydrological events. Natural phenomenons such as extremely hot weather or transport of contaminants during heavy rainfall events after drying-out of the soils force agriculture to adapt to impacts of climate change. Finally, lysimeters are also an essential tool to investigate the effects of climate change on soil hydrological processes (Groh et al. 2016).

Pesticide loads might be transported by water, soil and plant over years by dissolved substances directly or by disposing particle bound residues (Gevao et al. 2000). In addition, agricultural soils after being used for many years may contain multiple aged pesticide residues from applications of various pesticides that become stabilized by binding to the soil matrix. This may challenge the environmental risk assessment of the resulting mixture of long-term available pesticide residues in our agricultural soils.

To feed the growing world population, a number of innovations needs to be implemented to intensify agricultural production and simultaneously ensure environmental and human health protection. The safe use of pesticides appears as one of the biggest challenges of agricultural intensification. It is important to optimize the widespread precautionary use of pesticides. Appropriate mitigation practices such as pesticide application date shifts, applied dose reduction during rainy periods or tillage limitations in most erosion prone agricultural areas will help to avoid excessive concentrations in ground and surfacewaters. There is need for action than even after a stop of pesticide application, mobile metabolites can still reach ground and surfacewaters and thus should be considered for the evaluation of metabolite long-term dynamics.

Sustainable agriculture demands both conventional and organic farming to produce yields at affordable prices to ensure the livelihood of farmers along with efficient utilization of pesticides. Consequently, farmers should be supported to change their fertilization strategy. Organic farming limit the chemical applications but cannot be the only way to conventional farming. In addition, organic farming requires more land to produce the same quantity of food as conventional farming. Thus, it is quite difficult to ensure stable yields and quantity by reducing the use of conventional pesticides.

Another alternative to conventional pesticides for controlling plant pests are biopesticides. Biopesticides are naturally produced by living organisms like microorganisms, herbs and plants and thus are emerged as cost-effective and environment friendly alternatives to reduce pest damage without causing extensive damage to the surrounding environment. These alternatives to harmful pesticides can assist in changing the face of agriculture and make it more sustainable for future generations (Chandler et al. 2008; Kumar et al. 2019; Sharma et al. 2020).

The use of genetically modified crops would also reduce the environmental impacts associated with changes in pesticide use than most of these crops are herbicide or insecticide resistant. The cultivation areas of such plants are expanding globally every year especially in America and Asia (Brookes and Barfoot 2018). However, the release of genetically modified crops into the environment bore uncalculted risks for human health and thus is still prohibited in Austria.

Over the years, efforts have been made to develop nanopesticides that effectively protect crops against insect pests and diseases. Diverse materials for pesticide nanoformulations such as polymers, lipids, clays, metals and others were reported in excellent reviews (Kumar et al. 2019; Singh et al. 2020). The properties of nanomaterials suitable for their pesticide application include amongst others a large suface area, thermal stability, biodegradable nature and increased affinity to the target pest species. The application of nanotechnoly-based technologies offers solutions for agriculatural purposes to regulate the relases of active ingredients at the target site in designated manner. However, the use of nanopesticides in agriculture is still in the developmental stage.

There is a potential for optimizing and especially reducing the use of pesticides in agriculture, but research is needed to decide how this could be implemented. Pest monitoring systems and models should be developed to allowing forcasts for reducing the dosage of pesticides or the area that is treated. Integrated pest managemet concepts are needed to prevent not only the environment from potential harm but also to avoid the development of resistant pests, credibility and acceptance of plant protection concepts in public debates.

8 INDEXES

8.1 Literature

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8.2 Figures

8.3 Tables

APPENDIX

A1 Curriculum Vitae/Lebenslauf

Persönliche Daten

Name	Andrea Schuhmann
Geburtstag	20.01.1984
Geburtsort	Eisenstadt
Land	Österreich
Staatsbürgerschaft	Österreich
Familienstand	verheiratet
Universität	Universität für Bodenkultur Wien

Ausbildung

2009 – heute	Doktoratsstudium an der Universität für Bodenkultur Wien
2007 – 2009	Internationales Masterstudium „Natural Resources Management and Ecological Engineering, Universität für Bodenkultur Wien
2003 – 2007	Studium für Energie- und Umweltmanagement Fachhochschulstudiengänge Burgenland GmbH, Pinkafeld Thema der Diplomarbeit: "Biodegradation of maize oil in soil – Characterisation of microbial communities using PLFA technique"
1999 – 2003	Höhere Bundeslehranstalt für wirtschaftliche Berufe, Neusiedl/See

Beruflicher Werdegang

Mai 2016 - heute	Umweltbundesamt GmbH Koordination der Arbeitsgruppe Gaschromatographie
Okt. 2013 – Mai 2016	Umweltbundesamt GmbH Analytische Chemikerin in der Arbeitsguppe Flüssig- und Gaschromatographie
Juli 2009 – Okt. 2013	Umweltbundesamt GmbH Dissertantenstelle in den Arbeitsgruppen Pestizide und Flüssigchromatographie
Feb. – Juli 2007	Institut für Bodenforschung, Universität für Bodenkultur Wien Diplomarbeit im Rahmen des Forschungsprojektes "Characterisation of the microbial community during the degradation of vegetable oil in the soil"
Sept. 2006 – Jän. 2007	Institut für Bodenforschung, Universität für Bodenkultur Wien Studienbegleitendes Praktikum

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